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TOPIC REVIEW

Congress of neurological surgeons systematic review and evidence-based guidelines update on the role of emerging developments in the management of newly diagnosed glioblastoma

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Abstract

Target population These recommendations apply to adult patients with newly diagnosed or suspected glioblastoma.

Imaging

Question What imaging modalities are in development that may be able to provide improvements in diagnosis, and therapeutic guidance for individuals with newly diagnosed glioblastoma?

Recommendation Level III: It is suggested that techniques utilizing magnetic resonance imaging for diffusion weighted imaging, and to measure cerebral blood and magnetic spectroscopic resonance imaging of N-acetyl aspartate, choline and the choline to N-acetyl aspartate index to assist in diagnosis and treatment planning in patients with newly diagnosed or suspected glioblastoma.

Surgery

Question What new surgical techniques can be used to provide improved tumor definition and resectability to yield better tumor control and prognosis for individuals with newly diagnosed glioblastoma?

Recommendations Level II: The use of 5-aminolevulinic acid is recommended to improve extent of tumor resection in patients with newly diagnosed glioblastoma.

Level II: The use of 5-aminolevulinic acid is recommended to improve median survival and 2 year survival in newly diagnosed glioblastoma patients with clinical characteristics suggesting poor prognosis.

Level III: It is suggested that, when available, patients be enrolled in properly designed clinical trials assessing the value of diffusion tensor imaging in improving the safety of patients with newly diagnosed glioblastoma undergoing surgery.

Neuropathology

Question What new pathology techniques and measurement of biomarkers in tumor tissue can be used to provide improved diagnostic ability, and determination of therapeutic responsiveness and prognosis for patients with newly diagnosed glioblastomas?

Recommendations Level II: Assessment of tumor *MGMT* promoter methylation status is recommended as a significant predictor of a longer progression free survival and overall survival in patients with newly diagnosed with glioblastoma.

Level II: Measurement of tumor expression of neuron-glia-2, neurofilament protein, glutamine synthetase and phosphorylated STAT3 is recommended as a predictor of overall survival in patients with newly diagnosed with glioblastoma.

Level III: Assessment of tumor *IDH1* mutation status is suggested as a predictor of longer progression free survival and overall survival in patients with newly diagnosed with glioblastoma.

Sponsored by the American Association of Neurological Surgeons and Congress of Neurological Surgeons Joint Section on Tumors.

Reviewed for evidence-based integrity and endorsed by the American Association of Neurological Surgeons and Congress of Neurological Surgeons.

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Level III: Evaluation of tumor expression of Phosphorylated Mitogen-Activated Protein Kinase protein, EGFR protein, and Insulin-like Growth Factor-Binding Protein-3 is suggested as a predictor of overall survival in patients with newly diagnosed with glioblastoma.

Radiation

Question What radiation therapy techniques are in development that may be used to provide improved tumor control and prognosis for individuals with newly diagnosed glioblastomas?

Recommendations Level III: It is suggested that patients with newly diagnosed glioblastoma undergo pretreatment radiolabeled amino acid tracer positron emission tomography to assess areas at risk for tumor recurrence to assist in radiation treatment planning.

Level III: It is suggested that, when available, patients be with newly diagnosed glioblastomas be enrolled in properly designed clinical trials of radiation dose escalation, altered fractionation, or new radiation delivery techniques.

Chemotherapy

Question What emerging chemotherapeutic agents or techniques are available to provide better tumor control and prognosis for patients with newly diagnosed glioblastomas?

Recommendation Level III: As no emerging chemotherapeutic agents or techniques were identified in this review that improved tumor control and prognosis it is suggested that, when available, patients with newly diagnosed glioblastomas be enrolled in properly designed clinical trials of chemotherapy.

Molecular and targeted therapy

Question What new targeted therapy agents are available to provide better tumor control and prognosis for individuals with newly diagnosed glioblastomas?

Recommendation Level III: As no new molecular and targeted therapies have clearly provided better tumor control and prognosis it is suggested that, when available, patients with newly diagnosed glioblastomas be enrolled in properly designed clinical trials of molecular and targeted therapies

Immunotherapy

Question What emerging immunotherapeutic agents or techniques are available to provide better tumor control and prognosis for patients with newly diagnosed glioblastomas?

Recommendation Level III: As no immunotherapeutic agents have clearly provided better tumor control and prognosis it is suggested that, when available, patients with newly diagnosed glioblastomas be enrolled in properly designed clinical trials of immunologically-based therapies.

Novel therapies

Question What novel therapies or techniques are in development to provide better tumor control and prognosis for individuals with newly diagnosed glioblastomas?

Recommendations Level II: The use of tumor-treating fields is recommended for patients with newly diagnosed glioblastoma who have undergone surgical debulking and completed concurrent chemoradiation without progression of disease at the time of tumor-treating field therapy initiation.

Level II: It is suggested that, when available, enrollment in properly designed studies of vector containing herpes simplex thymidine kinase gene and prodrug therapies be considered in patients with newly diagnosed glioblastoma.

Keywords Glioblastoma · Imaging · Neuropathology · Surgery · Radiation therapy · Chemotherapy · Targeted therapy · Immunotherapy

Introduction

Rationale

Even in the most difficult diseases modern research techniques are making inroads into our understanding of the disease development in a manner that is improving or will impact our therapeutic approaches. Glioblastoma is no exception. The Cancer Genome Atlas project for glioblastoma has been a unifying work helping investigators from all over the world focus on the most promising avenues to attack this disease [1]. The literature on this matter is wide ranging provides considerable inspiration to those hoping to someday see this disease controlled [2, 3]. A few of these avenues are just reaching the point of refinement that allows meaningful application to humans. Though most of these are not ready for widespread and standard dissemination benefit can still be derived from their review. Clinicians involved in the management of newly diagnosed glioblastoma profit from learning of the trends in new therapeutic approaches so as to be better prepared if and when they come to general application. This guideline regarding emerging developments in the management of newly diagnosed glioblastoma is written to that end.

Objectives

To assess diagnostic and therapeutic techniques for the management of glioblastoma that have evolved since the last guidelines for new diagnosed glioblastoma were published to a point where human applications are being explored for value and efficacy.

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 newly diagnosed 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 topic at hand and conducted a systematic review of the literature relevant to emerging diagnostics and therapies in the management of newly diagnosed glioblastoma. Additional details of the systematic review are provided below and within the introduction and methodology chapter of the guideline [4].

Literature review

The task force collaborated with a medical librarian to search for articles published from July 1, 2005 to October 31, 2018 The PubMed and EMBASE data bases were searched. Strategies for searching electronic databases were constructed by the evidence-based clinical practice guideline taskforce members and the medical librarians using previously published search strategies to identify relevant studies. The EMBASE search delivered a greater breadth of literature and was primarily utilized. The search parameters were 'glioblastoma'/exp AND 'cancer therapy'/exp OR 'glioblastoma'/exp/dm_dt,dm_su,dm_th,dm_rt AND ('new diagnosis' OR 'newly diagnosed' OR 'initial diagnosis' OR 'first-line') AND [humans]/lim AND [english]/lim AND [embase]/lim AND [2007–2014]/py AND ('article'/it OR 'review'/it).

We supplemented searches of electronic databases with manual screening of the bibliographies of all retrieved publications. We also searched the bibliographies of recent systematic reviews and other review articles for potentially relevant citations. All articles identified were subject to the study selection criteria listed above. As noted above, the guideline committee also examines lists of included and excluded studies for errors and omissions. A strong effort is made to obtain a complete set of relevant articles as having a comprehensive set of relevant articles minimizes the chance that this guideline is based on a biased subset of articles. Use of the search strategy above and the secondary steps to capture more relevant articles yielded 1840 citations.

During the development process, multiple iterations of evidence tables and then the written review were conducted by the individuals of the writing panel, the CNS Committee and the Joint Guidelines Review Committee.

Study selection and eligibility criteria

1840 citations were manually reviewed by the team with specific inclusion and exclusion criteria as outlined below. Three independent reviewers reviewed and abstracted full text data for each article and the two sets of data were compared for agreement by a third party. Inconsistencies were re-reviewed and disagreements were resolved by consensus. Citations that addressed adult patients focusing on management of newly diagnosed glioblastoma were considered. To be included in our guideline, a publication had to meet the following criteria:

Inclusion criteria

- Published between July 1, 2005 and Octobert 31, 2018
- Published in English.
- Adult patients (age $\geq$ 18) with newly diagnosed glioblastoma were included in the study and the data on their outcomes could be separated from other histologies.
- Fully published (i.e., not in abstract form) peer-reviewed primary studies.
- Number of study participants with newly diagnosed glioblastoma $\geq$ 5.
- For studies with mixed histologic populations, baseline pretreatment and outcome information on study participants is provided for patients with newly diagnosed glioblastoma separate from other histologies.
- For studies with consideration of more than one treatment regimen, baseline pretreatment and outcome information on study participants is provided in a manner that can be separated by treatment.

Exclusion criteria

- in vitro only
- Animal only

We did not include systematic reviews, guidelines or meta-analyses conducted by others. These documents are developed using *different inclusion criteria* than those specified in our guideline. Therefore, they may include studies that do not meet our inclusion criteria. We did recall these documents if their abstract suggested that they might address

one of our recommendations, and we searched their bibliographies for additional studies.

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Data collection process

Those abstracts that met with the selection criteria mentioned above were retrieved in full text form. The adherence to the selection criteria were confirmed. To determine how the data could be classified the information in the full text manuscripts were then evaluated to determine whether they were providing results of therapy or were more centered on diagnostic or prognostic information. Agreement on these assessments and on the salient points regarding the type of study design and objectives, and conclusions and data classification was then reached by exchanging drafts and comments by e-mail. The information was then used for construction of the evidence tables.

Scientific foundation

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. In the paradigm for therapeutic maneuvers, evidence is classified into that which is derived from the strongest clinical studies (e.g., well-designed, randomized controlled trials), or Class I evidence. Class I evidence is used to support recommendations of the strongest type, defined as Level 1 recommendations, indicating a high degree of clinical certainty. Non-randomized cohort studies, randomized controlled trials with design flaws, and case–control studies (comparative studies with less strength) are designated as Class II evidence. These are used to support recommendations defined as Level 2 reflecting a moderate degree of clinical certainty. Other sources of information, including observational studies such as case series and expert opinion, as well as randomized controlled trials with flaws so serious that the conclusions of the study are truly in doubt are considered Class III evidence and support Level 3 recommendations, reflecting unclear clinical certainty. A summary of these categories of evidence can be viewed at https://www.cns.org/guidelines/guideline-procedures-policies/guideline-development-methodology.

Results

Imaging

Study selection and characteristics

Steps to accomplish data extraction included assessment of each abstract based on the above mentioned inclusion and exclusion criteria as could best be discerned. These same factors were used in detail for the subsequently chosen full text manuscripts. Additionally study design, number of institutions involved, the primary imaging modality used, the total number of patients per modality and group and the statistics used to compare them were extracted to complete the summary of the data in each manuscript.

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 97 were chosen as relevant to imaging for full text review and assessment. A total of 21 publications met the criteria for inclusion regarding the diagnostic value of imaging [5–25]. A total of 40 publications met the criteria for being useful for determining prognosis from imaging [26–65]. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Of the studies designed to diagnose glioblastoma with imaging five were done prospectively [5, 6, 9, 10, 19]. In none of these were the findings assessed in a blinded fashion against the known diagnosis or in a manner that allowed comparison parameters such as sensitivity and specificity. The other studies were retrospective in nature and thus being subject to the potential bias due to selective case choice for study and selective result reporting [7, 8, 11–18, 20–25]. Forty studies dealt with the ability of imaging to assess prognosis in patients with newly diagnosed glioblastoma. Seven were prospective in nature but the imaging assessments were not blinded allowing their interpretation by the investigator to possibly be biased.[28, 29, 31–33, 43, 48]. The others ten were retrospective in nature, leaving them subject to case selection bias, bias due lack or loss of information over time, the biases of the interpreting investigator in regard to the study and publication bias [26, 27, 30, 34–42, 44–47, 49–65].

Results of individual studies

The key results of the selected individual studies mentioned here are outlined in Tables 1 and 2.

Imaging for diagnosis

Positron emission tomography New agents labelled for detection by PET continue to be developed. Two prospective studies warrant mention. Tateishi et al. have demonstrated that ^{62}Cu-diacetyl-bis(N4-methylthiosemicarbazone) PET is able to differentiate glioblastomas from non-glioblastoma gliomas due to a higher SUV_{max} (1.68 + 0.94 and 0.98 + 0.52, respectively). The difference was significant (P = 0.03) [5]. Differentiating grade III tumors from glioblastoma by PET has been challenging. Hatakeyama et al. utilized ^{18}F-fluorothymidine (FLT) PET and were able show difference of FLT SUV_{max} and T/N ratio between grades III and IV gliomas was statistically significant (P < 0.01) [6] In a retrospective study of ^{11}C-acetate (ACE)-positron emission tomography in seven glioblastomas the SUV values were shown to be clearly higher than another set of grade II lesions [7] However this technique was not able to provide SUV values that were clearly different than grade III tumors. In none of these publications were the scans read in a blinded fashion and data was not available in a manner that allowed calculation of comparative parameters. Thus they yielded class III data.

Magnetic resonance imaging Differentiation of tumor progression from pseudoprogression during the early stages of therapy is a point of ongoing research. The use of normalized cerebral blood volume calculated from dynamic susceptibility contrast perfusion MRI was investigated retrospectively in 135 patients by Baek et al. [8]. The shape of the derived normalized cerebral blood volume histogram was found to be correlated strongly with the diagnosis of pseudoprogression. Specifically those cases with positive skewness that were leptokurtic were associated with pseudoprogression in 23 of 24 cases. The interpretation of the results were not blinded and the manuscript and did not provide information in a manner that allowed calculation of sensitivity and other associated parameters resulting in class III data.

Another study of perfusion parameters showed that diffusion-weighted MRI and MR spectroscopy using Cho:NAA and Cho:Cr ratios correlates with tumor grade, differentiating between GBM and lower grade glioma [9]. A pilot study of dynamic glucose-enhanced (DGE) MRI has also been performed to aid in detecting glioblastoma involved tissue from heatlhy white matter. The authors performed DGE MRI in 9 newly diagnosed glioblastoma patients and found higher concentrations of glucose in abnormal appearing tissue compared to healthy-appearing white matter [10].

Molecular subtyping of GBM by MRI parameters is also being investigated. Kong et al. in a single institution, retrospective study of 65 GBM patients evaluated pretreatment MRIs for 82 radiomic features and placed patients into 3 consensus clusters, which were correlated with molecular

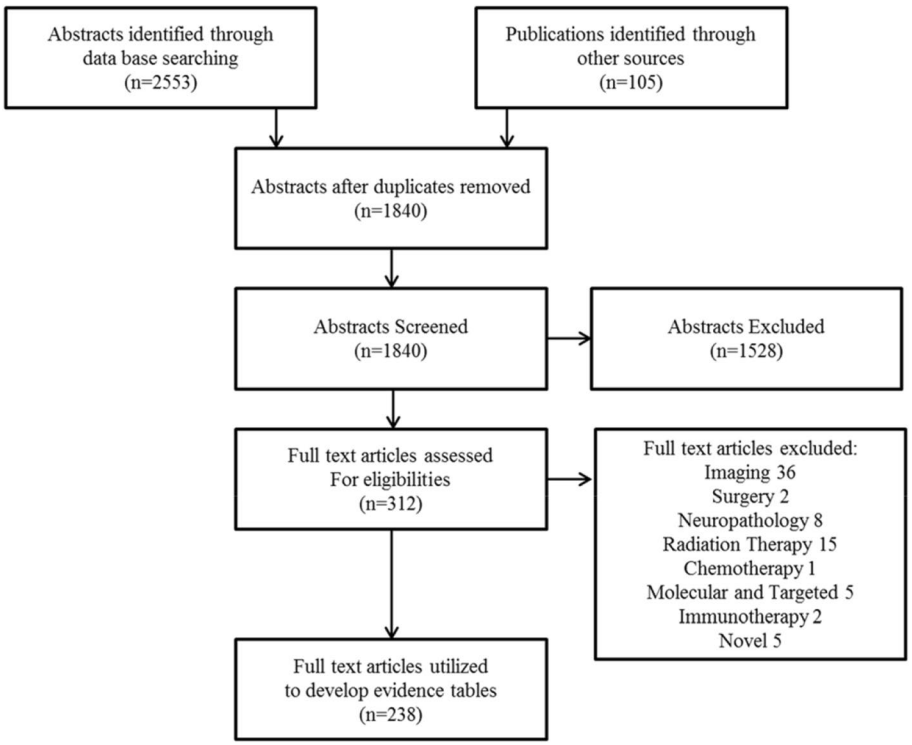

Fig. 1 Flow diagram reflecting literature review and selection

subclassification of their GBM. They found that radiomic feature clustering could be correlate with 3 subtypes: classical, proneural, and mesenchymal allowing for subtyping based on pretreatment MRI parameters [11]. Another study correlated a lower mean ADC with predicting neural subtype of GBM [12]. In one single center, retrospective study of 260 newly diagnosed GBM patients, the $EGFR^{A289D/T/V}$ mutation was associated with several MRI parameters including increased relative contrast enhancement, lower T1 signal values, increased T2 signal values, and a higher rCBV [13]. EGFRvIII expression has also been correlated with presurgical MRI findings, particularly the peritumoral heterogeneity index [14].

In another single institution retrospective study pretreatment MRIs of 18 GBM patients were evaluated for amide proton transfer-weight (APTw) metrics and this was correlated with MGMT promoter methylation status. In unmethylated patients, APTw parameters were significantly higher than in methylated patients ($p < 0.05$) indicating this maybe a non-invasive means of determining MGMT promoter methylation status [15]. Another retrospective single center study of 98 GBM patients evaluated pretreatment MRIs for 1665 radiomic features and found a combination of 36 features had an accuracy of 86.6% in predicting MGMT promoter methylation status [16].

Determination of IDH1 status in GBM based on MRI parameters is also being investigated. In a retrospective, single center study of 153 GBM patients greater than 33% non-contract-enhancing tumor along with a mass-like morphologic pattern was found to improve specificity and accuracy in predicting IDH1 status, though the results of this study did not reach significance [17]. Another retrospective, single center study of 44 GBM patients who underwent presurgical MR spectroscopy for 2-hydroxyglutarate (2-HG) found that 2-HG accumulation was higher in IDH1 mutant GBM compared to IDH wildtype GBM (3.191 vs 0 mM, $p < 0.001$) [18]. DTI phenotype has also been linked to IDH1 mutation status in one retrospective, single center study [19].

Pretreatment MRI features are also being used to differentiate the diagnosis of GBM from Primary CNS lymphoma (PCNSL). In a retrospective, single center study, 143 patients with GBM and PCNSL had their pretreatment MRIs evaluated for 127 radiomic features and these were correlated with pathologic diagnosis. This was then confirmed in a validation group. 15 features were found through logistic regression to correlate with diagnosis with an Area under the curve of 0.979, sensitivity of 0.938, and specificity of 0.944 in the discovery cohort then similar findings in the validation cohort [20]. Features on dynamic susceptibility contrast MRI have also been shown to have significant differences between PCNSL and GBM [21]. Image texture analysis by machine learning of diffusion weighted and perfusion MRI in a retrospective, single center study was also shown to differentiate between GBM and PCNSL [22]. Diffusion weight imaging parameters have also been shown in a retrospective, single center study to correlate with diagnosis of GBM vs PCNSL. PCNSL had a lower rADC, higher k^{trans}, and higher V^e than GBM [23].

BOLD based MRI has been used in a retrospective, single center study by Wiestler et al. to performed relative oxygen extraction fraction to differentiate between lower grade glioma and GBM. The authors used a random forest oblique machine learning classifier with this data to correctly identify WHO grade in 34 of 37 patients [24].

Another study investigated the usefulness of combining the input of multiple tumor-focused neurosurgeons, they call "crowd-sourcing," in helping determine resectability of a glioblastoma. In this pilot, retrospective study they found good interrater reliability in determining resection vs biopsy only. Greater variability was seen when determining if subtotal or gross total resection was the goal of surgery [25].

Imaging for prognosis

Positron emission tomography In a single institution, retrospective FDG PET study of 31 patients with newly diagnosed glioblastomas Colavolpe et al. measured the ratio between the tumor and contralateral maximal standardized uptake value (T/CL ratio), event free survival and overall survival. In this class III study they found that The T/CL ratio predicted overall survival in the glioblastoma subgroup ($P = 0.018$), independently of age, Karnofsky performance status, histological grade, surgery, and recursive partitioning analysis classification [26]. A larger biologic tumor volume on postoperative FET-PET Imaging was associated with a worse progression free survival and overall survival in one single center, retrospective study [27]. ^{18}F-FCho PET is being investigated for response to radiation. In a small single center study by Bolcaen et al., patients with GBM were followed prospectively with ^{18}F-FCho PET. They found that certain parameter changes in ^{18}F-FCho PET 1 month after radiation correlate with response at 6 months after therapy [28]. Features of hypoxia in residual tumor as seen on ^{18}F-FMISO PET in newly diagnosed GBM patients were found in one prospective study to correlate with a shorter overall survival [29]. Similar results were seen in a retrospective study [30].

Magnetic resonance imaging Three prospective studies analyzing magnetic spectroscopic imaging (MRSI) and dynamic susceptibility contrast imaging (DSCI) met inclusion criteria. In a prospective set of 14 newly diagnosed glioblastomas a magnetic resonance spectroscopic imaging of sites with lactate-to- N-acetyl-aspartate ratio of 0.4 or above in preradiotherapy residual tumor significantly predicted the site of relapse with 88.8% sensitivity and 97.6%

Table 1 Evidence for diagnosis based on imaging

Author/year	Study description	Data class	Conclusion
Binder et al. (2018)	A retrospective, single center study of 260 newly diagnosed primary GBM patient comparing OS in those baring EGFR mutations at alanine 289 (6% of study population) vs wildtype (WT) EGFR The contrast enhanced MRIs were then compared to determine differences in WT EGFR GBM vs EGFRA289D/T/V mutant patients. The authors further evaluated these differences in mice studies The outcome was OS as well as MRI parameters	III	The median OS in the WT patients was 15 months compared to the OS of 6 months in patient with the EGFRA289D/T/V mutation ($p=0.028$). The MRI parameters associated with the EGFRA289D/T/V mutations were increased relative contrast enhancement, lower T1 signal values, increased T2 values, and higher rCBV The authors concluded that EGFRA289D/T/V is a clinically significant mutation in patients with wildtype IDH1 primary GBM with a negative survival impact and MRI parameters that suggest hyperproliferation and increased invasion This study has a small sample size of affected patients and a retrospective design
Jiang et al. (2018)	A single center retrospective study of patients with newly diagnosed GBM undergoing pretreatment MRIs. The MRIs were processed for amide proton transfer–weight (APTw) metrics The APTw metrics were then analyzed to evaluate for correlation with MGMT promoter methylation status of the tumors	III	10 MGMT promoter methylated GBM and 8 unmethylated GBM patients were included. For unmethylated patients APTw parameters (variance, mean, 50th percentile, 90th percentile, and width) were higher than in MGMT promoter methylated patients ($p<0.05$) The authors conclude that APTw MRI metrics may be a non-invasive indicator of MGMT promoter methylation status in GBM patients This study is retrospective and has a small sample size
Lasocki et al. (2018)	A single center, retrospective study of MRIs on 153 newly diagnosed GBM to evaluate for parameters in the noncontrast-enhancing tumor (nCET) associated with IDH1 mutation status. In patients with > 33% nCET, MRIs were examined for morphologic patterns	III	In patients with > 33% nCET, a longer OS was observed with a mass-like morphologic pattern, though this did not reach significance. The combination of > 33% nCET and a mass-like pattern was found to improve specificity and accuracy in predicting IDH1 status The authors conclude the nCET area exhibits different morphologic patterns This study did not have statistically significant results and only 5 patients had IDH1 mutation and > 33%nCET
Natsumeda et al. (2018)	A single center, retrospective study of 44 consecutive GBM patients who underwent pre-operative MR spectroscopy for 2-hydroxyglutarate (2HG) followed by surgery and chemoradiation. Tissue samples were analyzed for IDH1 mutant status. MRS findings were then correlated with IDH1 status and OS	III	IDH1 mutant GBMs ($n=6$) had significantly higher 2HG accumulation (3.191 vs 0 mM, $p<0.001$) with a sensitivity of 100% and specificity of 92.59% in predicting IDH1 mutant status. No significant difference in OS was seen based on 2HG accumulation levels The conclusion was 2HG on MRS can aid in determining IDH1 mutant status This study is retrospective
Kim et al. (2018)	A single center, retrospective study of 143 patients with glioblastoma and primary CNS lymphoma to evaluate radiomic features that differentiate the diagnosis. The patients were separated into the discovery group (86) and validation group (57). 127 radiomic features were calculated	III	In the discovery group, 15 features were identified through logistic regression. In the discovery cohort, these features had an AUC of 0.979, sensitivity of 0.938, and specificity of 0.944 which was confirmed in the validation cohort ($AUC=0.956$) The authors conclude that radiomics-based approach can help distinguish PCNSL from GBM This is a retrospective study
Lee et al. (2018)	A single center, retrospective study of 89 newly diagnosed GBM and 30 PCNSL patients with pretreatment MRIs available that showed hypervascular foci to determine features on of vascular permeability and perfusion on Dynamic Susceptibility Contrast (DCS) MRI that could distinguish the diagnosis	III	PCNSL showed a significantly shorter MTT ($p<0.01$) and higher extraction fraction ($p<0.01$) and CBF ($p=0.01$) than GBM. No significant difference was seen in CBV Authors conclude vascular permeability on DSC-MRI further characterizes PCNSL This is a retrospective study

Table 1 (continued)

Author/year	Study description	Data class	Conclusion
Nakagawa et al. (2018)	A retrospective, single institution study of 70 patients with MRI including DWI and perfusion studies prior to surgical intervention with histopathology confirmed GBM (45) and PCNSL (25). MRI based image texture analysis (ITA) was then performed and then machine learning used to determine if differences between PCNSL and GBM	III	With analysis in machine learning, significant differences in rCBV, ADC, CET1, T2W values between PCNSL and GBM were found. Machine learning was shown to be superior to 2 board certified radiologists The authors concluded that MRI-based ITA machine learning maybe useful to differentiate GBM from PCNSL This is a retrospective study
Kong et al. (2018)	A retrospective, single center study of 65 MRIs of pretreatment GBM patients evaluating for 82 radiomic phenotypes placed into 3 consensus clusters which were then correlated with gene expression and molecular classification of GBM	III	The clusters (Groups 1–3) based on radiomic features were significantly associated with GBM classification: Group 1 with Classical, Group 2 with Proneural, and Group 3 with Mesenchymal
Bakas et al. (2017)	A retrospective study of 142 patient with primary GBM evaluating with preoperative MRI data to determine feature distinguishing tumors with EGFRvIII expression, as confirmed by histopathology, vs those without The outcome was patterns on MRI associated with EGFRvIII status	III	The Peritumoral Heterogeneity Index (PHI) was determined in a discovery cohort of 64 patient followed by a replication cohort of 78 patients. Significantly distinct distributions were found between WT patients and positive EGFRvIII patients. Accuracy, sensitivity, and specificity were all > 0.8 in both the discovery and replication cohorts The authors concluded that PHI is an imaging signature for the EGFRvIII status of primary GBM patients
Aggarwal et al. (2017)	A prospective study evaluating the ability of DWI and MRS to grade glial tumors. ADC values were obtained from DWI and Cho: NAA and Cho:Cr ratios were obtained The sensitivity, specificity, NPV, and PPV values of these features to determine grade was then calculated	III	GBM had significantly lower ADC values and higher Cho:NAA and Cho:Cr ratios than low grade or anaplastic astrocytoma. The sensitivity and specificity of these 3 features were all over 70% except the specificity of ADC was only 60% in predicting grade of tumor The authors conclude MRS and DWI have a role in grading brain tumors Radiologists were not blinded to pathology results, determination of tumor grade based on MRI not performed in prospective fashion
Paech et al. (2017)	A prospective observational study that included 9 GBM patients to determine if increased glucose concentration is seen within tumor tissue with T1 dynamic glucose-enhance (DGE) MRI	III	DGE = 2.02% within GBM which was significantly higher than the DGE = 0.08% seen in contralateral normal-appearing white matter ($p < 0.0001$) DGE = 2.02% within GBM which was significantly higher than the DGE = 0.08% seen in contralateral normal-appearing white matter ($p < 0.0001$) This study has a very small size and is pilot in form
Xi et al. (2017)	A retrospective, single center study of 98 GBM patients with pretreatment MRI available, which were evaluated for 1665 radiomic features then compared with MGMT promoter methylation status	III	A combination of 36 T1 and T2 features along with enhanced T1 features had an accuracy of 86.59% in predicting MGMT promoter methylation status The authors concluded radiomic features of MRI might predict MGMT methylation status This is a retrospective study with a small validation group of 20 GBMs

Table 1 (continued)

Author/year	Study description	Data class	Conclusion
Price et al. (2017)	70 patients with new diagnosis of GBM were prospectively observed after undergoing maximal surgical resection assisted by 5-aminolevulinic acid followed by chemoradiation then followed with MRI. IDH1 mutation status was determined in all patients. The DTI phenotype for each patient was then determined and compared to their IDH1 status	III	Based on DTI, For IDH1 wild-type patients, 42 patients were determined to be diffusely invasive, 14 locally invasive, and 5 minimally invasive. All 9 IDH1 mutant GBM patients showed a minimally invasive phenotype on DTI (significant difference in distribution, $p < 0.001$) The authors concluded that IDH1 mutant patients have a less invasive phenotype on DTI This study only has 9 patients in the group of interest
Lu et al. (2017)	A retrospective, single center study of 42 GBM patients and 18 PCNSL patients comparing DWI/ADC parameters to evaluate for differences to aid in pre-operative diagnosis	III	PCNSL had significantly lower rADC, higher Ktrans, and higher Ve than GBMs The authors conclude ADC can be useful for differentiation GBM and PCNSL This study is retrospective and small study patient numbers
Sonabend et al. (2017)	Pilot retrospective study of 20 newly diagnosed GBM patients whose MRI studies were evaluated by 13 academic neurosurgeons for resectability. The surgical goals (GTR, STR, or biopsy) and assessment of residual were then compared. The goal was determine feasibility of crowdsourcing; using a group for surgical decision making	III	Interrater reliability between resection vs biopsy was $k = 0.286$. Greater variability was seen between GTR and STR. Additionally a correlation between the surgical goal and EOR was found The authors conclude that crowdsourcing to predict volumetric EOR could be useful for determining resectability This is a small and retrospective study
Jajamovich et al. (2016)	Using 50 patients with MRI data available on TCGA, this study compared ADC histograms to GBM subtype to determine characteristics that predict subtype	III	A lower mean ADC was a significant predictor of neural subtype of GBM vs. non-neural ($p = 0.02$). Mesenchymal, classical, and proneural could not be significantly associated The authors conclude that ADC maps can predict neural subtype of GBM The study is retrospective and only 1 subtype was significantly identified
Wiestler et al. (2016)	A retrospective study of 37 newly diagnosed glioma patients, 27 GBM and 10 Grade II/III glioma using BOLD based MRI to measure relative oxygen extraction fraction (rOEF) and perfusion to determine differences in lower grade glioma and GBM	III	A random forest oblique machine learning classifier with the rOEF and perfusion data was able to correctly identify WHO grade in 34 of 37 patients The conclusion reached by the authors is multimodal MRI advances hold insight in underlying tumor biology This study is a pilot study that is retrospective in nature
Tateishi et al. (2014)	A prospective, single institution study of malignant brain tumors imaged with ^{62}Cu-diacetyl-bis(N^4-methylthiosemicarbazone) (^{62}Cu-ATSM)-PET. This included 10 newly diagnosed glioblastomas and 13 grade II and grade III lesions. These same patients were imaged with FDG-PET and L-methyl-[^{11}C]methionine (MET)-PET and then the imaging characteristics assessed to determine which method is most useful in providing a diagnosis of glioblastoma	III	The average ^{62}Cu-ATSM SUV_{max} values in GBM and non-GBM gliomas were 1.68 ± 0.94 and 0.98 ± 0.52, respectively. The ^{62}Cu-ATSM SUV_{max} was significantly higher for GBM than for non-GBM gliomas ($P = .03$). These differences are similar to those seen with (MET)-PET and superior to those identified by FDG-PET The authors note that (^{62}Cu-ATSM)-PET sees hypoxic regions in the tumor in better detail and may facilitate treatment planning for therapies dependent on this parameter The data provided yields class III evidence as the studies scanning technique is not assessed in blinded fashion against more commonly used imaging

Table 1 (continued)

Author/year	Study description	Data class	Conclusion
Baek et al. (2012)	This is a single institution, retrospective study of 135 newly diagnosed glioblastoma patients treated with concurrent radiation and temozolomide. Of these, 79 were found to have enlarged contrast-enhancing lesions after completion of radiation and then underwent another imaging evaluation 4–8 weeks later. These lesions were assessed by dynamic susceptibility contrast (DSC) perfusion MRI and perfusion parametric maps of relative normalized cerebral blood volume (nCBV) was calculated. The skewness and kurtosis of nCBV histograms were calculated for each first and second follow-up DSC MR perfusion image and then the percent change in each of these parameters was calculated. On the basis of histographic patterns of nCBV histograms, enrolled patients were classified into four categories: those cases with positive skewness that were leptokurtic (category 1) or platykurtic (category 2), and those with negative skewness that were leptokurtic (category 3) or platykurtic (category 4)	III	Pseudoprogression was observed in 23 of 24 (95.8%) patients in category 1, 10 of 15 (66.7%) in category 2, four of 20 (20.0%) in category 3, and 0 of 20 (0%) in category 4 (×2 test, P < .0001). The histographic pattern of nCBV was the best independent predictor (odds ratio, 3.51; P = .0032) for early tumor progression, rather than each percent change of skewness or kurtosis; the histographic pattern of nCBV represented the largest area under the receiver operating characteristic curve (0.934; 95% confidence interval: 0.855, 0.977), with a sensitivity of 85.7% and a specificity of 89.2% As a diagnostic test for pseudoprogression this represents class III data as the assessment was not blinded and it has not been applied prospectively in a matter where one could calculate sensitivity, specificity, positive and negative predictive values, and where applicable, likelihood ratios
Hatakeyama et al. (2008)	A single institution, prospective study of 41 newly diagnosed glioma patients with ^{11}C-methionine (MET) and ^{18}F-fluorothymidine (FLT) PET. Thirteen were glioblastoma patients and eight of the thirteen were studied with both types of PET	III	The difference of MET SUV_{max} and T/N ratio between grades II and IV gliomas was statistically significant (P < 0.001). The difference of FLT SUV_{max} and T/N ratio between grades III and IV gliomas was statistically significant (P < 0.01) The authors state that most, but not all, scans were done preoperatively and do not state which cases were studied when The timing of the PET studies was not well delineated and both study markers were not used in every patient, making statistical assessment more difficult This study provides class III data as the PET assessments here were not blinded and were not applied prospectively in a matter where one could calculate sensitivity, specificity, positive and negative predictive values, and where applicable, likelihood ratios
Yamamoto et al. (2008)	This is a retrospective, single institution study of ^{11}C-acetate (ACE)-positron emission tomography (PET) and ^{11}C-methionine (MET) and 2-deoxy-2-^{18}F-fluoro-D-glucose (FDG) in fifteen patients with newly diagnosed gliomas, of which seven were glioblastomas. The authors sought to investigate the usefulness of ACE-PET for the evaluation of brain glioma and differentiating high-grade gliomas, in comparison with MET and FDG-PET	III	The mean (±SD) ACE SUV in grade IV gliomas (3.75 ± 1.65) was significantly higher than that in grade II gliomas (1.40 ± 0.69; p < 0.02), but not significantly different from that in grade III gliomas (1.78 ± 0.30) The mean (±SD) MET SUV in grade IV gliomas (5.64 ± 0.99) was significantly higher than that in both grade II gliomas (2.91 ± 1.78; p < 0.02) but not significantly different from that in grade III gliomas (3.42 ± 1.08) The mean (±SD) FDG T/N ratio in grade IV gliomas (1.45 ± 0.52) was significantly higher than that in grade II gliomas (0.63 ± 0.08; p < 0.01) and grade III gliomas (0.71 ± 0.13; p < 0.04) The authors conclude that ACE-PET is potentially useful for diagnosis and grading of gliomas No prospective or blinded assessment of ACE-PET is provided. Sensitivity but not specificity or other statistical parameters can be calculated here; therefore this paper provides class III data

ACE ^{11}C-acetate; *DSC* dynamic susceptibility contrast; *FDG* fluorodeoxyglucose; *FLT* fluorothymidine; *GBM* glioblastoma; *MET* methionine; *nADC* normalized apparent diffusion coefficient; *nCBV* normalized cerebral blood volume; *PET* positron emission tomography; *PFS* progression free survival; *SUV_{max}* maximum intensity of the standard uptake value; *T/N ratio* the ratio of tracer uptake in the tumor to normal brain parenchyma; *TMZ* temozolomide

Table 2 Evidence for prognosis determination based on imaging

Author/year	Study description	Data class	Conclusion
Beig et al. (2018)	A retrospective study of GBM patients in the Cancer Imaging Archive (TCIA) investigating pre-treatment MRI scans for surrogate markers of hypoxia through 30 radiomic features. The authors determine a hypoxia enrichment score (HES) based on RNA data for 21 hypoxia-associated genes. Patients were divided into short-term (STS), mid-term (MTS), and long-term (LTS) survivors The hypoxia-radiomic surrogate markers were then placed through a training and validation set to determine prediction of OS	III	8 radiomic features were identified as associated with the HES. These features in both the training and validation sets were found to be able significantly differentiate between STS and LTS as well as between MTS and LTS The authors conclude that radiomic features of the MRI of treatment naïve GBM patients can predict extent of hypoxia and OS This study is limited by its retrospective nature and does not subgroup patients by molecular markers
Leu et al. (2018)	A single center, retrospective study of 61 patients with GBM who underwent pre-operative MRI to determine if tumor area (TA), as calculated by the ABC/2 method on contrast-enhancing T1 images, correlates with the primary endpoint, OS	III	The mean tumor volume was 18.2 mL. TA over 200 mm2 is associated with a worse prognosis (HR1.096, $p < 0.01$) Authors conclude that TA maybe a prognostic factor for patients with GBM This study is retrospective
Blomstergren et al. (2018)	Imaging from seventy GBM patients prior to surgery and treatment were retrospectively reviewed with their MRIs undergoingt quantitative radiological assessment (QRA) to determine residual tumor volume (RTV) and EOR. This was then correlated with primary endpoints:PFS and OS	III	In patients with EOR > 96% had a significantly increased PFS at 1 (HR of 2, $p = 0.017$), 1.5, and 2 years compared to < 96%. This was also seen with multivariate regression analysis in a 2-year OS model ($p = 0.043$) The authors conclude that EOR as determined by QRA can be a useful prognostic indicator This a retrospective study and therefore class III data
Juan-Albarracin et al. (2018)	A single institution, retrospective study of 50 patients with newly diagnosed GBM confirmed by histopathology then treated with standard chemoradiation who had pretreatment contrast enhanced MRI available for calculation of perfusion parameters These parameters were then correlated with the primary endpoint, OS	III	In both high-angiogenic tumors and low-angiogenic tumors, high rCBV-max and rCBFmax was significantly associated with a worse OS, with an average difference of 230 days in OS ($p < 0.05$) The authors conclude that preoperative vascular heterogeneity is associated with OS This study is limited by its retrospective design
Perez-Beteta et al. (2018)	A multicenter retrospective study of 165 GBM patients whose MRI were evaluated for geometric measures to determine surface regularity. A validation cohort from TCGA was used. This was correlated with OS	III	Surface regularity was found to significantly correlated with survival (HR 1.61, $p = 0.005$). Additionally, patients with surface regularity benefits more from complete resection than those with irregular surfaces The authors concluded surface regularity is a predictor of survival This is a retrospective study
Soike et al. (2018)	A retrospective, 2 center study of 82 patients with previously untreated GBM who underwent surgery, their tissue was evaluated for TCGA subtype, IDH1 status, and MGMT promoter methylation status, followed by standard chemoradiation to determine if molecular subtypes were associated with imaging response (IR) or pseudoprogression (PSP)	III	Patients with IR had an improved OS (29.4 months vs 14.5 months, $p < 0.01$). Patients with a proneural TCGA subtype were more likely to have an IR (60% vs 28%, $p = 0.03$). MGMT promoter methylation was associated with IR (58% vs 24%, $p = 0.032$) This is a retrospective study will small numbers in each group analyzed)
Dehkordi et al. (2017)	A retrospective, single center study of 33 newly diagnosed GBM patients who underwent dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) prior to any therapy. The DCE-MRI was used in Adaptive Neuro-Fuzzy Inference System (ANFIS) based on several parameters to evaluate its predictive ability for OS Outcome was survival time divided into three categories: Class 1 (short-term, < 6 months), Class 2 (medium-term, between 6–25 months), and Class 3 (long-term, > 25 months)	III	The ANFIS showed feasibility in function and predicting outcomes into 3 classes of survival. They also found age was a key significant factor associated with OS The authors concluded that development of a neuro-fuzzy-based model for predict outcome was possible This study is retrospective and the testing set had a limited number of images

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Bette et al. (2017)	A single center, retrospective study of 70 patients with newly diagnosed glioblastoma to investigate the relationship between the fractional anisotropy (FA) in the non-enhancing peritumoral region (NEPTR) and later tumor recurrence Five ROIs were taken in each patient and the FA was measured, final outcome was tumor recurrence	III	52.6% of ROIs later showed no tumor recurrence, 47.4% later measured tumor recurrence. FA was shown to be significantly lower in pre-operative NEPTR areas that later showed tumor recurrence The authors conclude lower FA in the NEPTR areas correlate with areas of later recurrence This a retrospective study
Amelot et al. (2017)	A retrospective, single center study of 234 GBM evaluating the pre-operative tumor invasiveness profile based on net proliferation rate and propensity to migrate to determine if GTR benefits only nodular tumors The outcome was OS	III	Overall patients undergoing GTR (median 2.8 years) has a significantly longer survival than partial resection (1.06 years) or biopsy only (0.622 years) ($p < 0.001$). When the GTR patients were separated by diffuse or nodular tumor invasiveness, no significant difference was seen in OS The authors concluded patients benefit from GTR with both nodular and diffuse GBM The study is retrospective
Choi et al. (2017)	A single center, retrospective study of 88 newly diagnosed GBM patients with known MGMT methylation status to determine if the area under the curve (IAUC) of their preoperative contrast-enhanced MRI correlates with PFS and OS	III	In patients with unmethylated MGMT promoter, a higher IAUC was significantly associated with a worse OS (IAUC30mean HR 3.04, $p = 0.008$, IAUC60mean HR 3.48, $p = 0.015$) and PFS (IAUC30mean HR 2.60, $p = 0.010$, IAUC60mean HR 2.15, $p = 0.015$). This was not found in MGMT promoter methylated patients The authors conclude that IAUC may be a useful prognostic factor in patients with unmethylated promoters This study is limited by its retrospective design and small size
Henker et al. (2017)	Single center study of 30 patients followed prospectively with newly diagnosed GBM who all underwent GTR followed by standard chemoradiation to determine pre-operative MRI features associated with prognosis. MRI parameters calculated included peritumoral edema (PTE), necrosis volume, necrosis-tumor ratio (NTR), and edema-tumor ratio (ETR) The primary endpoint was OS	III	NTR was found to be significantly associated with OS on univariate regression analysis ($p = 0.023$). Tumor volume ($p = 0.041$) and necrosis volume ($p = 0.039$) were also found to significantly influence OS on multiple linear regression analysis The authors conclude pre-treatment MRI necrosis and NTR are associated with a worse OS The parameters were determined in a retrospective fashion and as all patients were amendable to GTR, the results are not generalizable to all GBM patients
Krishnan et al. (2017)	Retrospective, single center study of 45 patients with GBM and post-surgical, pre-radiation MRIs in which restriction spectrum imaging (RSI), ADC-based hypercellularity volume fraction and intensities on contrast-enhanced and FLAIR were calculated. Primary endpoint was OS	III	On multivariate analysis, RSI on FLAIR was significantly associated with OS, higher intensity being correlated with shorter survival The authors conclude that post-surgical, pre-radiation RSI on FLAIR may be used for risk stratification in GBM patients This study is retrospective

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Bolcaen et al. (2017)	Eleven patients who underwent biopsy or debulking only of their GBM followed by treatment with temozolomide and radiation were followed prospectively. They underwent 18F-Fcho PET imaging before treatment, every 2 weeks during radiation and 1 month after radiation along with a thin-sliced MRI with and without contrast. RANO criteria were evaluated in each patient 6 months after treatment and they were divided into responders (R-partial and complete response) and nonresponders (NR-stable or progressive disease) They evaluated for differences in contrast-enhancing tumor volume (GdTV), metabolic tumor volume (MTV), and standardized uptake value (SUV) at all time points between R and NR	III	Four patients were responders (R) and 7 were nonresponders (NR). Of the calculations performed, the only significant differences ($p < 0.01$) seen was MTV x SUVmean 1 month after radiation therapy being higher in the NR group then R, the change in SUVmean between week 4 of radiation and 1 month following was higher in the R group then NR, and in the R group changes in GdTV group were higher than in the R between several time points. Using this, the authors determine a value of 7.6ccm of MTV x SUVmean as the cut-off to differentiate R and NR at 1 month after radiation. They also found several levels of decreased in GdTV that was sensitive and specific in differentiating R and NR The authors conclude MRI and 18F-FCho PET 1 month after radiation can predict 6 months response to therapy This study is limited by its small sample size and limited follow-up
Perez-Beteta et al. (2017)	A multi-center retrospective study of 117 GBM patients who pretreatment post-contrast MRIs were evaluated for several parameters include contrast enhancement (CE), maximal diameter, CE "rim", and geometric measures of the CE "rim." The primary endpoint was OS	III	OS was increased significantly with decreasing total volume, CE volume, geometric heterogeneity, and size of CE "rim." The authors conclude these MRI features are of prognostic significance This is a retrospective study
Mistry et al. (2017)	A retrospective, single center study of 207 GBM patients with pre-operative MRIs to determine correlation of contact with neurogenic zones: ventricular-subventricular (VSVZ), subgranular (SGZ), and corpus callosum (CC) with PFS and OS	III	On multivariate analysis, only contact with VSVZ was associated with a worse OS and PFS The authors conclude VSVZ contact is associated with a decreased survival This is a retrospective study
Muller et al. (2017)	A prospective observational study of 18 patients with newly diagnosed GBM. MRIs were taken before and after surgery and every 6 weeks during radiation then every treatment change. The T1W MRIs were then evaluated for cloudy-enhancement by subtracting the pre and post T1W MRIs. The primary endpoint was PFS	III	A decrease in volume of 21.4% of more of the cloudy-enhancement region was significantly associated with a longer PFS ($p = 0.038$) The authors conclude that cloudy-enhancement may be a method to determine response to therapy This study is retrospective and results were PFS
Liu et al. (2016)	A retrospective study of 69 GBM patients in one institution and 48 TCGA GBM patients evaluating presurgical MRI for angiogenic features on perfusion-weight imaging (PWI) and correlating angiogenic features with OS and response to antiangiogenic therapies. In each cohort, 2 groups were formed by consensus clustering with Cluster I being low angiogenic features and Cluster II having high angiogenic features	III	Cluster I patients had a significantly improved survival to Cluster II patients. Within Cluster II patients, those given antiangiogenic therapies (552.5d) had a significantly improved OS compared to those who did not (178d) ($p = 0.022$). This effect was not seen when Cluster I was included The authors conclude an angiogenic subtype of GBM may have a better response to antiangiogenic therapy This is a retrospective study of 2 small cohorts
Zhou et al. (2016)	A retrospective study on 2 datasets, 32 GBM patients from TCGA and 22 GBM patients from another institution with pretreatment and OS data available who had their MRIs evaluated by computational framework using spatial mapping and intratumoral groups to identify GBM tumor subregions and determine if these predict survival	III	In the datasets, this method of identifying GBM tumor subregions had a 87.50% and 86.36% accuracy in the 2 datasets for predicting survival group The authors conclude that this a novel method to predict survival This is a retrospective study

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Ingrisch et al. (2016)	A retrospective, single institution study of 66 pretreatment patients with GBM evaluating MRI radiomic analysis with OS. Patients were divided as high or low predicted risk based on random survival forests of the radiomic features	III	A significant difference ($p < 0.01$) was seen in OS curves between the high and low predicted groups The authors conclude that radiomic features of pretreatment MRI can stratify patients in low and high predicted mortality groups This study is retrospective
Toyonaga et al. (2016)	A single center, retrospective study of 32 newly diagnosed GBM patients who underwent presurgical MRI and FMISO PET scans. The imaging was evaluated for hypoxia features and hypoxia volume (HV), metabolic tumor volume in hypoxia (hMTV), and total lesion glycolysis in hypoxia (hTLG) were determined. The primary endpoints were PFS and OS	III	In multivariate analysis, hTLG and hMTV were found to be significantly associated with both OS and PFS The conclusion by the authors was that hMTV and hTLG as seen on FMISO PET are prognostic indicators in GBM This study is retrospective
Hojklint Poulsen et al. (2016)	A retrospective single center study of 146 GBM patients who underwent standard chemoradiation after surgery. FET PET scan were obtained post-operative and compared to OS	III	A large biological tumor volume (BTV) was associated with a worse OS and PFS The authors concluded that BTV is a strong prognostic factor This is a retrospective study
Gerstner et al. (2016)	A prospective, multicenter study of 42 patients with newly diagnosed glioblastoma with residual tumor after resection evaluating prognostic characteristics based on MRI and 18F-FMISO PET Images were obtained after diagnosis and 2 weeks prior to the start of chemoradiation. Conventional parameters were evaluated in relation to prognosis. OS was final outcome	III	Forty-two patients were included with a median OS of 408 days. SUVpeak and nCBF were found to be predictive of survival at 1 year and higher SUVpeak, ktrans were found to associated with a significantly shorter survival time The authors conclude that hypoxia based on 18F-FMISO PET and abnormal tumor vasculature on MRI are associated with a worse OS These prognostic factors have not been validated in a test group of patients
Choi et al. (2016)	A retrospective single center study of 112 newly diagnosed GBM patients with known MGMT methylation status in which ADC histograms of the pre-operative MRI were created and parameters compared with OS. 74 patients were included in the training set and 38 in the test set The outcome was PFS and OS	III	Lower ADC histogram parameters (mean ADC < 1335.1) were associated with a worse PFS and OS. This was found to be independent of MGMT methylation status This study showed a poorer prognosis for patients with low ADC histogram values This study is limited by its retrospective nature
Burth et al. (2016)	125 patients were included in this retrospective, single center study of patients who underwent surgical resection followed by medical and/or radiation therapy evaluating clinical and MRI parameters for their predictive abilities The outcome was PFS and OS	III	For OS, age, KPS, and EOR were found to be significantly associated with survival for clinical parameters and 10th percentile ADC and 90th percentile rCBV were significant for MRI parameters. For PFS, age, KPS, and EOR were found to significantly associated and 90th percentile rCBV for MRI parameters The authors concluded that MRI parameters can be used to aid in predicting OS but clinical parameters outperform these This study is limited by its retrospective nature
Chaddad et al. (2016)	MRI data from the Cancer Imaging Archive (TCIA) for 50 GBM patients prior to any treatment were retrospectively evaluated for 3 phenotypes: necrosis parts (vN), contrast enhancement/active tumor (vAT), and edema/invasion (vE) on T1 and FLAIR sequences These phenotypes were group using texture feature extraction from gray-level co-occurrence matrix (GLCM). The outcome was relation of phenotypes to OS	III	The GBM phenotype vAT was found to be significantly predict OS ($p < 0.01$). The phenotypes vN and vE did not significantly predict OS The authors concluded this novel texture feature extraction-based approach was able to divide GBM phenotypes, some of which predict patient OS This is a retrospective study testing a novel system of creating GBM phenotypes based on MRI texture features

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Deike et al. (2016)	Single institution retrospective study of patients with suspected new glioblastoma whose MRI presented the typical ring enhancement with a central necrosis were included. They analyzed two established malignancy indicators: minimum apparent diffusion coefficient (ADC) and maximum relative cerebral blood volume (rCBV) to define prognosis-related glioblastoma growth patterns. The intersection of the percentage of pixels within regions where both ADC values are less that the 30th percentile for the tumor (ADCmin) and CBV values were greater than the 70th percentile for the tumor (CBVmax) of all pixels of a tumor single ROI was calculated	III	The upper quartile of the normally distributed mean intersection of both ADCmin and CBVmax pixels in all patients was determined and called the big intersection (BI). Log-rank test exposed a significantly longer overall survival of patients with the BI (n = 16) compared to non-BI group (n = 49) (p = 0.0057) The authors conclude that the combination of diffusion and perfusion imaging allows visualization of different glioblastoma growth patterns that are associated with prognosis Because this was study was retrospective in nature and no prospective confirmatory group of patients was assessed this represents class III data
Akgoz et al. (2014)	A retrospective single institution study of 68 patients with newly diagnosed glioblastoma who received maximal surgical resection and RT, and (3) had interpretable spin-echo echo planar perfusion weighted imaging completed at two time points, (a) following surgery and prior to the initiation of RT and (b) within 6 months of the conclusion of RT	III	Multivariate analysis demonstrated that baseline tumor mean spin-echo echo planar perfusion normalized cerebral blood volume was predictive of PFS (p = 0.038) and OS (p = 0.004). Within the patient sample, baseline tumor mean spin-echo echo planar perfusion normalized cerebral blood volumes < 2.0 predicted longer patient PFS (median 47.0 weeks, p < 0.001) and OS (median 98.6 weeks, p = 0.003) compared with baseline mean spin-echo echo planar perfusion normalized cerebral blood volumes > 2.0 (median PFS 25.3, median OS 56.0 weeks) The authors concluded there appears to be a magnitude-dependent relationship between higher spin-echo echo planar perfusion normalized cerebral blood volumes and shortened survival The assessments were not blinded and no prospective validation set of patients is presented, yielding class III data
Bag et al. (2014)	A retrospective, single institution, pilot study of 28 newly diagnosed primary GBM patients, who were treated with resection followed by concurrent chemoradiation and adjuvant chemotherapy and who had satisfactory perfusion imaging preoperatively and after concurrent therapy	III	Perfusion parameters that were significantly (p < 0.05) associated with overall survival included post-treatment peritumoral relative cerebral blood flow (HR 2.02, 95% CI 1.151–3.52, p = 0.013) and post-treatment peritumoral relative cerebral blood volume (HR 2.192, 95% CI 1.310–3.670, p = 0.0028). The association of pretreatment perfusion parameters and overall survival was not statistically significant The assessments were not blinded and no prospective validation set of patients is presented, yielding class III data
Deviers et al. (2014)	A prospective, single institution analysis of 14 newly diagnosed glioblastoma patients who were treated with 60-Gy conformal fractionated radiation therapy (RT) and tipifarnib and underwent a combined magnetic resonance spectroscopic imaging (MRSI) and MRI examination after surgery or biopsy but before RT and then every 2 months after the end of RT until a relapse	III	The tumor-associated threshold value for lactate-to-N-acetyl-aspartate ratio of 0.4, using 3D-MRSI in pre-RT GBM patients could significantly (P < 0.01) predict the site of relapse with 88.8% sensitivity and 97.6% specificity The assessments were not blinded and no prospective validation set of patients is presented, yielding class III data

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Kim et al. (2014)	Single institution, retrospective study of 59 patients with newly diagnosed GBM who received standard concomitant chemoradiotherapy with TMZ and adjuvant TMZ for six cycles. MRI scans were obtained within 3 months and the data used to measure normalized cerebral blood volume (nCBV) and normalized apparent diffusion coefficient (nADC)	III	Twenty-seven patients had a measurable enhancing lesion and 32 patients lacked a measurable enhancing lesion. The median PFS of patients lacking measurable enhancing lesion was longer than for those with measurable enhancing lesions (17.6 vs 3.3 months, $P < 0001$). There was a significant, positive correlation between the 99th percentile nCBV value of a measurable enhancing lesion and the PFS ($P = .044$, $R^2 = .152$). In addition, the median PFS was longer in patients with a 99th percentile nCBV value ≥ 4.5 than it was in those with a value < 4.5 (4.4 vs 3.1 months, $P = .036$) The authors conclude the presence of an enhancing lesion seems to be an important factor for predicting the PFS in GBM patients The lack of blinded data interpretation and a validation set provides class III data
Zaw et al. (2014)	A retrospective single institution study of 22 patients with newly diagnosed glioblastoma receiving standard radiation, concurrent and adjuvant temozolomide with MRI scans with adequate diffusion data over at least three intervals: (a) post-surgical, pre-radiochemotherapy, (b) mid-radiochemotherapy, and (c) post radiochemotherapy not spanning more than a total of 8 months. The imaging data was then used to derive CIMPLE (cell invasion, motility, and proliferation level estimates) maps. "Full solution" and "no invasion" models of this technique were correlated with simple prognostic parameters	III	Proliferation rate maps from the ''no invasion'' approximation had significantly higher sensitivity (82 vs. 64%) and specificity (90 vs. 80%) for predicting 6 month progression free survival and was a better predictor of time to progression (log-rank; no invasion estimation, $P = 0.0134$; full solution, $P = 0.0555$) The authors recognize that the data is dependent on the assumption that diffusion weighted imaging correlates with tumor cellularity and can be altered by significant mass effect The lack of blinded data interpretation and a validation set warrants class III designation
Ellingson et al. (2013)	A retrospective, single institution study of 143 newly diagnosed glioblastoma patients undergoing standard surgical procedures and chemoradiation. Traditional and probabilistic functional diffusion mapping (fDM) was calculated using ADC maps acquired before and after combined radiation and temozolomide therapy	III	Probabilistic fDMs provided significant data that in a manner better than traditional fDMs at predicting 12-month PFS ($P = 0.0352$) and 24-month OS ($P = 0.0419$). Univariate log-rank analysis on Kaplan–Meier data also revealed that probabilistic fDMs could better separate patients on the basis of PFS and OS, compared with traditional fDMs. The lack of blinded assessment of this imaging analysis in a confirmatory set of patients leaves this as class III data
Li et al. (2013)	A single institution, retrospective analysis of 64 patients with newly diagnosed glioblastoma treated with surgery, radiation (RT) and various medical regimens. Standard anatomic MRI was acquired and used in conjunction with 3D ^{1}H magnetic resonance spectroscopic imaging (MRSI) The predictive values of MRSI parameters at baseline (after surgical resection but prior to RT and chemotherapy) and at post-RT in were assessed in relation to PFS6 and OS	III	Shorter PFS6 values were associated with a decrease in the ratio of N-acetyl aspartate to choline-containing compounds (NAA/Cho) in the region with a Cho-to- NAA index (CNI) > 3 at baseline and an increase of the CNI within elevated CNI regions (> 2) at two month follow-up (F2mo). Patients with higher normalized lipid and lactate at either time point had significantly worse OS. Patients who had larger volumes with abnormal CNI at F2mo had worse PFS6 and OS The lack of blinded assessment of this imaging analysis in a confirmatory set of patients leaves this as class III data

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Sunwoo et al. (2013)	This is a single institution, retrospective study of 26 newly diagnosed glioblastoma patients. Each was diagnosed by H&E technique and evaluated by Ki-67 labeling index. Each had undergone MRI including diffusion weighted imaging with standard b values (b = 1000 s/mm2) within 2 weeks before surgery. Additionally each case underwent assessment of MGMT promoter methylation status by methylation-specific polymerase chain reaction and methylation specific multiplex ligation-dependent probe amplification	III	A positive relationship was demonstrated between PFS and the mean ADC value (P = 0.001)
Colavolpe et al. (2012)	A single institution, retrospective study of 31 newly diagnosed glioblastoma patients. Pretreatment FDG PET data manipulation quantitatively measuring the ratio between the tumor and contralateral maximal standardized uptake value (T/CL). Event free survival (EFS) and overall survival were correlated with the PET data and standard prognostic factors	III	Median OS and EFS were 13.8 and 7.4 months, respectively, for glioblastomas. The T/CL ratio predicted OS in the glioblastoma subgroup (P = 0.018; HR = 2), independently of age, Karnofsky performance status, histological grade, and surgery, and independently of recursive partitioning analysis classification The authors conclude that by identifying high risk patients, FDG uptake may ultimately help to evaluate and adapt therapy in patients The lack of blinded assessment of this imaging analysis in a confirmatory set of patients leaves this as class III data
Ellingson et al. (2012)	A single institution, retrospective study of functional diffusion mapping in 143 newly diagnosed glioblastoma patients with (1) pathology-confirmation, (2) treatment with standard external beam radiotherapy (typically in 2 Gy fractions given once daily for 5 days over a 6-week period, totaling 60 Gy) and concomitant temozolomide (75 mg/m^2/day, 7 days per week during radiotherapy, followed by a 4-week break, then 6–12 cycles of adjuvant therapy at 150 mg/m^2/day to 200 mg/m^2/day), and (3) satisfactory (no gross geometric distortions or low signal-to-noise ratio in the raw DWI datasets) baseline (postsurgical, preradiochemotherapy) and minimum of 1 follow-up MRI scan approximately 4 weeks after radiochemotherapy Functional diffusion mapping was created by coregistering pre- and posttreatment apparent diffusion coefficient (ADC) maps and then performing voxel-wise subtraction. FDMs were categorized according to the degree of change in ADC in pre- and posttreatment fluid-attenuated inversion recovery (FLAIR) and contrast-enhancing regions	III	Patients with decreasing ADC in a large volume fraction of pretreatment FLAIR or contrast-enhancing regions were statistically more likely to progress earlier (P = 0.0003) and expire sooner (P < 0.0001) than in patients with a lower volume fraction The authors conclude that functional diffusion mapping is a sensitive imaging biomarker for predicting glioblastoma survival The lack of blinded assessment of this imaging analysis in a confirmatory set of patients leaves this as class III data

Table 2 (continued)

Author/year	Study description	Data class	Conclusion
Essock-Burns et al. (2011)	A prospective, single institution study of 35 glioblastoma patients treated with standard chemoradiotherapy with temozolomide and with the addition of enzastaurin (250 mg daily as an putative anti-angiogenic agent) In addition to anatomic and metabolic MR imaging, patients received dynamic susceptibility contrast (DSC) imaging prior to beginning therapy (postsurgical resection) and then serially at 1, 2, 4, 6, 8, 10, and 12 months after beginning therapy) to identify early predictors of radiographic response to antiangiogenic therapy and to evaluate changes in perfusion parameters that may be predictive of progression. Perfusion parameters, peak height (PH) and percent recovery toward normal, were calculated from the dynamic curves and correlated with PFS and OS	III	A series of observations were made: (1) Six month radiographic responders showed a significant improvement in percent recovery between baseline and 2 months into therapy, whereas 6-month radiographic nonresponders showed significantly increased PH between baseline and 1 month (P = .01) (2) At 2 months into therapy, greater percent recovery was predictive of better progression-free survival (multivariate Cox regression, P = .009,HR = 0.955, 95% CI = 0.926–0.987)) (3) Four months prior to progression, there was a significant increase in the standard deviation of the heterogeneity of the percent recovery within the tumor region (P < 0.04) The authors conclude DSC perfusion imaging provides valuable information about vascular remodeling as an early indicator of response to antiangiogenic therapy The lack of a blinded assessment of this imaging analysis in a confirmatory set of patients yields class III data
Laprie et al. (2008)	A single institution, prospective study of magnetic resonance spectroscopic imaging (MRSI) in nine newly diagnosed glioblastoma patients after surgery but before radiation or chemotherapy to assess its ability to predict the site of post-radiation tumor relapse. Standard MRI and investigational MRSI was then obtained every two months. Cho-to-NAA ratio of two or more (CNR2) was the minimal threshold for abnormal/tumor tissue	III	75% of the voxels within the T1 contrast enhancing and CNR2 region before therapy continued to exhibit CNR2 at relapse, compared with 22% of the voxels within the T1CE with normal CNR ($p < 0.05$). The location of new contrast enhancement with CNR2 corresponded in 80% of the initial T2 areas with CNR2 vs. 20.7% of the T2 voxels with normal CNR ($p < 0.05$) The authors conclude that metabolically abnormal regions detected prior to radiation are predictive of areas of radiation failure. They propose addition of MRSI data in defining radiation target volumes to Though this paper provides some prognostic information assessments were not blinded and there is no validation set of patients. Thus this is class III data

Abbreviations: 3D: three dimensional; ADC: apparent diffusion coefficient; Cho: choline; CI: confidence interval; CNI: choline to N-acetyl aspartate index; CNR: choline to N-acetyl aspartate ratio; CNR2: Cho-to-NAA ratio of two or more; DSC: dynamic susceptibility contrast; DWI: diffusion weighted imaging; EFS: event free survival; F2mo: two month follow-up; fDM: functional diffusion mapping; FLAIR: fluid-attenuated inversion recovery; GBM: glioblastoma; HR: hazard ratio; MGMT: O^6-methylguanine DNA methyltransferase; MR: magnetic resonance; MRI: magnetic resonance imaging; MRSI: magnetic resonance spectroscopic imaging; MSP: methylation-specific polymerase chain reaction; NAA: N-acetyl aspartate; nCBV: normalized cerebral blood volume; nADC: normalized apparent diffusion coefficient; OS: overall survival; PFS: progression free survival; PFS6: progression free survival at 6 months; PH: peak height; RT: radiation therapy; T1CE: T1 contrast enhancing; T/CL: tumor and contralateral maximal standardized uptake value; TMZ: temozolomide

Abbreviations: glioblastoma (GBM), overall survival (OS), Epidermal Growth Factor Receptor (EGFR), progression free survival (PFS), Magnetic Resonance Imaging (MRI), relative cerebral blood volume (rCBV), wildtype (WT), O(6)-methylguanine-DNA-methyltransferase (MGMT), noncontrast-enhancing tumor (nCET), The Cancer Imaging Archive (TCIA), hypoxia enrichment score (HES), dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI), short-term survivors (STS), mid-term survivors (MTS), long-term survivors (LTS), Central Nervous System (CNS), Area under the Curve (AUC), Primary Central Nervous System Lymphoma (PCNSL), Mean transit time (MTT), image texture analysis (ITA), Hazard Ratio (HR), isocitrate dehydrogenase (IDH), cerebral blood flow (CBF), gross total resection (GTR), near total resection (NTR), subtotal resection (STR), extent of resection (EOR), radiological assessment (QRA),residual tumor volume (RTV), tumor area (TA), ventricular-subventricular (VSVZ), subgranular (SGZ), corpus callosum (CC), fluoeo-ethyl-tyrosine (FET), positron-emission tomography (PET), fluoromisonidazole (FMISO), fludeoxyglucose (FDG), contrast-enhancing tumor volume (GdTV), metabolic tumor volume (MTV), standardized uptake value (SUV), Response Assessment in Neuro-Oncology (RANO), dynamic glucose-enhance (DGE), Diffusion Tensor Imaging (DTI), Karnofsky Performance Score (KPS), relative oxygen extraction fraction (rOEF), gray-level co-occurrence matrix (GLCM), Blood oxygenation level dependent (BOLD), hypoxia volume (HV), metabolic tumor volume in hypoxia (hMTV), total lesion glycolysis in hypoxia (hTLG), necrosis parts (vN), contrast enhancement/active tumor (vAT), edema/invasion (vE), fractional anisotropy (FA), The Cancer Imaging Archive (TCIA), contrast enhancement (CE), Apparent Diffusion Coefficient (ADC), biological tumor volume (BTV)

specificity [31]. Another prospective study of nine patients with MRSI looked at nine patients after surgery but prior to radiation. Seventy-five percent of the voxels within the T1 contrast enhancing region that had a Cho-to-NAA ratio of two or more before therapy continued to exhibit the same values at relapse suggesting this parameter may be used to predict location of eventual progression [32].

A single institution, prospective study using DSCI in 35 newly diagnosed glioblastomas beginning prior to radiation therapy measuring peak height and percent recovery toward normal provided a series of prognostic observations. This included (1) six month radiographic responders showed a significant improvement in percent recovery between baseline and 2 months into therapy, whereas 6-month radiographic nonresponders showed significantly increased peak height between baseline and 1 month ($P = 0.01$), (2) at 2 months into therapy, greater percent recovery was predictive of better progression-free survival (multivariate Cox regression, $P = 0.009$), and (3) four months prior to progression, there was a significant increase in the standard deviation of the heterogeneity of the percent recovery within the tumor region ($P < 0.04$) [33]. The observations were not made in a blinded fashion and were not validated in another set patients. Thus all three of these prospective studies provided class III data.

Seven retrospective studies evaluated various manipulations of diffusion weighted imaging data. Sunwoo et al. noted a positive relationship between preradiation ADC values and progression free survival [34]. Ellingson et al. described how the decreasing ADC values in regions of residual tumor correlated with earlier progression in an initial paper. They then went on to refine these observations in the same population by using probabilistic functional diffusion mapping showing as opposed to traditional diffusion mapping techniques. This technique was able to provide statistically significant predictions of 12 and 24 month progression free survival [35, 36]. Zaw et al. used diffusion data to derive cell invasion, motility and proliferation level (CIMPLE) maps. Data from these were used to create what they called "full solution" and "no invasion" models. They showed the no invasion approximation had the best ability to predict progression free survival [37]. Other studies have showed similar results that an ADC histogram with low mean values are associated with a worse prognosis [38–40]. In none of these studies were the imaging data observations blinded from the known patient clinical status and no independent validation set was studied to confirm the value of the findings. Thus, the four studies dealing with diffusion weighted imaging data yielded class III data. FLAIR and contrast-enhanced MRI can be used to calculate restriction spectrum imaging, higher intensities of this were associated with a shorter survival in one single center, retrospective study [41].

Retrospective studies have also evaluated various other MRI parameters in association with prognosis. Another retrospective study of presurgical MRI in 61 GBM patients calculated the tumor area by the ABC/2 method and found an area greater than $200mm^2$ was associated with a worse prognosis (HR 1.096, $p < 0.01$) [42]. A large tumor volume has been associated with a worse outcome in additional studies, along with increase necrosis volume [43]. In MGMT promoter unmethylation GBM patients, a higher area under the curve of presurgical contrast-enhanced MRI was correlated with a worse progression free survival and overall survival ($p < 0.05$) [44]. Another study associated increased surface irregularity with a worse overall survival [45]. Contact of lesions with the ventricular-subventricular zone on pretreatment MRI was also associated with a worse progression free survival and overall survival in a single center, retrospective study [46].

Post-operative MRI was also associated with progression-free survival and overall survival, Blomstergren et al. reviewed 70 GBM patients' findings that an extent of resection of greater than 96% is associated with an increased progression free survival and overall survival ($p = 0.017$, $p = 0.043$, respectively) [47]. An observational prospective study of 18 GBM patients also correlated "cloudy-enhancement," visualized by subtracting ore-contrast T1 weight images from post-contrast T1 weighted images, with progression free survival linking a decrease of more than 21.4% of the "cloudy enhancement" with a longer progression free survival [48].

Specialized manipulation of MRI data has been shown to provide prognostic data. Kim et al. showed that measurement of the normalized cerebral blood volume provided a significant positive correlation with progression free survival [49]. Bag et al. noted the relative cerebral volume (rCBV) derived from perfusion imaging correlated with overall survival [50]. The association of high rCBV with overall survival showed in another retrospective, single center study by Juan-Albarracin et al. [51]. Baseline postoperative tumor mean spin-echo echo planar perfusion normalized cerebral blood volume was predictive of progression free survival ($p = 0.038$) and overall survival ($p = 0.004$) [52]. Deike et al. carried out a statistical manipulation of combined diffusion and perfusion data. They demonstrated patterns of ADC decrease and cerebral blood volume increase that were best associated with prognosis [53]. For the same reasons noted above in the other prognostic studies using MRI, this was class III data.

Beig et al. performed a retrospective review of GBM patients in the Cancer Imaging Archive to determine radiomic features associated with a hypoxia enrichment score (HES) based on RNA data. They found 8 radiomic features significantly associated with the HES and that these features could differentiate with long-, medium-, and short-term

survivors [54]. Another study showed the feasibility of an Adaptive Neuro-Fuzzy Inference System that evaluated pretreatment contrast-enhanced MRI parameters to predict OS [55]. Low fractional anisotropy (FA) of the contrast-enhancing region on MRI was correlated with an improved overall survival in one single center, retrospective study [56]. Fractional anisotropy (FA) of the pretreatment MRI in the non-enhancing peritumoral region has also been associated with later tumor recurrence, areas of recurrence showed significantly lower FA [57].

Other MRI parameters have been shown to not affect OS. Amelot et al. compared MRIs of 234 pretreatment GBMs for invasiveness and divided patients under gross total resection into diffuse or nodular tumors, there was no significant difference in overall survival. The authors concluded both patterns benefit from gross total resection of contrast-enhancing tumor [58]. Similarly, several other pretreatment MRI parameters have also been investigated in retrospective studies and linked to overall survival [59–62].

Imaging response (IR) and pseudoprogression on MRI was correlated with TCGA subtype, IDH1 status, and MGMT promoter methylation status in a 2 center, retropsective study of GBM patients. Soike et al. found that a proneural TCGA subtype was associated with a significantly higher rate of IR (60%, $p = 0.03$) as well as MGMT promoter methylation (58%, $p = 0.032$). IR was associated with a better overall survival of 29.4 months compared to 14.5 months, $p < 0.01$ [63]. MRI features have also been used to predict response to therapy. A retrospective study divided patients into 2 groups based on angiogenic features on the perfusion-weight pretreatment imaging. They noted patients with low angiogenic features had a better overall survival compared to the high angiogenic group with or without antiangiogenic therapies. In the high angiogenic group, those treated with antiangiogenic therapies had an overall improved survival to those who were not ($p = 0.02$) [64].

In a study of magnetic resonance spectroscopic imaging Li et al. found magnetic resonance spectroscopic imaging predicted shorter six month progression free survival values. A decrease in the ratio of N-acetyl aspartate to choline-containing compounds (NAA/Cho) in the regions with a Cho-to- NAA index (CNI) > 3 at baseline and an increase of the CNI within elevated CNI regions (> 2) at two month follow-up is where the most valuable predictive data could be derived. For reasons of lack of blinding and a validation set this is class III data [65].

Synthesis of results

New PET agents including ^{62}Cu-diacetyl-bis(N4-methylthiosemicarbazone), ^{18}F-fluorothymidine and ^{11}C-acetate were reported in a manner that allowed consideration in this guideline. However, the presence of only a single report fitting inclusion criteria for each, and the disparate nature of their mechanisms precludes creation of specific recommendations regarding them.

Utilization of MRI signal measurement techniques for diagnosis and prognosis offer useful additions to the images themselves when diagnosing new or progressing disease. This is particularly true when applied in the form of MRSI, diffusion weighted imaging, and cerebral blood volume calculations. Specialized manipulation of MRI data has also shown promise in aiding in prognosis and diagnosis, particularly in differentiating glioblastoma from primary CNS lymphoma. Molecular subtyping is also being correlated with specific MRI findings. As with the PET studies, the techniques are quite different from each other and even within them the parameters measured are yet to be settled on in any consistent form. The promising nature of these modalities certainly suggests that more work be done to crystallize and validate them and this is reflected in the recommendation.

Surgery

Study selection and characteristics

Steps to accomplish data extraction included assessment of each abstract based on the above mentioned inclusion and exclusion criteria as could best be discerned. These same factors were used in detail for the subsequently chosen full text manuscripts. Additionally study design, number of institutions involved, the primary surgical treatment modality used, the total number of patients per modality and group and the statistics used to compare them were extracted to complete the summary of the data in each manuscript.

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 10 were chosen as relevant to surgery for full text review and assessment. A total of 8 publications met the criteria for inclusion regarding the therapeutic value of surgery [66, 67, 70–73, 75, 76]. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Of the fourteen studies that met criteria for inclusion in the evidence tables one was a prospective randomized study, three prospective, single institution studies and nine retrospective, single institution studies, and one retrospective multiple institution study. The retrospective single and multiple institution studies are subject to the usual risks of bias related to selection, misclassification, survivorship and publication bias and recognition that in data collected in this manner correlation does not imply causation [66–72]. In the prospective stuies by Zhu et al., Yoneda et al., and Neira et al. the data collected and reported may be influenced by

selection bias, attrition bias and bias of change in methods over time [73–75]. The secondary evaluation of the randomized, prospective study reported by Pichelmeier et al. includes the possibility of selection and ascertainment bias, hidden agenda bias and variability due to random error related to problems with unintentional data entry oversight and neglect [76]. Of the three studies that met criteria for inclusion in the evidence tables one was a prospective randomized study, one a prospective, single institution study and one was a retrospective, single institution study. The study by Aldave et al. is a retrospective analysis of fluorescence guided surgery subject to weaknesses related to selection, misclassification, survivorship and publication bias and recognition that in data collected in this manner correlation does not imply causation [66]. In the prospective study by Zhu et al. the data collected and reported may be influenced by election bias, attrition bias and bias of change in methods over time [73]. The secondary evaluation of the randomized, prospective study reported by Pichelmeier et al. includes the possibility of selection and ascertainment bias, hidden agenda bias and variability due to random error related to problems with unintentional data entry oversight and neglect [76].

Results of individual studies

The key results of the selected individual studies are outlined in Table 3.

Pichelmeier et al. provide a post hoc analysis of just the glioblastomas from the 2006 report of the use of 5-ALA in malignant brain tumors by Stummer et al. [76, 77]. They applied the RTOG recursive partitioning analysis (RPA) technique to the population comparing survival in those who underwent resection with 5-ALA to those who underwent resection with white light only [78]. Overall, more complete resections were accomplished in the 5-ALA group. Also the median survival and 2 year survival rates in the RPA groups IV and V were longer when 5-ALA was used for the surgical resection at the time of initial diagnosis. By breaking up the population into subgroups the power of the observations is diminished. The post hoc nature of the analysis, though ingenious, does not equate to a well-designed randomized trial and thus this is class II data. The Stummer et al. paper, from which the data was extracted, did not separate glioblastomas from other histologies in their data analysis and therefore it does not meet the criteria for inclusion in this guideline [77].

In a prospective single center study Neira et al. evaluated 32 patient undergoing fluorescein-guided surgical resection of newly diagnosed glioblastoma. They found that intensity of fluorescein correlated with MRI contrast enhancing regions of tumor. The frequency of gross total resection was greater with use of the dye, though not to a statistically significant extent [75].

From a retrospective review of a group of 118 tumor cases in which fluorescence-guided tumor resection was undertaken in newly diagnosed lesions Aldave et al. extracted 52 glioblastomas with complete resection of enhancing tumor by postoperative MRI [66]. The median overall survival was 27.0 months in patients with nonresidual fluorescence ($n=25$) and 17.5 months for the group with residual fluorescence ($n=27$, $p=0.015$). The neurological complication rate was 18.5% in patients with nonresidual fluorescence and 8% for the group with residual fluorescence ($p=0.267$). This information supports the prior suggestions that removal of all fluorescent tissue is beneficial to survival and raises previously mentioned concerns that resection of all fluorescing tissue in regions of eloquent tissue needs to be undertaken judiciously. The retrospective and nonblinded nature of this data produces class III data.

Additional studies of 5-ALA to augment the extent of resection, all retrospective in nature provide for support for improved extent of resection and in some cases improved periods of progression free survival [69, 72]. In a prospective study serving as an adjunct to what has been learned from 5-ALA Yoneda et al. provided evidence that extent of fluorescence correlates with tumor region cellularity and MIB-1 index [74]. In a very small retrospective study ($n=8$) Lee et al. have assessed near-infrared imaging with indocyanine green and concluded it was feasible but required more study [71].

Arguing that a greater extent of resection is beneficial, Esquenazi et al. retrospectively analyzed 86 patients undergoing some form of surgery for newly diagnosed glioblastoma. They found that "supratotal" resection, defined as resection beyond enhancing margins on the scan, was associated with significantly better overall survival and one and two year progression free of survival [70]. In another retrospective study of 70 patients with newly diagnosed glioblastoma the use of intraoperative MRI was shown to allow a surgeon to more closely approximate the planned extent of resection compared to cases done without intraoperative MRI. However, this had no effect on overall survival [67]. In a retrospective study of a novel surgical adjunct Rozumenko et al. assessed laser surface thermal therapy. Overall survival was 18.4 month in the laser surface thermal therapy group ($n=28$) and 14.3 months in the control group ($n=63$), $p=0.03$. These findings are subject to the usual bias limitations of a retrospective study. As with all such studies, additional prospective comparative study was recommended [68].

In a single institution prospective study Zhu et al. compared diffusion tensor imaging to direct subcortical stimulation for identification of the pyramidal tracts in brain tumor surgery [73]. Eighteen glioblastomas were included in the

overall group of 58 patients. The authors found a high concordance rate between the two techniques with a sensitivity and specificity of 93% for diffusion tensor imaging. In the overall group 10% of cases had persistent worsening of neurologic function when using diffusion tensor imaging to guide dissection. The lack of control cases and the case to case variability in the acquisition and use of diffusion tensor imaging leaves this as class III data.

Synthesis of results

The class II and class III data on 5-ALA suggest that it should be considered as an assistive modality to improving extent of resection in operable newly diagnosed glioblastomas. Development of modalities to improve the safety and extent of surgical resection such as diffusion tensor imaging tractography and intraoperative MRI are potentially important to long term preservation of neurologic function. Enrollment of individuals in studies to show that these and other novel techniques can be meaningfully disseminated are important for their future development.

The class II that is supported by the class III data on 5-ALA suggest that where approved, it should be considered as an assistive modality to improving extent of resection in operable newly diagnosed glioblastomas. Development of modalities to improve the safety of surgical resection such as diffusion tensor imaging tractography are critical to long term preservation of neurologic function. Enrollment of individuals in studies to show that these techniques can be meaningfully disseminated are important for their future development.

Neuropathology

Study selection and characteristics

Steps to accomplish data extraction included assessment of each abstract based on the above mentioned inclusion and exclusion criteria as could best be discerned. These same factors were used in detail for the subsequently chosen full text manuscripts. Additionally study design, number of institutions involved, the primary neuropathology evaluation used, the total number of patients studied and group and the statistics used to compare them were extracted to complete the summary of the data in each manuscript.

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 56 were chosen as relevant to neuropathology for full text review and assessment. A total of 2 publications met the criteria for inclusion regarding the diagnostic value of test or marker [79, 80] A total of 46 publications met the criteria for being useful for determining prognosis from pathology information. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Of the 48 studies that met criteria for inclusion in the evidence Tables 4 and 5 ten were simple prospective studies [81–87, 106–108, 118]. These studies were at risk of being influenced by selection bias, attrition bias and bias of change in methods over time. The remaining 38 publications were retrospective in nature and were subject to the possibility of selection and ascertainment bias, hidden agenda bias and variability due to random error related to problems with unintentional data entry oversight and neglect [80, 88–105, 109–117, 119–124].

Evaluation of tumor markers as a means for glioblastoma diagnosis

Two retrospective studies assessed tumor markers as a means of obtaining the diagnosis of glioblastoma. In a manuscript by Young et al. it was shown that restricted water diffusion in MRI correlated with epidermal growth factor receptor amplification thus suggesting glioblastoma. The data evaluation was not carried out in a blinded fashion thus yielding class III data [79]. More recently Manterola et al. studied miRNA expression levels in the serum microvesicles of patients with newly diagnosed glioblastoma. The expression levels of 1 small noncoding RNA (RNU6-1 was most upregulated compared to normal, $P < 0.001$) and 2 microRNAs (miR-320, $P = 0.0067$, and miR-574-3p, $P = 0.0055$) were significantly associated with a glioblastoma diagnosis. In addition, RNU6-1 was consistently an independent predictor of a glioblastoma diagnosis. This well done study included a validation set but was done retrospectively and in a nonblinded fashion resulting in class III data [80].

Results of individual studies

The key results of the selected individual studies are outlined in Tables 4 and 5.

Evaluation of tumor markers for determination of glioblastoma prognosis

MGMT promoter methylation

Prospective studies assessing prognosis The presence of *MGMT* promoter methylation emerged as a significant predictor of a longer progression free survival and overall survival in patients with newly diagnosed with glioblastoma. In a prospective study of 67 newly diagnosed glioblastomas by Felsberg et al. MGMT promoter hypermethylation was

Table 3 Evidence for surgery

Author/year	Study description	Data class	Conclusion
Coburger et al. (2018)	A multi-center retrospective study of 70 patients with new diagnosis of GBM who underwent planned subtotal resection followed by concurrent chemoradiation A senior neurosurgeon reviewed all pre-operative imaging in a blinded fashion and determined the potential EOR. The post-operative images were then reviewed for actual EOR. The actual EOR was then subtracted from the potential EOR calculating the change in EOR. The study then compared results between use of intra-operative MRI (iMRI) vs not The primary outcome was change in EOR	III	33 patients under surgery with use of iMRI. The group that underwent iMRI was found to have a significantly lower change in EOR (4% vs 22%, $p=0.008$) The authors conclude that use of iMRI allows for actual EOR to more closely correlate with the intended EOR when patient with GBM are undergoing planned STR The study is limited by its retrospective nature. OS is also included and was found to be 11 months in the iMRI patients and 10 months in the patients it was not used ($p=0.049$) though this data is likely confounded by a large number of patients with MGMT promoter methylation in the iMRI group
Neira et al. (2017)	Single center, prospective case series of 32 patients undergoing fluorescein-guided surgical resection of newly diagnosed glioblastoma The authors compared visual intensity of the fluorescein to histopathological findings in contrast (CE) and non-contrast enhancing (NCE) areas. These cases were compared to 32 historical controls. The outcomes evaluated for was presence of fluorescein in correlation to histopathological findings as well as GTR rates	III	For CE regions, fluorescein sensitivity was 0.879 and none of the CE were normal on histopathology. For NCE areas, fluorescein intensity had a sensitivity of 0.694 and specificity of 0.667. In patients for whom GTR was planned, 29 in the cases series and 22 of the historical controls, 93.1% of the fluorescein group and 77.3% in the control group had GTR with mean contrast enhancement resection of 99.7% and 96.8% respectively, no significant difference was found The authors conclude fluorescein-guided surgery helps guide surgical resection beyond the contrast-enhancing regions of the tumor These cases were compared to historical controls, no significant difference in GTR rates were found, and sensitivity and specificity for NCE < 70%
Esquenazi et al. (2017)	Single center, retrospective review of 86 consecutive newly diagnosed GBM patients undergoing surgical resection by one senior neurosurgeon The senior neurosurgeon, when GTR was considered possible, performed a "supratotal" resection through a subpial technique. The patients were divided into 3 groups: GTR (the "supratotal"), near total resection (NTR), and subtotal resection. The outcome was OS	III	25 patients underwent GTR, 13 NTR, and 48 subtotal resections. OS was 54 months in the GTR patients ($p<0.01$), 16.5 months in the NTR ($p=0.05$), and 13.2 months in the subtotal resection group The authors conclude that the surgeon's subpial technique to a "supratotal" resection is associated with increased OS Single surgeon study of a small number of patients with no comparison to other techniques for GTR
Lee et al. (2016)	Retrospective, single center case series on use of near-infrared (NIR) imaging with indocynanine green (ICG) during glioma surgery, 8 cases of newly diagnosed GBM were included The outcome was ability to visualize tumor, as confirmed by histopathology, using NIR	III	NIR has a sensitivity, specificity, PPV and NPV of 96.3%, 42.9%, 86.7%, and 75% respectively for newly diagnosed GBM patients The authors conclude that NIR shows real-time visualization of contrast-enhancing tumors and further study is needed to establish usefulness
Della Puppa et al. (2016)	A single center, retrospective review of 121 patients who underwent resection of newly diagnosed GBM followed by chemoradiation investigating the combined use of 5-ALA and carmustine wafers The patients were divided in 3 groups: Group 1—5-ALA guided resection and placement of carmustine wafers, 20 patients, Group 2—carmustine wafers alone, 42 patients, and Group 3—use of 5-ALA alone, 59 patients The primary outcome was OS	III	Group 1 had the longest OS of 22 months, significantly greater than Group II at 18 months, and group 3 at 21 months (all $p<0.01$). Complete resection was associated with a longer OS ($p<0.00001$). Group 1 had the greatest percentage of patients with GTR (80%), followed by group 3 (76%), then group 2 (47%) The authors conclude use of 5-ALA along with carmustine wafers is safe and the addition of 5-ALA increases OS. Complication rates were comparable in Group 1 and Group 2 (20% vs 19%%) This study is limited by its retrospective nature

Table 3 (continued)

Author/year	Study description	Data class	Conclusion
Aldave et al. (2013)	A retrospective, single institution review of 118 patients with high grade gliomas operated upon with 5-aminolevulinic acid (5-ALA). A subgroup of 52 patients with complete resection of enhancing tumor at the time of early postoperative MRI were chosen for analysis. Radiation and chemotherapy follow-up were provided in all with some receiving additional investigative therapy	III	At surgery, residual fluorescence was left in those cases where resection of that region was deemed likely to produce new neurological deficits. The median overall survival was 27.0 months in patients with nonresidual fluorescence (n = 25) and 17.5 months for the group with residual fluorescence (n = 27) (P = .015). The neurological complication rate was 18.5% in patients with nonresidual fluorescence and 8% for the group with residual fluorescence (P = .267) The assessment of residual fluorescence was not blinded and not verification set of patients was explored, yielding a class III evidence for this publication
Zhu et al. (2012)	Single institution prospective cohort study assessing the correlation between diffusion tensor imaging tractography (DTI) and direct subcortical stimulation (DsCS) in identifying the pyramidal tracts (PT) in 58 patients with newly diagnosed brain lesions, including 18 glioblastomas (18 GBM; 26 low-grade gliomas; 3 AA; 4 AO; 1 DNET; 3 CNS lymphomas; 1 CNS metastasis; 2 cavernous malformation). The PT were delineated with DTI and integrated with the intraoperative image guidance platform. Intraoperatively, when the resection area was < 20 mm from the PT based on DTI, direct subcortical stimulation was performed repeatedly at an initial intensity of 10 mA with stepwise increase to a maximum of 16 mA	III	The authors demonstrated that there is a high concordance rate between DsCS and DTI in identifying the PT with a sensitivity and specificity of 93% for DTI. The median intercept between positive DsCS sites and DTI imaging of the PT was 5.2 mm (range 2.0–14.7 mm). Postoperative MRI revealed gross total resection in 69% of patients with a 1-month postoperative motor worsening in 10% of patients. This study provides class III data as there is no control group and both DTI and DsCS are subject to multiple variables making the conclusion drawn in this study highly subjective
Pichelmeier et al. (2008)	This is a multi-center, randomized, prospective phase III study of 5-aminolevulinic acid (ALA) in patients undergoing surgery for newly discovered malignant gliomas consistent with glioblastoma. Ultimately 243 patients were found to have glioblastoma. Patients from the study were redistributed based on the criteria put forth by the RTOG-RPA classification. This resulting in patients being classified in RPA classes III, IV or V	II	A significantly larger number of "complete" resections (defined as absence of contrast enhancing tumor on early postoperative MRI) could be achieved using fluorescence-guided resections with ALA–induced tumor fluorescence, compared to conventional microsurgery (65% vs. 36%, p < 0.001). Stratified for degree of resection, survival of patients with complete resections was clearly longer in RPA classes IV and V (17.7 months vs. 12.9 months, p = 0.0015, and 13.7 months vs. 10.4 months, p = 0.0398; 2-year rates: 21.0% vs. 4.4% and 11.1% vs. 2.6%, respectively), but was not in the small subgroup of RPA class III patients (19.3 vs. 16.3 months, p = 0.14). Survival of patients from the ALA study is correctly predicted by the RTOG-RPA classes This is a post hoc analysis of a subset of patients from a larger study. This limits power but yields class II data

5-ALA 5-aminolevulinic acid; *AA* anaplastic astrocytoma; *ALA* aminolevulinic acid; *CNS* central nervous system; *DNET* dysembryoplastic neuroepithelioma; *DsCS* direct subcortical stimulation; *DTI* diffusion tensor imaging; *mA* milliamperes; *PT* pyramidal tracts; *RPA* Recursive Partitioning Analysis; *RTOG* Radiation Therapy Oncology Group

associated with longer time to progression (p = 0.0001) and longer overall survival (p = 0.0004). The methylation assessment was carried out in a blinded fashion but no validation set was explored. Thus the data in in this study is class II in nature [81]. More recently Thon et al. evaluated a somewhat smaller group of patients (n = 56) prospectively confirming these findings. Patients who were methylated had significantly longer PFS and OS of 15.0 and 20.3 months vs 6.1 (p = < 0.001) and 7.3 months (p = < 0.001). The assessment of outcome was not blinded to MGMT status and no validation was accomplished, resulting in class III evidence [82]. Although a number of other studies included cases collected in a prospective manner the assessments of responses were not blinded to *MGMT* status and no separate set of cases were collected for a validation of this finding [83–87]. Thus these studies yield class III data.

Retrospective studies assessing prognosis Lalezari et al. carried out a study of 418 patients in a retrospective manner while blinding patient outcome to those that interpreted MGMT expression by immunohistochemistry (IHC), and MGMT promoter methylation by methylation-specific PCR (MSP) and bisulfite sequencing of 24 neighboring CpG sites. They found that patients with lower IHC staining for MGMT had shorter progression free survival and overall survival. Progression free survival was longer for cases with individuals that were hypermethylated (over 3 CpG sites methylated) [88] The lack of a validation cohort in this study results in a class II study.

There are also a series of reports that are class III and retrospective in design from the beginning and reach the same conclusions as above [34, 89–100]. Various authors point out that these observations are largely made in patients who were treated with standard radiation therapy with concurrent and then adjuvant temozolomide. Beyond simple yes or no answers to *MGMT* promoter methylation various authors have tried to look into this status in greater details. Felsberg et al., as part of a publication mentioned above, retrospectivelyassessed *MGMT* promoter methylation diagnostic methods including mRNA and protein expression, the presence of *MGMT* sequence polymorphisms in 67 cases and found that polymerase chain reaction based analyses produced the most significantly predictive data in relation to progression free survival and overall survival [81]. Etcheverry et al. studied CpG site methylation levels in 40 newly diagnosed glioblastomas and identified 4 CpG sites (*FNDC3B, TBX3, FSD1, and DGKI*) as independent OS predictors [85]. In a retrospective study of 73 glioblastomas Tanaka et al. looked at the quantity *MGMT* RNA and found more than 1000 copies of *MGMT* mRNA per mg of tumor RNA was associated with longer progression free survival [101].

In a retrospective study combining a local data base with the TGCA Nguyen et al. looked at 303 patients for *MGMT* promoter methylation status, I*DH1* genotype and correlated them with the presence or absence of *hTERT* mutation. *hTERT* mutation alone had no particular effect on survival parameters. However, *hTERT* mutation presence seemed to augment overall survival in patients with *MGMT* promotor methylation (28.3 months) compared to 15.9 months in patients with *hTERT* mutations and an unmethylated MGMT promoter status (p < 0.0001) [102].

IDH1 mutation and prognosis

In a prospective, multi-institutional study of 301 patients with newly diagnosed glioblastoma receiving a variety of treatments those with *IDH1* mutations were associated with prolonged PFS (RR, 0.42; 95% CI, 0.19 to 0.91; P = 0.028) but did not reach significance in terms of prolonged OS (RR, 0.43; 95% CI, 0.15 to 1.19; P = 0.10). This information provided class III data as the interpretation of the molecular studies was not blinded and there was not attempt to pursue a validation set [86]. In a retrospective study of 237 newly diagnosed glioblastomas from a clinical study Hartman et al. found that the uncommon glioblastoma (7/2%) with *IDH1* mutation had a better overall survival than those that did not have the mutation [103].

Phosphorylated mitogen-activated protein kinase (p-MAPK)

In a retrospective study of 268 newly diagnosed glioblastomas and then an additional 60 similar patients from a separate site Pelloski et al. found that elevated p-MAPK expression was strongly associated with poor response to radiotherapy (P < 0.001) [104]. Patil et al. looked at 188 newly diagnosed glioblastomas retrospectively and separated them into low, medium and high tiers of p-MAPK expression with median survivals of 32.4, 18.2, and 12.5 months, respectively. Using multivariate analysis they showed a 2.4-fold hazard of death among patients with intermediate p-MAPK expression over low p-MAPK expression (hazard ratio [HR], 2.4; P = 0.02). Also, high-expression patients were 3.9 times more likely to die, compared with patients with low p-MAPK expression (HR, 3.9; P = 0.007) [105]. Though both of these studies are class III in nature, their significant findings suggest marker may be strongly prognostic and a prospective study may add significantly to the literature.

EGFR amplification

Srividya et al. assessed 140 newly diagnosed glioblastoma patients prospectively for *EGFR* amplification and

Table 4 Evidence for neuropathology tumor markers as a means of diagnosis of glioblastoma

Author/year	Study description	Data class	Conclusion
Manterola et al. (2014)	A multi-institutional, retrospective study of 75 patients with newly diagnosed glioblastoma patients. 55 healthy donors were utilized. Sera were collected from each individual and tested for the differences in the miRNA expression levels of serum microvesicles. Sera from an initial training group of 25 patients was analyzed first. These were compared to a group of the healthy controls paired by age and sex. Then a validation group of an additional 50 glioblastoma cases was studied	III	The expression levels of 1 small noncoding RNA (RNU6-1 was most upregulated compared to normal, P < .001) and 2 microRNAs (miR-320, P = 0.0067, and miR-574-3p, P = 0.0055) were significantly associated with a GBM diagnosis. In addition, RNU6-1 was consistently an independent predictor of a GBM diagnosis The authors conclude that that this RNA signature could serve as a differential diagnostic marker for glioblastoma This study was well done with a test and validation set but the validation set was not interpreted in a blinded fashion yielding class III data
Young et al. (2013)	A multi-institutional, retrospective analysis of 147 newly diagnosed glioblastomas correlating MR imaging features and sequences (sagittal and axial T1-weighted images; axial T2-weighted images; axial gradient recalled-echo [GRE] or SWI; axial DWI with ADC maps; and contrast coronal, sagittal, and axial T1-weighted images) with epidermal growth factor receptor gene amplification status by fluorescent in situ hybridization	III	DWI proved to be most useful and a total of 142 cases had adequate imaging for quantitative analysis. Restricted water diffusion correlated with epidermal growth factor receptor amplification (P = .04). Quantitative DWI analysis found that all ADC measurements correlated with epidermal growth factor receptor amplification, with the highest correlations found with ADC_{ROI} (P = .0003) and ADC_{mean} (P = .0007) With the restricted population utilized, the semiquantitative nature of in situ hybridization analysis for EGFR amplification and relative quantitative data available from DWI sensitivity, specificity, positive and negative predictive values, and likelihood ratios could be calculated. However the analysis was not assessed in a blinded fashion. In terms of DWI data being diagnostic for EGFR amplification status, this yields class III data

DWI diffusion weighted imaging; *EGFR* epidermal growth factor receptor; *GRE* gradient recalled-echo; *miRNA, miR* micro-ribonucleic acid; *MR* magnetic resonance; *RNA* ribonucleic acid; *ROI* region of interest

expression. They found a strong positive correlation between amplification and expression ($p < 0.0001$). They found a statistically significant interaction between *EGFR* overexpression and age. This quantity predicts progressively shorter survival in an age dependent manner but the data was not assessed in a blinded fashion and no validation set was reported, making this class III data [106].

Insulin-like growth factor-binding proteins

Insulin-like growth factor-binding protein (IGFBP) isoforms 2, 3 and 5 were assessed by Santosh et al. using real time quantitative PCR and immunohistochemistry prospectively in a group of 136 newly diagnosed glioblastoma patients. In a multivariate analysis IGFBP-3 expression was found to be associated with shorter survival in glioblastoma ($p = 0.007$). The non-blinded nature of the data assessment and lack of a validation set yields class III data in this case [107].

10q23/PTEN deletion

In a prospective study of 73 newly diagnosed glioblastoma patients treated with radiotherapy and concurrent and adjuvant temozolomide Srividya et al. analyzed 10q23/PTEN deletion by fluorescence in situ hybridization. They found that individuals with this deletion were generally older and had a shorter survival ($p < 0.05$) [108]. In a retrospective study of 155 patients treated with radiotherapy and concurrent and adjuvant temozolomide chemotherapy Carico used immunohistochemistry to assess PTEN expression. PTEN loss was not prognostic of overall survival (HR 1.31, CI: 0.85–2.03, $p = 0.22$) [109]. The contradictory results of the information from these two studies impedes the ability to

Table 5 Evidence for neuropathology tumor markers as a means of determining prognosis for glioblastoma

Author/year	Study description	Data class	Conclusion
Gurrieri et al. (2018)	A retrospective, single center 108 consecutive GBM patients Tumor specimens underwent pyrosequencing for MGMT promotor methylation status. Methylation status, based on average methylation level of ten CpG sites, was divided into unmethylated (UM < 9%), Indeterminate Methylation (IM 9–29%), and highly methylated (HM > 29%). Outcomes were PFS and OS	III	Median overall survival for all patients was 14.4 months and progression free survival was 13.1 months. Fifty-one patients were UM with OS of 13.2 (95% CI: 8.3–15.2) months and PFS 7.97 (95% CIL 6.0–9.5)months, 24 patients had IM with an OS of 15.8 months (95% CI: 9.8–30.8) and PFS of 11.6 months (95% CI: 7.37–24.3), and 33 patients with HM with an OS of 19.5 months (95% CI: 11.5–47.1) with a PFS of 15 months (95% CI: 10.0–24.9). They additionally found that patients treated under the Stupp protocol had a better OS and PFS in all groups Authors conclude that MGMT status is an independent prognostic marker for GBM A well-designed study that is limited by a lack of consensus in the community on the most accurate way to determine MGMT promoter methylation status or the number of CpG islands that should be analyzed
Romano et al. (2018)	A single center, retrospective review of 21 patients with glioblastoma who underwent gross total resection followed by concomitant chemo-radiation. Immunohistochemical analysis was performed for p-ATM (phospho-Ataxia Telangectasia Mutated) and p53 Immunohistochemistry was evaluated by two pathologists. For p-ATM staining, no signal was "0", the percent positive cells less than or equal to 10% was considered "+", 10–50% was "++", > 50% was "+++". For p53 expression, > 10% was considered over-expressed and less than this was normal expression. There was a minimum of 2 years follow-up for each patient. The outcome evaluated was OS	III	Eighteen cases had p-ATM expression with 6 cases of "+", 4 of "++" and 8 of "+++". For p53, 10 cases showed overexpression. Patients with overexpression of ATM showed a worse OS than those with low ATM expressions (23 months vs 14 months, p = 0.022). The expression of p53 did not significantly affect overall survival (HR 0.809, p = 0.675). Four subgroups were compared with normal expression of p53 and low p-ATM expression having the longest survival of 36 months, followed by 20 months for p53OE/ATM-low, 14 months for p53OE/ATM-high, then 10 months for p53NE/ATM-low The authors conclude that p-ATM expression has prognostic value in GBM patients which may be related to radiosensitization properties. Additionally, they conclude p53 when measured with ATM expression has prognostic value due to the results of their subgroup analysis This is not a well-designed study for prognosis and the author's conclusions on the additional prognostic value in determining p53 status is not well supported. A larger sample size under current WHO diagnostic criteria would strengthen the conclusions found in this study
Shu et al. (2018)	A retrospective single center review of 304 adult wild-type IDH GBM patients The authors used MGMT promoter methylation, TERT promoter mutations, MRI-features, along with known prognostic factors to perform a survival analysis to determine if TERT promoter mutations and MGMT promoter methylation are prognostic biomarkers. Several statistical analyses were performed for the final survival analysis including random survival forest analysis, Kaplan–Meier analysis, Cox proportional hazard regression, and LASSO regression	III	The random forest analysis showed 4 subgroups (MGMT, p = 0.00154, age, p = 0.00238, edema, p = 0.03998, ADC value p = 0.02874) with significantly different OS. TERT promoter mutations themselves did not show a significantly different OS This study is limited in its retrospective nature but shows a survival benefit with the presence of MGMT promoter methylation

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Vasaikar et al. (2018)	A retrospective study of 25 patients with glioblastoma for the survival analysis The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) database were analyzed to evaluate for endothelin B receptor (ETBR) expression. Expression levels were then determined in the 25 included patients and correlated with OS	III	In expression data from TCGA and the GEO database, mRNA expression of ETBR was higher in glioblastoma tissue compared to normal aging brain tissue. In the 25 patients studied, 64% were found to have low ETBR expression and 36% high expression. OS was significantly shorter in patients with high ETBR expression compared to those with low The authors conclude ETBR expression is higher in GBM than control brain tissue, higher levels are correlated with a worse outcome, and this is a potentially drugable target This is a small, retrospective study and survival analysis only included Swedish patients
Urbschat et al. (2017)	In this retrospective study, 72 patients with a new diagnosis of GBM were included They were divided into 2 matched cohorts of 36 patients each. Group A received standard Stupp regime. Group B underwent standard Stupp regime along with carmustine wafer implantation. Each tumor was tested for MGMT methylation status and Comparative genomic hybridisation (CGH) for chromosomal changes The outcome measure was OS	III	No significant difference in OS was found in Group A and Group B, median OS was 267 days Overall, presence of MGMT promotor methylation was associated with a longer OS (10.7 months vs 6.6 months, $p=0.041$) but its presence did not affect OS in each subgroup. In CGH analysis of Group B, amplification of 4q12 was associated with a shorter OS ($p=0.00835$) and loss of chromosome 10 and loss of 13q were associated with a longer OS ($p=0.0123$, $p=0.0364$ respectively). These affects were not seen in Group A The authors conclude MGMT promotor methylation is associated with a better prognosis overall. They also conclude that based on presence of chromosomal alterations, there may be subsets of patients who benefit from carmustine wafers and others who fair worse with use of carmustine wafers This study is limited by its small size given number of chromosomal alterations examined and its retrospective nature
Boonyawan et al. (2017)	A retrospective, single institution study of 122 patients treated with concurrent chemoradiation (CRT) followed by adjuvant temozolomide (TMZ) for new diagnosis of glioblastoma Platelet and lymphocyte counts were taken before and after CRT as well as during adjuvant TMZ. A greater than 30% increase in counts after CRT was considered the "high platelet" group. Overall survival in each group was compared	III	In the "high platelet" count cohort when comparing from before and after CRT was found to have a significantly lower overall survival of 11 months compared to 28 months in the "low platelet" group ($p=0.0062$, HR 3.4 (1.6–7.5). This was not found when comparing lymphocyte counts The authors concluded that elevation in platelet counts after CRT may be used as a poor prognostic marker This study included a small number of patients in the group of interest (12). A multivariate analysis was performed for other known prognostic factors: Age, KPS, IDH1 status, MGMT status, and extent of resection. The study had a well-designed control of lymphocyte counts to rule out general bone marrow effects

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Cesarini et al. (2017)	A retrospective, single institution study of 69 patients with GBM who underwent surgical resection followed by RT and TMZ Immuno-reactivity of type 5 phosphodiesterase (PDE5) was determined within tumor specimen for each patient. The outcome was OS	III	Fifty percent of patients' tumors showed strong PDE5 immuno-reactivity. Multivariate analysis for other prognostic factors were included (EGFRvIII expression, age, Ki67 index, KPS, and MGMT status) There was a correlation between those patients whose tumors showed high PDE5 expression and overall survival (15 months with high expression vs 10 months with low expression, $p = 0.0028$). The authors perform several in vitro tests to evaluate the underlying mechanism behind PDE5 prognostic effect Further investigations may help show the expression of PDE5 in tumor tissue and its prognostic indication
Mikkelsen et al. (2017)	A retrospective, single institution study of 106 patients with new diagnosis of glioblastoma and had 2 pre-operative MRIs available greater than 14 days apart The patients are divided into 2 groups: faster than expected and slower than expected Gompertzian growth, Histopathological slides were reviewed by a medical student then confirmed by a pathologist. Features evaluated for included: necrosis, microvascular proliferation, pseudopalisades, cellular density, atypia, mitotic count, vascular features (thromboses, hemorrhage, pseudorosettes), vascular density, secondary structures of Scherer, desmoplasia, leukocytes, GBM subtypes, and cell types. Multivariate analysis was the performed	III	The median tumor volume in all patients increased from 17.7 mL to 27.5 mL between the first and second scans. Half of patients had slower than expected growth, and half faster than expected growth. In patients with higher than expected tumor growth, increased thromboses ($p = 0.015$) and high cellular density were found (0.013) The authors conclude that high cellular density and thromboses are histopathologic features associated with radiologic faster growth in patients with GBM This study is limited by its retrospective nature. The study design is weak as pathology slides were not blinded and reviewed by more than one independent pathologist
Nguyen et al. (2017)	A retrospective review including 2 cohorts of IDH wildtype primary GBM 303 patients were included in the UCLA/Kaiser cohort and 190 from The Cancer Genome Atlas cohort IDH1 genotype and MGMT promoter methylation status were determined. PCR amplification of hTERT promoter was performed on all UCLA/Kaiser cohort tumors and expression levels determined. For TCGA cohort, this data was extracted from published data and only those with hTERT mutation and MGMT promoter status were included. OS was assessed in both cohorts and PFS was evaluated in the UCLA/Kaiser cohort	III	In the UCLA/Kaiser cohort, hTERT mutation was detected in 75% of patients. In patients with hTERT promoter mutation the OS was 18.5 month versus 17.8 months in those without. When MGMT promoter methylation status and hTERT mutation status were evaluated together, patients with the hTERT mutation (hTERT.MT) and MGMT methylation (MGMT.M) had the best overall survival of 28.3 months whereas the poorest was seen in hTERT.MT and unmethylated MGMT at 15.9 months ($p < 0.0001$). In TCGA cohort, hTERT.MT-MGMT.M patients had an OS of 17.8 months compared to the 13.9 months ($p = 0.0026$) in hTERT.MT-unmethylated MGMT patients No significant difference in overall survival was seen in the hTERT wildtype patients when comparing MGMT promoter methylation status The authors found that hTERT promotor mutations alone were not associated with prognosis. In patients who have both hTERT mutations and methylation of the MGMT promoter, a survival benefit is seen This is a well-designed study limited by its retrospective nature

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Molitoris et al. (2017)	A retrospective, multi-institutional study of new GBM patients who underwent maximal safe resection followed by radiation therapy and temozolomide after routine analysis for MGMT methylation status Prognostic variables were taken for each patient including age at diagnosis, gender, KPS, MGMT status, and EOR. There were 2 cohorts evaluated the "entire cohort" (n = 486) which included patients with unknown MGMT methylation status (n = 177) and the validation cohort which included only those patients with a known MGMT methylation status (n = 309) The overall survival for each patient was then used a nomogram of the prognostic factors in the "validation" cohort	III	In the "validation cohort" significant ($p < 0.01$) predictors of survival included Age, KPS, MGMT status, EOR, and the nomogram predicted survival (NPS). In the multivariate analysis for 1 year overall survival only the 1-year NPS continued to be significant (HR 0.31, $p < 0.01$). At 2 years, patients in the highest quintile of the 1-year NPS groups had a survival of 63% compared to 7% in the lowest quintile group The authors concluded that the 1-year NPS adequately represented all factors in their prognostic significance This study evaluated a nomogram that included MGMT methylation status to predict overall survival at 1 year. Further prospective studies would confirm its prognostic value
Thon et al. (2017)	A prospective single center study of MGMT promoter methylation status as a prognostic marker in 56 patients with new diagnosis of unresectable glioblastoma, confirmed by biopsy, who underwent concurrent chemoradiation The outcomes measured were OS, PFS, and PRS (post-recurrence survival)	III	Fifty-six patients met criteria for this study. 30 had MGMT promoter methylation present and 26 were unmethylated. At final analysis, 98.2% had progression and 94.6% had died, 1 patient was lost to follow-up. Patients who were methylated had significantly longer PFS and OS of 15.0 and 20.3 months vs 6.1 ($p = < 0.001$) and 7.3 months ($p = < 0.001$). The PRS was significantly longer in methylated patients as well (4.5 vs 1.4 months, $p < 0.001$). In the methylated group, 20% of patients survived past 4 years whereas none did in the unmethylated group. MGMT status was the strongest predictor of survival in multivariate analysis The authors conclude that MGMT methylation status is an independent prognostic marker for patients with unresectable glioblastomas treatment undergoing concurrent chemoradiation This small study with long-term follow-up indicates that MGMT promoter methylation is a positive prognostic marker in patients undergoing biopsy only followed by radiation and temozolomide. It is uncertain if this is due to improved response to therapy or is an independent positive marker. Additionally, it is uncertain if treating physicians were blinded to MGMT status
Yuan et al. (2017)	A retrospective single institution study of 48 primary GBM patients The patients were divided into long- and short-term survivors (more or less than a 450 day survival) MGMT promoter methylation status was determined in each patient. Of the 2 compared groups, 6 tumors from each group underwent miRNA microarray and qRT-PCR analysis. Upregulated miRNAs found in long term survivors were then evaluated in all 48 patients. The outcome was OS	III	Thirty-one patients were included in the short-term survivor group (mean OS = 300 days) and 17 in the long-term survivor group (mean OS = 827 days, $p < 0.0001$). Presence of MGMT promoter methylation was associated with a longer overall survival (467 vs 328 days, $p = 0.0004$). Four miRNAs were found to be upregulated in long-term survivors—let-7 g-5p, miR-139-5p, miR-17-5p, and miR-9-3p The authors conclude that the four-miRNAs found to be upregulated in this study may potentially be a new prognostic marker. Additionally, they showed that MGMT promoter methylation is associated with a better prognosis This retrospective small study of miRNAs shows a potential new prognostic marker that will require validation in prospective studies

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Wang et al. (2016)	This is a retrospective study which included 41 GBM patients with MGMT promoter methylation as a training set to determine gene signatures correlated with prognosis in these patients The patients are placed in "high" and "low" risks groups based on their gene signature. The genes found were then tested in the CGGA (Chinese Glioma Genome Atlas) and TCGA (The Cancer Genome Atlas). The outcome was overall survival	III	The authors found a 3-gene signature associated with a worse prognosis determined to be the "high" risk group—FPR3, IKBIP, and S100A9 expression. The "low" risk group in the training set had a median OS of 1074 days vs 372 days in the "high" risk group ($p=0.0033$). In the CGGA set and TCGA the median OS of the "low" risk vs "high" risk groups were 1074 vs. 374 days ($p=0.0001$) and 489 vs 342 days ($p=0.0315$) respectively The manuscript concludes that the three-gene signature described has prognostic value for GBM patients with MGMT promoter methylation This is not a well-designed study and difficult to read due to poor writing. Further and better designed studies would strengthen the results of this study
Evans et al. (2016)	Prospective, single institution study of 16 patients with newly diagnosed glioblastoma proven by surgery. Patients provided blood samples before, twice during and at the end of standard chemoradiation. Microvesicles were isolated from the samples using serial centrifugation and stained for surface markers (Annexin V, CD41, anti-EGFR, and CD235) and analyzed by flow cytometry. These results were compared to time to progression and overall survival	III	Increases in Annexin V positive microvesicle levels during chemoradiation therapy were associated with earlier recurrence and shorter overall survival in newly diagnosed glioblastoma patients The authors concluded this effect was “dramatic”, with over a four-fold increase in the hazard ratio for an individual at the 75th versus the 25th percentile of Annexin V positive microvesicle levels The interpretation of the test results were not blinded and there was no separate validation set, and thus this is class III data
Zhang et al. (2016)	A single institution, retrospective study of 70 patients with newly diagnosed glioblastoma. Tumor tissue samples were assessed for interferon-induced protein with tetratricopeptide repeat 1 (IFIT1); the expression of which inhibits the expression of O-6-methylguanine-DNA methyltransferase (MGMT). MGMT was also measured by the immunohistochemistry. Expression was measured by semiquantitative analysis	III	The $\chi 2$ test indicated that the expression of IFIT1 and MGMT was negatively correlated ($r=-0.288$, $P=.016$). Univariate and multivariate analyses confirmed high IFIT1 expression as a favorable prognostic indicator for progression-free survival ($P=.005$ and .017) and overall survival ($P=.001$ and .001), respectively The authors conclude that IFIT1 is predictive biomarker for this patient population. The retrospective nature of the data, lack of blinding of the result interpretation and the lack of a prospective validation set yields class III data
Lin et al. (2014)	A single institution, retrospective analysis of 90 newly diagnosed glioblastomas treated in a variety of manners. Tumor specimens were subjected to phospho(p)-STAT3 (Tyr705) immunohistochemistry. The specimens were then reviewed by 3 pathologists blinded to outcome. These results, as well as standard prognostic parameters were compared to PFS and OS	II	Univariate survival analysis revealed significant correlations of high p-STAT3 expression with shorter PFS ($P=0.012$) and OS ($P=0.009$). Multivariate survival analysis confirmed high p-STAT3 expression as a significant prognostic indicator for shorter PFS (HR 2.158, $P=0.019$) and OS (HR 2.120, $P=0.031$), independent of age, KPS and chemoradiotherapy The authors conclude that STAT3 might be a promising therapeutic target in GBM The blinded nature of p-STAT3 assessment without going to a validation set yields class II data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Kanemoto et al. (2014)	This is a retrospective, multi-institutional study of 85 newly diagnosed glioblastomas treated with radiation therapy with or without some form of chemotherapy. The authors quantitatively analyzed the methylation status of the entire MGMT promoter region in patients with tissue qualifying for the study. The patients were split into a 52 patient training set and a 32 patient test set. Progression free survival and overall survival were calculated. The genomic DNA was subjected to bisulfite treatment and then analyzed by Sanger sequencing with coverage of 96 clones per sample. This analysis quantitatively revealed the degree of methylation of each cytidine phosphate guanosine (CpG) site. Based on these data, they constructed a prognostic prediction system for glioblastoma patients using a supervised learning method for a training set. This was validated in the test set of 32 samples	III	The data from the training set demonstrated excellent prognostic ability with OS and PFS (OS, $p=0.0381$; PFS, $p=0.00122$) Thus, the diagnostic accuracy of their system was better than that of the methylation specific PCR based approach (OS, $p=0.993$; PFS, $p=0.113$). In the test set the significance was also derived for OS ($p=0.0476$) and PFS ($p=0.0376$) The authors conclude a precise assessment of the methylation status of the MGMT promoter may improve the prediction of disease progression. They used a test set, though it was extracted from the same population of patients as the training set and the response data was not blinded from the investigators. This and the overall retrospective nature of the study design results in this data being class II
Manterola et al. (2014)	A multi-institutional, retrospective study of 75 patients with newly diagnosed glioblastoma patients. 55 healthy donors were utilized. Sera were collected from each individual and tested for the differences in the miRNA expression levels of serum microvesicles. Sera from an initial training group of 25 patients was analyzed first. These were compared to a group of the healthy controls paired by age and sex. Then a validation group of an additional 50 glioblastoma cases was studied	III	The expression levels of 1 small noncoding RNA (RNU6-1 was most upregulated compared to normal, $P<.001$) and 2 microRNAs (miR-320, $P=0.0067$, and miR-574-3p, $P=0.0055$) were significantly associated with a GBM diagnosis. In addition, RNU6-1 was consistently an independent predictor of a GBM diagnosis The authors conclude that that this RNA signature could serve as a differential diagnostic marker for glioblastoma This study was well done with a test and validation set but the validation set was not interpreted in a blinded fashion yielding class III data
Tanaka et al. (2014)	A multi-institutional, retrospective quantitative measurement of MGMT mRNA in 140 newly diagnosed gliomas. Of these 73 were glioblastomas and of those 45 were treated with radiation therapy with concurrent and then adjuvant temozolomide. Progression free survival and overall survival were correlated with the mRNA quantity	III	In the 73 GBMs, a significant prognostic factor for progression-free survival was fewer than 1000 copies/mg RNA of MGMT mRNA ($p=0.0150$). Of 45 patients with GBMs that had been treated with temozolomide and radiation, progression-free survival was significantly longer for those whose GBM had fewer than 1000 copies/mg RNA of MGMT mRNA than for those whose GBM had more than 1000 copies/mg RNA ($p=0.0090$) The authors concluded MGMT mRNA might be useful as a prognostic factor and for predicting the results of therapy for GBMs treated by temozolomide The data was assessed in a blinded fashion and no validation set of cases
Lalezari et al. (2013)	This is a retrospective analysis of 418 newly diagnosed glioblastomas from two institutions primarily treated with temozolomide and radiation in various combinations. The investigators determined MGMT expression by IHC, and MGMT promoter methylation by methylation-specific PCR (MSP) and bisulfite sequencing of 24 neighboring CpG sites. Cases with over 3 CpG sites methylated were designated as hypermethylated. This data was correlated with OS and PFS	II	Patients with $<30\%$ IHC staining for MGMT had PFS of 10.9 months and OS of 20.5 months, compared with PFS of 7.8 months ($P<.0001$) and OS of 16.7 months ($P<.0001$) among patients with $\geq 30\%$ staining. PFS was also higher among hypermethylated patients than among hypomethylated patients (11.5 months vs. 7.9 months; log-rank, $P=.0001$). By Kaplan–Meier analyses, combined testing of MGMT MSP and IHC enabled identification of a long-term survival group when methylation and low expression were observed in tandem ($P<.0001$) The pathologists interpreting the MGMT analyses were blinded to the clinical data improving the value of this data but a validation set is not noted. These points in addition the retrospective data collection leads to the conclusion this is class II data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Lee et al. (2013)	A single institution, retrospective study of 36 newly diagnosed glioblastomas extracted from a series of 211 gliomas. They were treated with standard surgical techniques followed by radiation and concurrent and adjuvant temozolomide. They investigated the expression of CD151 (one of the tetraspanin superfamily members, was the first member of the tetraspanins to be identified as a positive effecter of metastasis) by immunohistochemistry, and O6-methylguanine-DNA methyltransferase (MGMT) methylation analysis using real-time methylation-specific PCR and correlated with PFS and OS	III	Overexpression of CD151 was observed in a significant proportion (55.6%) of glioblastomas. CD151 overexpression was closely associated with MGMT methylation (P = 0.014), and it was a prognostic factor for predicting worse overall survival (OS; P = 0.002) and progression-free survival (PFS; P = 0.043). It was also found that combination of CD151 overexpression and MGMT methylation better stratified the patients' OS (P = 0.001) and PFS (P = 0.009). In multivariate analysis, CD151 overexpression was an independent prognostic factor for predicting OS over MGMT methylation (P = 0.012)
McDonald et al. (2013)	This is a retrospective, single institution review of 78 newly diagnosed glioblastoma patients. Bisulphite modification followed by CpG pyrosequencing was performed to assess the percentage level of MGMT promoter methylation for each tumor specimen. The authors point out the importance of the variant T genotype of the c.-56 C > T promoter-enhancer SNP (rs16906252) in various cancers and therefore evaluate it in these glioblastomas. DNA isolated from tumor tissue was used for genotyping of the c.-56 C > T SNP (rs16906252). This data was correlated with survival	III	The study confirmed that MGMT methylation is associated with a significant survival benefit in glioblastoma patients treated with temozolomide (either concurrently with radiotherapy or sequential treatment). The study demonstrated that a promoter variant, the c.-56C > T (rs16906252) single nucleotide polymorphism (SNP) located within a cis-acting enhancer element at the proximal end of MGMT, is associated with the presence of MGMT promoter methylation in de novo glioblastoma. Furthermore, the authors show that the overall survival of patients carrying both the above mentioned SNP and MGMT methylation showed a strong survival benefit (p = 0.025) when compared to either molecular event on its own Assessments were not blinded and the authors recommend a validation study. This represents class III data
Patil et al. (2013)	A single institution, retrospective analysis of 188 patients. Patients surviving less than 30 days and without satisfactory tissue for activated mitogen-activated protein kinase (p-MAPK) were excluded. This yielded 108 cases for evaluation Nuclear p-MAPK expression. Evaluation of p-MAPK immunostaining was performed in at least 3 separate fields of the tumor, each containing ≥ 50 tumor cells. Immunoreactive nuclei were quantified with the mean and standard deviation of reactive nuclei in each of the 3 fields. The results were categorized in the following levels: low (0%–10%), medium (11%–40%), and high (41%–100%). This information was correlated with survival data	III	Median overall survival among all patients was 19.5 months. Activated MAPK expression levels of < 10%, 11%–40%, and ≥ 41% were observed in 33 (30.6%), 37 (34.3%), and 38 (35.2%) patients, respectively Median survival for low, medium, and high p-MAPK expression was 32.4, 18.2, and 12.5 months, respectively. Multivariate analysis showed 2.4-fold hazard of death among patients with intermediate p-MAPK over low p-MAPK expression (hazard ratio [HR], 2.4; P = .02); high-expression patients were 3.9 times more likely to die, compared with patients with low p-MAPK (HR, 3.9; P = .007) The assessment of the results was not blinded and no validation set was evaluated, leaving this as class III data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Reynes et al. (2013)	A single institution, prospective study of 22 patients with newly diagnosed glioblastoma and 40 concurrent normal controls accrued over 18 months. All patients received standard radiation, concomitant and adjuvant temozolomide. Venous blood samples were obtained from patients within two weeks before the start of radiochemotherapy and during the last week of concomitant treatment. Endothelial cells were measured by an immunomagnetic technique and immunofluorescence microscopy. Microparticles were quantified by flow cytometry. Microparticle-mediated procoagulant activity was measured by endogen thrombin generation and by phospholipid-dependent clotting time. Methylation status of MGMT promoter was determined by multiplex ligation-dependent probe amplification	III	Pretreatment levels of circulating endothelial cells and microparticles were higher in patients than in controls ($p < 0.001$). After treatment, levels of microparticles and thrombin generation decreased, and phospholipid-dependent clotting time increased significantly. A high pretreatment endothelial cell count, corresponding to the 99th percentile in controls, was associated with poor overall survival. MGMT promoter methylation was present in 27% of tumor samples and was associated to a higher overall survival (66 weeks vs 30 weeks, $p < 0.004$) The authors conclude this exploratory study suggests an association between postsurgical higher CEC count and shorter survival in patients with glioblastoma. The lack of blinded assessments and lack of a validation set yield class III data
Rosati et al. (2013)	Single institution, retrospective study of 83 patients with newly diagnosed glioblastoma. Immunohistochemical expression of glutamine synthetase was scored semi-quantitatively on the basis of cell number, staining intensity, and distribution of immunoreactive cells. These findings were compared to patient demographics, seizure history, treatment type and treatment outcomes	II	Glutamine synthetase expression patterns in neoplastic cells were inversely correlated to the presence of epilepsy ($P < 0.0001$ for intensity and $P < 0.009$ for homogeneity of GS distribution, respectively). Absent/low intensity of Glutamine synthetase expression was significantly associated with a longer survival in both univariate (19 vs 8 months; $P = 0.0005$) and multivariate ($P = 0.003$) analyses The authors conclude glutamine synthetase is a valuable biomarker for epilepsy and overall survival. The study parameter was assessed in a blinded fashion but no validation set of cases were evaluated yielding class II data
Sunwoo et al. (2013)	This is a single institution, retrospective study of 26 newly diagnosed glioblastoma patients. Each was diagnosed by H&E technique and evaluated by Ki-67 labeling index. Each had undergone MRI including diffusion weighted imaging with standard b values ($b = 1000$ s/mm^2) within 2 weeks before surgery. Additionally each case underwent assessment of MGMT promoter methylation status by methylation-specific polymerasechain reaction and methylation specific multiplex ligation-dependent probe amplification	III	The mean ADC revealed a positive relationship with MGMT promoter methylation ratio ($P = 0.015$) and was also significantly different according to MSP-determined methylation status ($P = 0.011$). Median PFS was significantly related with methylation ratio ($P = 0.017$) and MSP-derived methylation status ($P = 0.025$) A positive relationship was demonstrated between PFS and the mean ADC value ($P = 0.001$). The 5th percentile ADC values showed a significant negative relationship with Ki-67 labeling index ($P = 0.036$) The authors recognize the need for further investigation. The lack of ability to calculate usual statistical predictive parameters and lack of blinded assessment of this imaging analysis leaves this as class III data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Young et al. (2013)	A multi-institutional, retrospective analysis of 147 newly diagnosed glioblastomas correlating MR imaging features and sequences (sagittal and axial T1-weighted images; axial T2-weighted images; axial gradient recalled-echo [GRE]; axial DWI with ADC maps; and contrast coronal, sagittal, and axial T1-weighted images) with epidermal growth factor receptor gene amplification status by fluorescent in situ hybridization	III	DWI proved to be most useful and a total of 142 cases had adequate imaging for quantitative analysis. Restricted water diffusion correlated with epidermal growth factor receptor amplification (P = .04). Quantitative DWI analysis found that all ADC measurements correlated with epidermal growth factor receptor amplification, with the highest correlations found with ADC_{ROI} (P = .0003) and ADC_{mean} (P = .0007) With the restricted population utilized, the semiquantitative nature of in situ hybridization analysis for EGFR amplification and relative quantitative data available from DWI sensitivity, specificity, positive and negative predictive values, and likelihood ratios could be calculated. However the analysis was not assessed in a blinded fashion. In terms of DWI data being diagnostic for EGFR amplification status, this yields class III data
Carico et al. (2012)	This is a single institution, retrospective study of 155 patients with newly diagnosed glioblastoma. Tumor tissue was analyzed for PTEN expression through IHC and EGFR amplification status as revealed by fluorescence in-situ hybridization. This data was compared to survival information. The focus was to assess this marker in patients in the "temozolomide" era as opposed to data in the literature on patients treated prior to common use of temozolomide	III	Median survival of 20.0 months (95% CI: 15.0–25.5) and 18.2 months (95% CI: 13.0–25.7) was observed in PTEN retained and PTEN loss patients, respectively (p = .71). PTEN loss was not prognostic of overall survival (HR 1.31, CI: 0.85–2.03, p = .22). EGFR alterations were more common in patients with PTEN loss Though this is a negative observation, this is of interest given information on patient sets treated prior to the temozolomide era suggesting the import of PTEN The histologic data was not assessed in a blinded fashion and no validation set was assessed. This produces class III data
Karim et al. (2012)	This is a prospective study of standard radiation and temozolomide in 31 patients with newly diagnosed glioblastoma. Each underwent analysis of MGMT protein immunostaining, MGMT methylation-specific PCR assay, and Ki-67 expression using immunohistochemistry expressed as a labeling index. This information was compared to PFS and OS	III	The MGMT-methylated patients had a higher median time to progression of 13 months (range 8 to 18 months, 95% CI of 9.36 to 12.9), and OS of 24 months (range 12 to 31 months, 95% CI of 16.1 to 21.32), while the unmethylated patients had a median time to progression of 6.5 months and a median OS of 12 months, such correlations were highly significant (P = 0.0001). MGMT immunoexpression failed to show significant correlation with MGMT promotor methylation or the outcome of the patients. Patients with Ki-67 < 17% had a median time to progression of 16 months and median OS of 24 months compared to 7 and 12.5 months respectively for the patients with Ki-67 ≥ 17% (P < 0.05) The histologic assessments were not performed in blinded fashion and no validation set was undertaken, yielding class III data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Motomura et al. (2012)	A multi-institutional, retrospective analysis of tissue from 79 newly diagnosed glioblastoma patient. These were assessed by immunhistochemical staining for Olig2, IDH1-R132H, p53, PDGFRA, PDGFB, synaptophysin, p16, EGFR, Hes-1, Nestin, VEGF, YKL-40, CD4, podoplanin, GFAP, and Ki-67. This data was correlated with survival. An independent set of 401GBM mRNA expression profiles was compiled from theOncomine Premium Research Edition to assess subtype reproducibility	III	Consensus clustering identified four distinct GBM subtypes: Oligodendrocyte Precursor (OPC) type (characterized by highly positive scores of Olig2, PDGFRA, p16, p53 and synaptophysin), Differentiated Oligodendrocyte (DOC) type (high positivity for the oligodendroglial marker Olig2 and negativity for p53 and p16), Astrocytic Mesenchymal (AsMes) type (strongly associated with strong expressions of nestin, CD44 and podoplanin, with a high glial fibrillary acidic protein score) and Mixed type (frequent expressions of p16, EGFR and Hes-1, as well as a p53 were predominant). The median overall survival of OPC-type patients was significantly longer than that of the AsMes-type patients (19.9 vs 12.8 months, P = 0.041) The histologic and data base assessment was not blinded to outcome. Also, the validation set was limited as IDH1- R132H, p53, p16 and Ki-67 were not available in the 401 GBM mRNA expression profiles. Thus this represents class III data
Niyazi et al. (2012)	A retrospective, single institution study of 79 newly diagnosed glioblastomas treated with standard concurrent radiation and temozolomide and adjuvant temozolomide. The patients received a total dose of 60 Gy in 6 weeks, in a once-daily schedule of 2 Gy per fraction for a total of 30 fractions. Patients were treated using megavoltage equipment, such as linear accelerator beams with minimal nominal energy of 6 MV. In 54 patients MGMT methylation status was evaluated. They were followed with [^{18}F]Fluoroethyltyrosine ([^{18}F]FET) and additional MRI for recurrence Recurrences were defined as ''in-field'' if more than 80% of the tumor recurrence resided within the prescription 95% isodose surface, and ''marginal'' if 20–80% of the lesion was inside the 95% isodose surface. All others were defined as outside the field or "ex-field"	III	Median follow-up was 595 days. 41.5% (12/29) of the MGMT methylated population had no relapse, 37.9% (11/29) had an in-field-recurrence and 20.7% (6/29) an ex-field/marginal recurrence, whilst 28.0% (7/25) of the MGMT unmethylated population had no relapse, 64.0% (16/25) had an in-field-recurrence and 8.0% (2/25) an ex-field/marginal recurrence (p = 0.15) In MGMT methylated patients, the 1-year/2-year OS was 93.1%/ 78.1% compared to 64.9%/7.3% in MGMT unmethylated patients Median PFS was 642 days for MGMT methylated patients compared to 231 days in MGMT unmethylated patients (p < 0.001) This assessment of MGMT in relationship prognosis for recurrence after radiation was not done in a blinded fashion and no validation set of patients was assessed, leaving this as class III data
Pallud et al. (2012)	This is a single institution, retrospective analysis of 308 consecutive newly diagnosed primary glioblastoma patients. Included in the pathology analysis of the tumor samples was immunohistochemistry for neurofilament protein (NFP) clone 2F11, which reacts with the phosphorylated forms of the 70-kilodalton component of this protein. This was correlated with standard demographic parameters and survival	II	Median overall survival and progression-free survival were 13.0 and 7.6 months, respectively, for NFP-positive GBMs, and 7.0 and 5.1 months, respectively, for NFP negative GBMs (p = 0.02). Based on multivariate analysis NFP immunoexpression was found to be an independent factor associated with overall survival The authors conclude NFP may help identify a distinct subgroup of primary GBMs with a favorable prognosis, which should be considered in the design of future targeted therapies The pathology review of the specimens was blinded from clinical parameters and outcome. No validation set has been addressed and thus this is class II data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Ohka et al. (2011)	A multi-institutional, retrospective study including 51 primary and 3 secondary glioblastomas. Tumor DNA was extracted and modified with bisulfate. Pyrosequencing technology was used to determine the methylation status of the CpG island region of the MGMT promoter and LINE-1. The investigators used the touchdown PCR method for the MGMT promoter and the conventional PCR method for LINE-1. This data was correlated with survival statistics	III	The authors report LINE-1 methylation is proportional to *MGMT* promoter methylation in low grade gliomas ($r=0.336$, $p=0.011$) but not in glioblastomas. By Kaplan–Meier survival curves higher LINE-1 methylation is a favorable prognostic factor in primary GBMs, even compared to *MGMT* promoter methylation ($p=0.010$ vs. 0.015) The methylation data was not evaluated in a blinded fashion and the authors note validation of their findings is necessary. Thus this represents class III data
Srividya et al. (2011)	Tumors from a prospective cohort of adult patients with newly diagnosed glioblastoma ($n=73$), treated uniformly with temozolomide radiochemotherapy at a single institution, were examined for 10q23/PTEN deletion by fluorescence in situ hybridization	III	10q23/*PTEN* homozygous deletion was frequent in patients > 45 years of age ($P+=0.034$) and the median age of patients harboring *PTEN* homozygous deletions was significantly higher than those with the retained status ($P=0.019$). 10q23/*PTEN* homozygous deletion was associated with shorter survival in the entire cohort as well in patients > 45 years ($P<0.05$) The marker analysis was not assessed in a blinded fashion. The authors note their study should be validated by others. Therefore this information represents class III data
Svendsen et al. (2011)	This is a retrospective, dual institution study of tumor tissue specimens from 74 individuals with newly diagnosed glioblastoma. Quantification of the progenitor marker neuron-glial-2 (NG2) was undertaken and correlated with survival parameters	II	NG2 expression was graded in a semiquantitative and blinded fashion as low: 0/+ or high: + +/+ + + Kaplan–Meier survival analysis demonstrated a shorter median survival of 8 months for the NG2 high ($n=38$), compared to the NG2 low ($n=36$) expressing patients, that had a median survival of 12.5 months. The difference in survival was statistically significant whether NG2 was highly expressed on tumor cells or the associated vasculature (Log Rank $test_{5.196}$, $df=1$, $p=0.03$ and $p=0.02$). The information was not confirmed with a validation cohort This produces class II data
Ducray et al. (2010)	A multi-institutional retrospective study of tumor tissue to assess the genomic characteristics associated with the response of GBMs to either first-line chemotherapy (BCNU or temozolomide) or radiation therapy (without temozolomide). For the comparative genomic hybridization (CGH) array study, 67 samples were available: 21 responders to radiotherapy, 18 non-responders to radiotherapy, 11 responders to first-line chemotherapy and 17 non-responders to first-line chemotherapy. The gene expression array study was performed on 56 samples (including 37 samples common to the CGH study): 19 responders to radiotherapy, 15 non-responders to radiotherapy, 12 responders to first-line chemotherapy and 10 non-responders to first-line chemotherapy. The tissue DNA was analyzed with the human genome-wide CIT-CGHarray. The RNA was assessed with the Genechip Human Genome U133 Plus 2.0 Expression array. Immunohistochemistry was carried out with formalin-fixed paraffin-embedded samples testing for the presence of CD3, CD20 and CD68. DNA methylation status of the MGMT promoter was determined by bisulfite modification and subsequent nested methylation specific PCR. This histologic and molecular data was correlated with response	III	Differential expression of microenvironment genes (non-specific inflammatory response genes as well as in genes involved in the B cell-mediated response and T cell activation, and genes induced by hypoxia) and *p16* locus deletion are associated with responses to radiation therapy and to first-line chemotherapy, respectively, in GBM A large amount of differential data is available from this study and the authors suggest it should be used in a prospective manner to predict responses in the future and to design therapy The molecular data was not assessed in a fashion blinded from the response data. A validation set has not been completed. Thus this is class III prognostic data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Etcheverry et al. (2010)	Prospective cohort study assessing methylation and gene expression profiles in 40 patients with newly diagnosed glioblastoma as well as an assessment of CpG site methylation levels and relationship to survival in 50 patients uniformly treated with TMZ/RT + TMZ therapy. DNA methylation profiling was performed using the Infinium HumanMethylation27 beadchip which interrogates > 27,000 CpG sites within the promoter regions of 14,475 genes	III	The authors identified 616 differentially methylated (440 hypermethylated; 176 hypomethylated) CpG sites between glioblastoma and normal brain samples. Univariate Cox analysis identified 476 CpG sites that were significantly associated with overall survival, including the MGMT promoter and SOX10 promoter. Cox multivariate analysis including MGMT promoter methlation status identified 4 CpG sites as independent OS predictors. These sites were located within the promoter regions of the FNDC3B, TBX3, FSD1, and DGKI genes. The authors state that this represents the largest assessment of genome-wide methylation status in uniformly treated glioblastoma patients with identification of several genes whose expression may be tightly regulated by epigenetic mechanism. This is an observational study with no blinded assessments and no validation set, yielding class III data
Hartmann et al. (2010)	A retrospective analysis of tumor tissue from patients enrolled in a multi-institutional study entitled "NOA-04 Randomized Phase III Trial of Sequential Radiochemotherapy of Anaplastic Glioma With Procarbazine, Lomustine, and Vincristine or Temozolomide" and from a prospective translational cohort study of the German Glioma Network. This included 237 glioblastomas and 145 anaplastic astrocytomas. They sequenced the isocitrate dehydrogenase 1 gene (IDH1) at codon 132 and correlated this data with standard demographic parameters and survival	III	7.2% of glioblastomas carried an IDH1 mutation. Co-evaluation of histological diagnosis and IDH1 status showed that both factors contribute to prognosis. The 17 glioblastoma patients with IDH1 mutation had a longer overall survival than those without it ($p = 0.014$). The authors concluded IDH1 mutation and IDH1 expression status were of greater prognostic relevance than histological diagnosis according to the current WHO classification system. The review of data in relationship to outcomes was not blinded and there was not validation set and thus this is class III data
Santosh et al. (2010)	A two institution, prospective study of 136 newly diagnosed adult glioblastoma patients among a larger group of gliomas. All underwent standard radiation and temozolomide therapy. Tumor tissue was subjected to real time quantitative PCR and immunohistochemistry was for Insulin-like growth factor –binding protein (IGFBP) isoforms 2, 3 and 5. These results were correlated with demographic parameters and survival measurements in the patient population	III	Multivariate analysis revealed IGFBP-3 expression (hazard ratio, 1.021; $P = 0.030$) and patient age (hazard ratio, 1.027; $P = 0.007$) to be associated with shorter survival in glioblastoma The relationship between these biomarkers and clinical outcomes were not assessed in a blinded fashion. As is common with such studies, the authors recognize need for additional studies for confirmation and determination of relationship to therapy. This yields class III data
Srividya et al. (2010)	This is a prospective study of tissue from 140 glioblastoma patients treated with standard chemoradiotherapy. The tumors were examined for EGFR amplification by fluorescence in situ hybridization and EGFR/p53 expression by immunohistochemistry. This data was correlated with survival parameters	III	A strong positive correlation between EGFR amplification and EGFR overexpression ($\rho = 0.5157$; $p < 0.0001$; CI 0.3783 to 0.6309) and a negative association of EGFR amplification ($\rho = 0.3417$; $p < 0.0001$; CI -0.4842 to -0.1816) and EGFR overexpression ($\rho = -0.3095$; $p < 0.001$; CI -0.4561 to -0.1465) with p53 immunopositivity was observed. Multivariable Cox proportional hazards models revealed a statistically significant interaction between EGFR overexpression and age to be associated with shorter survival (HR: 1.001; $p < 0.0001$; CI 1.000 to 1.002), thus predicting a higher hazard with increasing age The authors conclude the prognostic value of EGFR overexpression is age-dependent, and there is a propensity for a higher hazard with increasing patient age The molecular data was not evaluated in a manner blinded to the survival and no validation set was assessed. Thus this is class III data

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Colman et al. (2009)	This is a retrospective, multi-institutional study of tumor samples from newly diagnosed glioblastomas assessing the predictive power of a biomarker panel for outcome. An analysis was performed using GBM microarray data from 4 independent data sets. An examination of the genes in 136 newly diagnosed glioblastoma specimens consistently associated with patient outcome, revealed a consensus 38-gene survival set. Application to formalin fixed-paraffin embedded samples using real time reverse-transcriptase polymerase chain reaction assays in a retrospective set of 68 newly diagnosed glioblastomas resulted in a 9-gene subset which appeared robust in these samples. This 9-gene set (*PDPN, AQP1, YKL40, RTN1, EMP3, GPNMB, IGFBP2, OLIG2, LGALS3*) was then validated in an additional independent sample set of 101 newly diagnosed glioblastomas. A metagene score was calculated for each sample by subtracting the sum of the values of the good-prognosis genes from the sum of the values of the poor-prognosis genes. The samples were ranked by metagene score and divided into 2 groups based on results from recursive partitioning analysis	III	Using the nine gene profile, Kaplan–Meier curve analysis showed a significantly better progression free survival ($p=0.0007$) and overall survival ($p=0.0055$) in cases with a low metagene score The authors point out plans to assess this profile prospectively Validation work was clearly carried out in this report but the study material was retrospective and not done a manner where marker analysis was blinded from outcomes. Thus this study yields class III information
Felsberg et al. (2009)	This is a single institution, prospective study of newly diagnosed glioblastoma undergoing resection, radiation and chemotherapy with temozolomide started after radiation. 67 cases had satisfactory tissue for correlative analysis. Specimens from each case were investigated for *MGMT* promoter methylation, mRNA and protein expression, as well as presence of *MGMT* sequence polymorphisms. In addition, they screened for genetic aberrations of the *EGFR*, *TP53*, *CDK4*, *MDM2*, and *PDGFRA* genes as well as allelic losses on chromosomal arms 1p, 10q, and 19q	II	A significantly longer time to progression ($P=0.0001$) was observed after initiating chemotherapy and a longer overall survival ($P=0.0004$) occurred in patients with *MGMT*-hypermethylated tumors. On multivariate analysis the addition of near-complete tumor resection to MGMT promoter methylation resulted in better time to progression ($P=0.0002$) and overall survival ($P=0.0001$). None of the other measured parameters were either linked to the *MGMT* methylation status or associated with survival in their series of primary glioblastoma patients. Each immunohistochemical staining was scored while blinded to the clinical and molecular information. *MGMT* methylation promoter status assessment methods were compared retrospectively. No validation set of patients was done or is proposed. This yields class II data
Gerstner et al. (2009)	A retrospective, single institution study of 64 patients with newly diagnosed glioblastoma and aged 70 or older. *MGMT* methylation-specific PCR was performed using PCR primer sets specific for methylated and unmethylated *MGMT* promoter sequences. Kaplan Meier curves and the log-rank test were used to compare median progression free and median overall survival in patients who had methylated *MGMT* vs unmethylated *MGMT*	III	In patients who received an alkylating agent, median overall survival in methylated patients was 489 days vs 263 days in unmethylated patients ($p=0.0021$). In an analysis including all 64 patients, median progression free survival in methylated patients was 328 days vs 173 days in unmethylated patients ($p=0.0241$) and median overall survival was 345 days in methylated patients and 223 days in unmethylated patients ($p=0.0178$) The *MGMT* analysis was not blinded to clinical outcome and no validation set was assessed to test this finding. Thus the information in this paper represents class III findings

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Weller et al. (2009)	A prospective, multi-institutional study of 301 patients with newly diagnosed glioblastoma with treatment ranging from surgery alone to surgery, radiation and temozolomide. The tumors were investigated for TP53 mutation, p53 immunoreactivity, epidermal growth factor receptor, cyclin-dependent kinase CDK 4 or murine double minute 2 amplification, CDKN2A homozygous deletion, allelic losses on chromosome arms 1p, 9p, 10q, and 19q, O^6-methylguanine methyltransferase (MGMT) promoter methylation, and isocitrate dehydrogenase 1 (IDH1) mutations. This data was then correlated with demographic parameters, progression free survival and overall survival	III	Multivariate analysis revealed younger age, higher performance score, *MGMT* promoter methylation, and temozolomide radiochemotherapy as independent factors associated with longer OS. *MGMT* promoter methylation was associated with longer PFS (relative risk [RR], 0.5; 95% CI, 0.38 to 0.68; $P < .001$) and OS (RR, 0.39; 95% CI, 0.28 to 0.54; $P < .001$) in patients receiving temozolomide. *IDH1* mutations were associated with prolonged PFS (RR, 0.42; 95% CI, 0.19 to 0.91; $P = .028$) and a trend for prolonged OS (RR, 0.43; 95% CI, 0.15 to 1.19; $P = .10$) The molecular information was not interpreted in a blinded manner and no validation set is presented, resulting in the data from this study being class III
Pelloski et al. (2006)	A retrospective analysis of 268 newly diagnosed glioblastoma patients at one institution. Inclusion required adequate tissue for immunohistochemical staining for p-Akt, p-mTOR, p-p70S6K, and p-MAPK assessment and adequate MRI assessments of surgical resection and response to radiation therapy (regression/stable vs. progression). Overall survival was also assessed. Results were validated in an analysis of 60 patients with newly diagnosed glioblastoma enrolled in two clinical trials at a second institution	III	Elevated p-MAPK expression was most strongly associated with poor response to radiotherapy ($P < 0.001$), a finding corroborated in the validation cohort ($P < 0.001$). For survival, higher expressions of p-mTOR, p-p70S6K, and p-MAPK were associated with worse outcome (all $P < 0.03$) Markers were scored separately and the molecular profile was determined while blinded to clinical information The details on the therapy provided in addition to radiation therapy are not provided in the initial group. In the validation group chemotherapy or a radiation therapy sensitizing agent was provided making interpretation of this study as an assessment of radiation response alone less clear The blinded assessment of (1) radiation response and (2) marker status improves the value of this study. The incomplete clinical treatment data and the retrospective nature of the primary cohort and the validation cohort (even though it was a separate population) results in this being class III data
Wrensch et al. (2006)	Retrospective analysis of 873 newly diagnosed glioma patient registered in the San Francisco Bay Surveillance Epidemiology and End Results (SEER) database examining the relationship between survival and gene polymorphisms, IgE and IgF levels against 4 herpes viruses, and genetic alterations in TP53, EGFR, and MDM2. 519 newly diagnosed glioblastoma patients were included in the study of which 230 had genotyping or serologic data available	III	Using registry data, the authors determined treatments for each patient and Cox regressions for survival were adjusted for age, resection vs biopsy, radiation, and chemotherapy. The authors determined that polymorphisms in ERCC1 C8092A (HR 0.72; $P = 0.0004$) and GSTT1 (HR 1.64; $P = 0.0004$) correlated with survival in all glioma patients, although these polymorphisms did not meet their criteria for statistical significance for glioblastoma patients alone. Glioblastoma patients with elevated total IgE levels experienced longer survival (9 months) compared to patients with normal or borderline total IgE (HR 0.62; $P = 0.0007$). The authors suggest that the relationship between immunologic factors and glioblastoma survival should be further explored. The retrospective nature of this study renders the data class III

Table 5 (continued)

Author/year	Study description	Data class	Conclusion
Watanabe et al. (2005)	A single institution, prospective study of 45 cases with tissue samples of a 58 patient study of interferon-beta, ACNU and radiation after surgery. 29 cases were newly diagnosed glioblastomas. They analyzed the MGMT promoter methylation and TP53 mutation status in each of the 29 cases. Progression free survival and overall survival were analyzed	III	The presence of MGMT methylation emerged as a significant predictor of a longer progression free survival ($p=0.0099$) and overall survival ($p=0.008$) when exclusively analyzing the 29 patients with glioblastomas The authors conclude these findings highlight the importance of MGMT methylation as a specific predictive factor for responsiveness to nitrosourea chemotherapy The assessments of were not carried out in a blinded fashion and there is no validation set, yielding class III data

ACNU: 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3–2(2-chloroethyl)-3-nitrosourea; ADC: apparent diffusion coefficient; BiSEQ: bisulfite sequencing; CI: confidence interval; DWI: diffusion weighted image; EGFR: epidermal growth factor receptor; GBM: glioblastoma; GRE: gradient recalled-echo; IDH1: isocitrate dehydrogenase 1; IHC: immunohistochemistry; MDM2: murine double minute 2; MGMT: O6-methylguanine DNA methyl-transferase; HR: hazard ratio; MSP: methylation-specific PCR; OS: overall survival; p-Akt: phosphorylated protein kinase B; PCR: polymerase chain reaction; PET: positron emission tomography; PFS: progression free survival; p-MAPK: phosphorylated mitogen-activated protein kinase; p-mTOR: phosphorylated mammalian target of rapamycin; p-p70S6K: phosphorylated 70 kDa ribosomal protein S6 kinase; PTEN: Phosphatase and tensin homolog; RNA: ribonucleic acid; ROI: region of interest; RR: relative risk

create a recommendation. Additionally, neither study was done in a blinded manner and there were no validation sets thus resulting in class III data.

Miscellaneous markers and studies

There certainly are other marker studies are relatively unique and well done and yielding class II data and are mentioned here. In a retrospective study where the analysis was studied in a blinded fashion, Svendsen et al. evaluated the neuron progenitor marker neuron-glia-2. They found high expression of this element to be associated with a shorter median survival in newly diagnosed glioblastoma patients than those with low or no expression [110]. A retrospective, but blinded, analysis of neurofilament protein by immunohistochemistry in this patient group was carried out by Pallud et al. They found that median overall survival and progression-free survival were 13.0 and 7.6 months, respectively, for neurofilament protein positive glioblastomas, and 7.0 and 5.1 months, respectively, for neurofilament protein negative glioblastomas ($p=0.02$) [111]. Absent or low glutamine synthetase expression within tumor as determined by immunohistochemistry is associated with longer survival [112]. High expression of phosphorylated STAT3 in tumor as determined by immunohistochemistry is associated with shorter survival [113].

A number of interesting and ingenious reports comprising class III data are available that serve as preliminary information on markers and quantities that may eventually prove of value. For various reasons expanded upon in the evidence table these studies could not be upgraded to class II and could not be used to set forth a clear recommendation. For instance, glioblastoma patients with elevated total IgE levels experienced longer survival (9 months) compared to patients with normal or borderline total IgE levels (HR 0.62; $P=0.0007$) [114]. Colman et al. reported on microarray technique assessing the expression of broad set of genes in newly diagnosed glioblastoma and were able to narrow the assessment down to a 9 gene set whose expression could be scored in a manner that was predictive of progression free survival and overall survival. The gene was validated in second retrospective set of patients [115]. In an expansive tumor tissue comparative genomic hybridization array DNA analysis by Ducray et al. it was observed that differential expression of microenvironment genes and *p16* locus deletion is associated with responses to radiation therapy and to first-line chemotherapy, respectively, in GBM [116]. Motomura et al. carried out a screening study with immunohistochemistry of 17 different markers in 79 newly diagnosed glioblastomas. The results allowed them to divide the patients into four groups. Those with highly positive scores for Olig2, PDGFRA, p16, p53 and synaptophysin were termed the "oligodendrocyte precursor type" and had

better survivals than the other groups. The assessments were not blinded. An analysis of a limited group of markers was done in a larger validation set [117]. Reynes et al. found that higher postsurgical circulating endothelial cell counts were associated with shorter survival in patients with newly diagnosed glioblastoma [83]. In patients with Ki-67 < 17% by immunohistochemistry Karim et al. found a median time to progression of 16 months and median OS of 24 months as compared to 7 and 12.5 months respectively for the patients with Ki-67 ≥ 17% (P < 0.05) [84]. Evans et al. have published a pilot study on the use of blood-borne microvesicles using flow cytometry to assess Annexin V for phosphotidyl serine, CD41 for platelets, anti-EGFR for tumor cells, and CD235 for red blood cells. Increases in Annexin V positive microvesicle levels during active radiation and temozolomide therapy were associated with earlier recurrence and shorter overall survival (P = 0.009) [118]. In a study of interferon-induced tetratricopeptide repeat 1 (IFIT1), an inhibitor of MGMT production, Zhang et al. demonstrated elevated expression of this protein was associated with prolonged progression free survival and overall survival [119]. More detailed information on each of these studies is summarized in Table 5.

In more recent publications a study of endothelin B receptor (ETBR) from 25 patients from the The Cancer Genome Atlas Vasaikar et al. overall survival was significantly shorter in patients with high ETBR expression compared to those with low [120]. Romano et al. evaluated p-ATM in 21 patients and found over expression was a negative prognostic factor for overall survival, possibly related to it's altering tumor radiosensitivity [121]. Cesarini et al. published a retrospective, single institution study of 69 patients with GBM who underwent surgical resection followed by RT and TMZ and analyzed mmuno-reactivity of type 5 phosphodiesterase (PDE5). There was a correlation between those patients whose tumors showed high PDE5 expression having a longer mean overall survival of 15 months with high expression compared 10 months in those individuals with low expression, p = 0.0028) [122]. More detailed information on each of these studies is summarized in Table 5.

Though most of the studies mentioned in this portion of the discussion are related to molecular markers or related factors, additional information arising from standard pathology labs have also been studied. Boonyawan et al. retrospectively studied the platelet and lymphocyte counts before, during and after concurrent chemoradiation in 122 patients with newly diagnosed glioblastoma. In the "high platelet" count cohort (> 30% increase from baseline) when comparing from before and after CRT was found to have a significantly lower overall survival of 11 months compared to 28 months in the "low platelet" group (p = 0.0062, HR 3.4 (1.6–7.5) [123]. In a retrospective of study emphasizing tumor histology Mikkelsen et al. divided 106 newly diagnosed glioblastomas into "faster" and "slower" growing tumors based on serial preoperative imaging. The authors conclude that high cellular density and thromboses are histopathologic features associated with "faster" radiologic growth in patients with glioblastoma [124].

Synthesis of results

Tumor *MGMT* promoter methylation status has become a common point of discussion in brain tumor boards in terms of prognosis and likely response to standard cytotoxic chemotherapy. The number of studies to that end reflects this fact and the numerous techniques for making this evaluation all reach similar conclusions. Less prognostic data using *IDH1* mutation for glioblastomas is available but the class II and III data suggests this parameter can predict survival. Fewer publications are available in regard to neuron-glia-2, neurofilament protein, glutamine synthetase and phosphorylated STAT3 but they are well done and also yield class II data suggesting their value in determination of prognosis. p-MAPK protein expression, *EGFR* expression, and insulin-like growth factor-binding protein-3 expression, though less of a topic in most prognostic discussions, have some supporting data and this information may be of use in panels of prognostic glioblastoma markers.

Radiation therapy

Study selection and characteristics

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 45 were chosen as relevant to radiation for full text review and assessment. A total of 30 publications met the criteria for inclusion regarding radiation therapy [111, 125–153]. Additionally study design, number of institutions involved, the primary neuropathology evaluation used, the total number of patients studied and group and the statistics used to compare them were extracted to complete the summary of the data in each manuscript. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

There is only one phase III randomized trial, all the rest selected publications were retrospective or non-randomized phase I or II studies, there is substantial risk of bias [142]. For example, four studies evaluated markers for recurrence and prognosis after radiation treatment [111, 129, 130, 136]. Three of these studies were retrospective in nature leaving them subject to case selection bias, bias due lack or loss of information over time, the biases of the interpreting investigator in regard to the study and publication bias [111, 129,

130]. The other study was a phase I study [136]. It suffers hidden agenda bias, and variability due to random error related to problems with unintentional data entry oversight and neglect. Sixteen studies evaluated altered fractionation or dose of radiation for GBM [127,131–133, 135, 136,138–148]. of which only one was a phase III trial [142], five were phase I trials [127, 132, 135, 136, 139] one was phase I/II trial [131], five were phase II [133, 143–146] and the other four were retrospective studies [138, 141, 147, 148]. All of these were subject to bias related patient selection, loss or lack of information collection, data interpretation, and choice to publish. Seven studies dealt with the new radiation techniques for the treatment of glioblastoma [125, 128, 134, 137, 140, 149, 150]. There was only one single arm, nonrandomized phase I study [125], four single arm phase II trials, the rest were all retrospective studies, and suffered all bias and limitations associated with retrospective studies as mentioned above [128, 134, 137, 140].

Results of individual studies

The key results of the selected individual studies are outlined in Tables 6 and 7.

Markers for recurrence and prognosis after radiation treatment

Several studies evaluated the value of pretreatment PET in predicting GBM recurrence [92, 129, 136]. Lee et al. reported a post hoc analysis of a phase I study to determine if increased uptake on MET-PET imaging obtained before radiation and temozolomide treatment is associated with the site of subsequent recurrence of GBM [129]. A total of 26 patients were included in the study. The uptake of MET-PET appears to identify areas at highest risk for recurrence. However, since this is a post hoc analysis of a single arm phase I trial, the determination of potential sites of recurrence was not done in a blinded fashion and no validation set of patients assessed, it only provided class III data. Tsien et al. showed suboptimal coverage of tumor defined by MET-PET resulted in a higher risk of recurrence [136]. Similarly, as this is a secondary endpoint in a phase I study, determination of sites of recurrence is not blinded with no validation data set, thus only provided class III data. Most recently, Niyazi et al. also reported their single institution experience on the predictive value of FET-PET on GBM recurrence [92]. The authors reported the FET-PET assessed recurrence pattern was influenced by MGMT methylation status. Unfortunately, this is a retrospective study, and the determination of recurrence was not blinded. It only provided class III data.

Rivera et al. performed a retrospective review to evaluate the predictive utility of MGMT promotor methylation status to radiation treatment. The study included 225 patients with GBM treated with radiation without concurrent chemotherapy. There is a survival benefit in patients with methylated MGMT. The author concluded MGMT promotor methylation appears to be predictive biomarker of radiation response [130]. However, this is a retrospective study, and its data assessment was not blinded and was without a validation data set. Thus it provided class III data. Additional information on markers for prognosis related to radiation therapy is available in Table 6.

Radiation therapy: assessment of efficacy

Altered fractionation and radiation dose escalation There is only one phase III randomized trial, RTOG 9006, compared hyperfractionated radiation with BCNU with standard radiation with BCNU [142]. It compared 72 Gy in 1.2 Gy BID vs 60 Gy in 2 Gy daily with BCNU. 694 patients were enrolled and randomized. However, there was no significant differences in OS, median survival time, or PFS between groups. There was no significant difference between the arms on overall acute or late treatment-related toxicity. This is class I data, however hyperfractionated radiation failed to show any benefit compared to conventional fractionated radiation treatment.

Tsien, et al. reported a single arm phase I dose escalation study of 3D conformal radiotherapy with concurrent chemotherapy for patients with newly diagnosed GBM [136]. Radiation dose was escalated in 4 groups, 66 Gy, 72 Gy, 78 Gy, and 84 Gy. The study included two groups (Group 1: PTV2 < 75 cm3; Group 2: PTV2 ≥ 75 cm3). A total of 209 patients were enrolled, of which 209 patients were evaluated for acute toxicity, and 180 patients were evaluated for late toxicity. No dose limiting toxicity was observed in either group, and doses were escalated to 84 Gy in both groups. Median survival in group 1 was 11.6–19.3 m and in group 2 was 8.2 – 13.9 m. However, since it is a single arm phase I study, it yields class III data. Subsequently, the authors also reported the results of a phase I dose escalation study using intensity modulated radiation therapy (IMRT) and concurrent temozolomide [136]. The MTD was found to be 75 Gy. Nonetheless, due to the phase I nature of the study, it yielded class III data.

Monjazeb et al. also reported a phase I dose escalation study using a hypofractionated concurrent IMRT boost [135]. They were able to safely treat patients to 80 Gy without dose limiting toxicity. However, due to the phase I design, it only provided class III data. Another group reported their single institution experience of moderate dose escalation with high dose radiation (61–76 Gy in 2 Gy per fraction) with concurrent temozolomide in 128 patients with GBM [141]. The treatment was well tolerated; however the OS and PFS were similar to standard dose treatment

patients. The retrospective, nonrandomized design of this study yielded class III data.

Several studies evaluated altered fractionation radiation in the management of GBM. Beauchesne, et al. performed a phase I/II trial to evaluate the safety, tolerability, and efficacy of an ultrafractionation regimen (0.75 Gy 3 times daily to a total dose of 67.5 Gy) in patients with newly diagnosed GBM [131]. A total of 40 patients were enrolled and started radiation, and 34 patients were included after exclusions for early or unrelated deaths or serious hematologic toxicity. The median overall survival was 16 months. When compared retrospectively with the EORTC/NCIC trial evaluating temozolomide, the result is superior to treatment with radiation alone or chemoradiation [152]. Despite the phase II design, it is a small, single arm, non-randomized trial, with significant selection and survivorship bias with comparison to historical data only. It yields class III data.

On the other hand, several studies evaluated hypofractionation radiation schedule [132, 133, 139]. Chen, et al. performed a phase I trial to determine the maximal tolerated biologic dose intensification of radiotherapy using fractional dose escalation with temozolomide in patients with newly diagnosed GBM [132]. Radiation doses were escalated from 3 to 6 Gy per fraction to 60 Gy using a 3 + 3 design. A total of 16 patients were enrolled. With a median follow up of 14.4 months, there was no dose limiting toxicity at any dose level. The median overall survival was 16.2 months. It only provided class III data due to the phase I in design. Reddy, et al. reported a single arm phase II trial of patients with newly diagnosed GBM treated with hypofractionated intensity modulated radiation with concurrent temozolomide [133]. PTV1 (enhancing lesion and cavity with 5 mm margin) received 60 Gy in 6 Gy fractions, while PTV2 (abnormality on T2 with 5 mm margin) received 30 Gy in 3 Gy fractions. A total of 24 patients were enrolled. The median overall survival was 16.6 m with no grade 3 or higher non-hematological toxicity. Nonetheless, since it is a small single arm, non-randomized trial, it only yielded class III data. The authors also reported the pattern of failure in the trial, and suggested hypofractionated radiation may alter the pattern of failure with more distant failure [153]. However, it only provided class III data as a post hoc analysis of a single arm, non-randomized trial. Moreover, Ciammella et al. reported their single institution experience of hypofractionation radiation for patients with newly diagnosed GBM.[138] The radiation treatment consisted of 25 Gy in 5 Gy fractions. There was no grade 3–4 neurotoxicity, with favorable PFS, and OS when compared to historical control. However, the retrospective nature of the study only provided class III data. Lastly, Ammirati et al. also reported a phase I study of hypofractionated radiation with concurrent temozolomide in patients with high grade glioma [139]. All patients received 52.5 Gy in 3.5 Gy fractions, and concurrent temozolomide dose escalated from 50 mg/m^2 to 65 mg/m^2 and 75 mg/m^2. Only 9 patients with GBM were enrolled, and trial was escalated to final dose with no irreversible grade 3 or higher toxicity. Due to the phase I nature of the study design, it yielded class III data. There are several phase II trial evaluated hypofractionated radiation treatment [143–146]. However, all these were single arm trials, as a result, only provides class II evidence, and warrant further evaluation. There were two retrospective studies compared hypofractionated radiation vs conventional fractionated RT. Wang et al. focused on elderly population, and showed there is no significant difference with hypofractionated radiation and conventional radiation treatment [149]. And Navarria performed a propensity score matched analysis, showed there is no significant difference between these two radiation regimens [144, 147]. However, these are retrospective studies, only provide class III evidence.

Sheu et al. compared IMRT and VMAT techniques, which showed similar result, without any significant difference between techniques. The retrospective, nonrandomized design of this study yielded class III data [148].

Randolph et al. performed retrospective study compared timing of initiation of radiation in patients with newly diagnosed GBM. They showed there was a modest improvement in both PFS and OS in patients who received biopsy or STR with time to RT > 28 days. However, it is a retrospective, non-randomized study, only provide class III data [151].

Duma et al. evaluated targeting white matter pathways adjacent to, and leading away from, the original contrast-enhancing tumor site (termed leading-edge radiosurgery [LERS]) with single-fraction stereotactic radiosurgery as a boost to standard therapy could limit the spread of glioma cells and improve clinical outcomes [150]. A total of 174 patients treated with radiosurgery to the leading edge (LE) of tumor cell migration were reviewed. The 2-, 3-, 5-, 7-, and 10-year actual overall survival rates after LERS were 39%, 26%, 16%, 10%, and 4%, respectively. They concluded LERS is a safe and effective upfront adjunctive therapy for patients with newly diagnosed BM. However, it is a retrospective, non-randomized study, only provide class III data.

Novel radiation techniques

Reardon et al. performed a feasibility study of radioimmune therapy with ^{131}I labeled murine antitenascin monoclonal antibody 81C6 (^{131}I-81C6) into surgical cavity to achieve a patient specific 44 Gy boost to the 2 cm resection margin in patients with newly diagnosed GBM [126]. The patients then received standard radiation treatment and chemotherapy. 16 patients were enrolled. Feasibility was demonstrated but the pilot study nature of the study yielded class III data.

Chen et al. performed a phase I study to evaluate the feasibility of gross total resection and permanent I-125 brachytherapy followed by hyperfractionated radiotherapy

Table 6 Evidence for radiation therapy: markers for prognosis

Author/year	Study description	Data class	Conclusion
Niyazi et al. (2012)	A retrospective, single institution study of 79 newly diagnosed glioblastomas treated with standard concurrent radiation and temozolomide and adjuvant temozolomide. The patients received a total dose of 60 Gy in 6 weeks, in a once-daily schedule of 2 Gy per fraction for a total of 30 fractions. Patients were treated using megavoltage equipment, such as linear accelerator beams with minimal nominal energy of 6 MV. In 54 patients MGMT methylation status was evaluated. They were followed with [^{18}F]Fluoroethyltyrosine ([^{18}F]FET) and additional MRI for recurrence Recurrences were defined as ''in-field'' if more than 80% of the tumour recurrence resided within the prescription 95% isodose surface, and ''marginal'' if 20–80% of the lesion was inside the 95% isodose surface. All others were defined as outside the field or "ex-field"	III	Median follow-up was 595 days. 41.5% (12/29) of the MGMT methylated population had no relapse, 37.9% (11/29) had an in-field-recurrence and 20.7% (6/29) an ex-field/marginal recurrence, whilst 28.0% (7/25) of the MGMT unmethylated population had no relapse, 64.0% (16/25) had an in-field-recurrence and 8.0% (2/25) an ex-field/marginal recurrence ($p = 0.15$) In MGMT methylated patients, the 1-year/2-year OS was 93.1%/ 78.1% compared to 64.9%/7.3% in MGMT unmethylated patients Median PFS was 642 days for MGMT methylated patients compared to 231 days in MGMT unmethylated patients ($p < 0.001$). The authors concluded MGMT methylation was associated with improved outcome This assessment of MGMT in relationship prognosis for recurrence after radiation was not done in a blinded fashion and no validation set of patients was assessed, leaving this as class III data
Tsien et al. (2011)	A prospective, single institution phase I study to determine the maximum-tolerated dose (MTD) of intensity modulated radiation therapy (IMRT) with concurrent temozolomide in patients with newly diagnosed glioblastoma ($n = 38$). Twenty-two of 32 patients with pretreatment ^{11}C methionine positron emission tomography (MET-PET) uptake showed uptake beyond the contrast-enhanced MRI	III	Suboptimal IMRT coverage of the tumor defined by MET-PET (defined as less than 95% isodose coverage of MET-PET tumor) resulted in a higher risk of subsequent noncentral failure ($P < 0.001$). This method of determining potential sites of recurrence was not done in a blinded fashion and no validation set of patients was assessed, leaving this as class III data
Rivera et al. (2010)	A retrospective, single institution study of the relationship between MGMT methylation status and outcome in newly diagnosed glioblastoma patients treated with radiation therapy alone prior to adaptation of concurrent/adjuvant temozolomide as standard therapy. 225 patients with sufficient banked tissue were selected Additional patient and data characteristics: 53 patients eventually received some form of adjuvant chemotherapy and the remaining 172 patients did not receive any chemotherapeutic agents until after the first tumor recurrence. 183 cases were identified as having (1) no adjuvant therapy prior to the assessment of radiation response, and (2) pre- and postradiotherapy magnetic resonance imaging studies available for assessment and comparison Methylation-specific, quantitative real-time polymerase chain reaction following bisulfite treatment on isolated DNA was used to assess MGMT promoter methylation status	III	Unmethylated tumors were twice as likely to progress during radiation treatment. The median time interval between resection and tumor progression of unmethylated tumors was also nearly half that of methylated tumors(15 vs. 31 weeks, $P = .009$, log-rank). Promoter methylation was also found to confer improved overall survival in patients who did not receive adjuvant alkylating chemotherapy (63 weeks in the MGMT methylated vs. 51 weeks in the unmethylated tumors, $P = .019$, log-rank). Multivariable analysis reveals the MGMT promoter methylation status to be an independent prognostic factor when accounting for age, performance status, and extent of resection The data was not assessed in a blinded fashion and no validation set was provided. Thus this is class III data
Lee et al. (2009)	A single institution, prospective study of 11C-methionine positron emission tomography to determine if increased uptake of this tracer was associated with eventual treatment failure in patients with newly diagnosed glioblastoma. Patients were part of a radiation dose escalation study given with temozolomide	III	Twenty six patients had adequate pretreatment and post-treatment PET and MRI studies for inclusion. Nineteen of 26 had appreciable (> 1 cm3) volumes of increased MET-PET activity before treatment. Five of 19 patients had PET-positive areas that were not fully encompassed within the high-dose region, and all five patients had failures outside the primary treatment region. Among the 14 patients with adequately covered PET positive areas, only two had treatment failures outside the primary treatment area This method of determining potential sites of recurrence was not done in a blinded fashion and no validation set of patients was assessed, leaving this as class III data

IMRT: intensity modulated radiation therapy; MET: ^{11}C methionine; MGMT: O6-methylguanine DNA methyl-transferase; MTD: maximum-tolerated dose; PET: positron emission tomography

Table 7 Evidence for radiation therapy: assessment of efficacy

Author/year	Study description	Data Class	Conclusion
Sheu et al. (2018)	A single institution retrospective study of Volumetric modulated arc therapy (VMAT) as compared to intensity modulated radiation therapy (IMRT) in GBM patients	III	88 patients included. With a median follow up of 27 m. The overall survival, freedom from progression, and freedom from new or worsening toxicity rates were not different between the 2 treatment groups. There was no difference in incidences of alopecia, erythema, nausea, worsening or new onset fatigue, or headache during radiation, or temozolomide dose reduction for thrombocytopenia or neutropenia. The mean time of treatment (TOT) was significantly reduced by 29% ($P < .01$) with VMAT (mean TOT: 10.3 min) compared with IMRT (mean TOT: 14.6 min) The authors conclude treatment with VMAT results in similar oncologic and toxicity outcomes compared with IMRT and may improve resource utilization by reducing treatment time The retrospective, nonrandomized design of this information yields class III data
Ali et al. (2018)	Phase III randomized trial. From 1990–1994, patients with malignant gliomas were randomized between hyperfractionated radiation (HFX) of 72.0 Gy in 60 fractions given twice daily and 60.0 Gy in 30 fractions given once daily. All patients also received BCNU	I	694 patients randomized There was no significant difference between the arms on overall acute or late treatment-related toxicity No statistically difference in OS, or PFS The authors conclude there is no benefit of HFX Large phase III randomized trial yields class I data
Mallick et al. (2018)	Phase II randomized trial newly diagnosed GBM patients were randomized to conventional fractionated radiotherapy (CRT) or HART. CRT arm (60 Gy in 30 fractions over 6 weeks @ 2 Gy/per fraction) or simultaneous integrated boost intensity modulated radiotherapy in HART arm (60 Gy in 20 fractions over 4 weeks @ 3 Gy/per fraction to high-risk planning target volume (PTV) and 50 Gy in 20 fractions over 4 weeks @ 2.5 Gy/per fraction to low-risk PTV)	II	89 pts enrolled median follow-up of 11.4 months No difference in OS, PFS Authors conclude HART is comparable to CRT in terms of survival outcome Small sample size phase II trial yields class II data
Navarria et al. (2018)	Retrospective propensity score matched analysis newly diagnosed glioblastoma patients treated with CRT 60 Gy/30 fractions or HFRT 60 Gy/15 fractions A propensity score matching analysis (PSM) was performed using a logistic regression that considered age, KPS, extent of surgery, MGMT and IDH status	III	267 patients were included. After 1:1 matching, 82 patients resulted in each group No statistically significant differences were recorded between the two radiation therapy treatments performed The authors conclude short course of radiation therapy would seem comparable to CRT in terms of outcome The retrospective, nonrandomized design of this information yields class III data
Scoccianti et al. (2018)	Multicenter phase II trial assessing the efficacy and the toxicity of hypofractionated radiotherapy with SIB plus temozolomide in patients with glioblastoma dose was 52.5 Gy in 15 fractions of 3.5 Gy and 67.5 in 15 fractions of 4.5 Gy to the SIB volume	III	Median overall survival (OS) was 15.1 months median progression-free survival (PFS) was 8.6 months trial confirms that hypofractionated radiotherapy with SIB and association with temozolomide may be a reasonable and feasible option for good prognosis patients with GBM Small, single arm, phase II trial resulted in class III data

Table 7 (continued)

Author/year	Study description	Data Class	Conclusion
Fariseli et al. (2017)	prospective, open-label, monocentric, nonrandomized, single arm, phase II study primary endpoint was the proportion of progression-free patients at 12 months, and the secondary endpoints were overall survival (OS) and toxicity radiation was delivered 3 times daily, 2 Gy/fraction, for 5 consecutive days, and the total dose was 60 Gy	III	35 pts The primary endpoint failed to be applied; Macdonald criteria could be used in 16 (46%) patients with local or intracerebral recurrence (group A) The OS was 22 months, and OS probabilities at 12, 18, and 24 months were 82%, 59%, and 44%, respectively The authors concluded improve local control and OS, a more aggressive treatment schedule should be explored Small, single arm, phase II trial resulted in class III data
Navarria et al. (2017)	Single arm phase II trial Patients with newly diagnosed GBM consisted of 60 Gy, in daily fractions of 4 Gy given 5 days per week for 3 weeks he primary endpoints were overall survival (OS), progression free survival (PFS), and incidence of radiation induced brain toxicity Secondary endpoint was the evaluation of neurocognitive function	II	A total of 97 patients were included No severe toxicity occurred and the neuropsychological evaluation remained stable the median OS time, 1,2-year OS rate were 15.9 months, 72.2% and 30.4% authors concluded HFRT with concomitant and adjuvant TMZ chemotherapy is an effective and safe treatment Non-randomized design of this study results in level II evidence
Wang et al. (2017)	Retrospective review Compared hypofractionated RT vs standard fractionated RT in elderly pts (≥ 60) SFRT (60 Gy/30 fractions or 59.4 Gy/33 fractions) versus HRT (40 Gy/15 fractions)	III	158 patients were treated with SFRT versus 26 with HRT No difference in OS between HRT and SFRT. For patients receiving TMZ, there was no survival difference between those treated with HRT and those treated with SFRT Conclusions: Elderly GBM patients receiving HRT and those receiving SFRT had similar OS The retrospective, nonrandomized design of this information yields class III data
Duma et al. (2016)	Retrospective study Targeting white matter pathways adjacent to, and leading away from, the original contrast-enhancing tumor site (termed leading-edge radiosurgery [LERS]) with single-fraction stereotactic radiosurgery as a boost to standard therapy could limit the spread of glioma cells and improve clinical outcomes	III	The median overall survival was 23 months (mean 43 months) from diagnosis. The 2-, 3-, 5-, 7-, and 10-year actual overall survival rates after LERS were 39%, 26%, 16%, 10%, and 4%, respectively Authors concluded LERS is a safe and effective upfront adjunctive therapy for patients with newly diagnosed GBM However, single center retrospective study results in level III evidence
Randolph et al. (2016)	Retrospective review o further evaluate if a delay in the start of radiation therapy (RT) affects patient outcomes for glioblastoma (GBM)	III	May 1999 to May 2010, a total of 161 patients included Median time from surgery to start of RT was 20 days for biopsy alone, 28 days for subtotal resection (STR) and 28 days for gross total resection (GTR) delay > 28 days did not result in a difference in PFS when compared to no delay (6.7 vs. 6.9 months, $p = 0.07$). PFS was improved in biopsy or STR patients with a > 28 day delay to start of RT (4.2 vs. 6.7 months, $p = 0.006$). OS was also improved in patients receiving biopsy or STR with a > 28 day delay to start of RT (12.3 vs. 7.8 months, $p = 0.005$) The study showed OS and PFS were not different between time to RT > 28 days compared to < 28 days However, single center retrospective study results in level III evidence

Table 7 (continued)

Author/year	Study description	Data Class	Conclusion
Beauchesne et al. (2016)	An eight center, prospective, phase II study of concurrent ultra-fractionated radiotherapy (three doses of 0.75 Gy spaced apart by at least 4 h were delivered daily, 5 days a week for six consecutive weeks for a total of 67.5 Gy) and temozolomide treatment (75 mg/m^2 for 7 days a week) in inoperable glioblastoma patients (n = 40). After a 4-week break, chemotherapy was resumed for up to six cycles of adjuvant temozolomide treatment, given every 28 days, according to the standard 5-day regimen	III	Complete responses were seen in four patients, and partial responses were reported in seven patients. The median survival from the initial diagnosis was 16 months The authors conclude the technique has tolerable toxicity, is well accepted by patients and had encouraging survival rates As a therapeutic study the lack of concurrent control population results in this being class III data
Ammirati et al. (2014)	A prospective phase I study to assess the scope and tolerability of hypofractionated intensity modulated radiation therapy with concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma (n = 9). All patients received peri-radiation TMZ; 1 week before the beginning of radiation therapy, 1 week after radiation therapy and for 3 weeks during radiation therapy. Standard 75 mg/m^2/day dose was administered to all patients 1 week post- radiation therapy. Dose escalation was commenced at level I: 50 mg/m^2/day, level II: 65 mg/m^2/ day and level III: 75 mg/m^2/day for 4 weeks. Hypofractionated intensity modulated radiation therapy was delivered at 52.5 Gy in 15 fractions to the contrast enhancing lesion (or surgical cavity) plus the surrounding edema plus a 2 cm margin. Toxicity and outcomes were followed	III	Three patients were accrued at each temozolomide dose level. Median follow-up was 10 months (range, 1–15). Median progression free survival was 3.9 months (95% confidence interval [CI]: 0.9–7.4; range, 0.9–9.9 months) and the overall survival was 12.7 months (95% CI: 2.5–17.6; range, 2.5–20.7 months). Time spent in a KPS ≥ 70 was 8.1 months (95% CI: 2.4–15.6; range, 2.4–16 months). No instance of irreversible grade 3 or higher acute toxicity was noted. The authors concluded hypofractionated intensity modulated radiation therapy at 52.5 Gy in 15 fractions with peri-radiation therapy temozolomide at a maximum tolerated dose of 75 mg/m^2/day for 5 weeks is well tolerated. They made the usual suggestion of expansion to a larger multicenter study to validate these observations. The phase I nature of this study with no concurrent comparator cohort renders class III data
Kageji et al. (2014)	A retrospective, single institution study of boron neutron capture therapy in newly diagnosed glioblastoma patients. 17 received BSH (sodium borocaptate) based intraoperative BNCT and 6 received BPA (boronophenylalanine) based nonoperative BNCT over the interval of 1998 through 2005. A comparative group of 34 individuals receiving standard temozolomide chemoradiation at the same institution from 2006 through 2009 were chosen as controls. Survival parameters were followed	III	The median survival time of those receiving BNCT was 19.5 months with 2, 3 and 5 year survival rates of 31.8%, 22.7% and 9.1%, respectively. The mean survival time for the controls was 13.5 months and the 2 and 3 year survival rates were 27.5% and 16.5%, respectively. Utilizing recursive partitioning analysis and other techniques, it was determined there was no difference in survival between BNCT and control patients. The authors chose to emphasize the relative equivalence of the two therapies. The retrospective nature of the data collection in both groups yields class III data
Badiyan et al. (2014)	A single institution, retrospective study of moderate dose escalation using high-dose radiation therapy (HDRT, 61–76 Gy at 2 Gy per fraction, n = 128) in the setting of concurrent temozolomide (TMZ) in patients with newly diagnosed glioblastoma multiforme (GBM), compared with standard-dose radiation therapy (SDRT, 60 Gy at 2 Gy per fraction, n = 81)	III	Overall median follow-up time was 1.10 years, and for living patients it was 2.97 years. Actuarial 5-year overall survival (OS) and progression-free survival (PFS) rates for patients that received HDRT versus SDRT were 12.4% versus 13.2% (P = .71), and 5.6% versus 4.1% (P = .54), respectively. The retrospective, nonrandomized design of this information yields class III data
Ciammella et al. (2013)	A single institution, retrospective investigation of the effects of hypofractionated radiation therapy for patients with glioblastoma (n = 67). Hypofractionated XRT was delivered to a total dose of 25 Gy in 5 fractions prescribed to 70% isodose. Response to treatment, OS, PFS, toxicity and patterns of recurrence were evaluated	III	No grade 3–4 acute or late neurotoxicity was observed. With median follow-up of 14.9 months, the median OS and PFS were 13.4 and 7.9 months, respectively. The authors concluded the favorable overall survival, low rates of toxicity and satisfying good quality of life results warrant further analysis of optimal fractionation schedules for the treatment of glioblastoma. The retrospective and selective nature of the information in this study yields class III data

Table 7 (continued)

Author/year	Study description	Data Class	Conclusion
Waters et al. (2013)	A single institution, prospective study of immediate postoperative radiation followed by standard radiochemotherapy with temozolomide in newly diagnosed glioblastoma (n = 11). Brachytherapy was carried out on postoperative day 2–3, with 45–60 Gy delivered to a 1 cm margin	III	Nine of eleven patients had adequate post-treatment MRI studies. Two of these nine patients (22%) developed new regions of contrast enhancement prior to radiochemotherapy. Two patients developed reversible neurological deficits possibly referable to the brachytherapy. The median survival of all patients was 15.6 months (range 5–46.4 months). The 2-year overall survival was 42.4%. Median survival after pathologic diagnosis for patients with RPA class 4 was 26.5 months, RPA class 5 was 14.7 months, and RPA class 6 was 9.2 months. The authors conclude the technique is safe The phase I and non-comparative nature of this data provide class III data
Gupta et al. (2012)	A single institution, retrospective study of subventricular zone radiation dose in 40 newly diagnosed gliobastoma patients who had been treated with postoperative conventionally fractionated focal conformal radiotherapy plus chemotherapy. Survival was assessed	III	Multivariate analysis identified recursive partitioning analysis class, Karnofsky performance status, and mean ipsilateral subventricular zone dose as independent predictors of survival. Increasing mean dose to the ipsilateral subventricular zone dose was associated with significantly improved overall survival, but not progression free survival. The retrospective nature of this study yields class III data
Monjazeb et al. (2012)	A single institution, prospective Phase I dose escalation trial for newly diagnosed glioblastoma multiforme patient (n = 21) using a hypofractionated concurrent intensity-modulated radiotherapy (IMRT) boost. Radiotherapy consisted of daily fractions of 1.8 Gy with a concurrent boost of 0.7 Gy (total 2.5 Gy daily) to a total dose of 70, 75, or 80 Gy. Concurrent chemotherapy was not permitted. Dose limiting toxicities were defined as irreversible Grade 3 or any Grade 4–5 acute neurotoxicity attributable to radiotherapy	III	All patients experienced Grade 1 or 2 acute toxicities. Acutely, 8 patients experienced Grade 3 and 1 patient experienced Grade 3 and 4 toxicities. Of these, only two reversible cases of otitis media were attributable to radiotherapy. Thus using a hypofractionated concurrent IMRT boost, they were able to safely treat patients to 80 Gy without any dose-limiting toxicity. It was recommended the study be repeated with the additional use of temozolomide. The phase I nature of the report yields class III data
Reddy et al. (2012)	A single institution, phase II study of hypofractionated intensity-modulated radiotherapy (hypo-IMRT) with concurrent and adjuvant temozolomide (TMZ) in adult patients with newly diagnosed glioblastoma (n = 24). They were required to have a KPS ≥ 60 and residual enhancing tumor with a greatest diameter of ≤ 6 cm. Radiation consisted of a total dose of 60 Gy in 10 fractions (6 Gy/fraction) to the T1-weighted margin with a 5 mm margin and to the T2 abnormality on T2-weighted MRI with 5-mm margin to 30 Gy in 10 fractions (3 Gy/ fraction). Concurrent TMZ was given at 75 mg/m^2/day for 28 consecutive days. Adjuvant TMZ was given at 150 to 200 mg/m^2/day for 5 days every 28 days thereafter. Standard survival and toxicity parameters were followed	III	There was no grade 3 or greater nonhematologic toxicity. The median overall survival was 16.6 months (range, 4.1–35.9 months). Six patients underwent repeated surgery for suspected tumor recurrence; necrosis was found in 50% to 100% of the resected specimens The authors concluded the regimen was comparable to current standard therapy The lack of a concurrent comparative cohort yields class III data
Chen et al. (2011)	A prospective Phase I radiation dose-per-fraction escalation study with intensity modulated radiation therapy (IMRT) in conjunction with standard concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma (n = 16)	III	The median survival was 16.2 months (range, 3–33). One patient experienced vision loss in the left eye 7 months after IMRT. Four patients underwent repeat surgery for suspected tumor recurrence 6–12 months after IMRT and 3 had radionecrosis. The phase I nature of the study yields class III data

Table 7 (continued)

Author/year	Study description	Data Class	Conclusion
Tsien et al. (2011)	A prospective, single institution phase I study to determine the maximum-tolerated dose (MTD) of intensity modulated radiation therapy (IMRT) with concurrent temozolomide in patients with newly diagnosed glioblastoma (n = 38). Progression free survival and overall survival was measured	III	IMRT doses of 66 to 81 Gy were delivered over standard 6 week intervals. Late CNS grade III toxicity was observed at 78 (2 of 7 patients) and 81 Gy (1 of 9 patients). None of 22 patients receiving 75 or less Gy developed RT necrosis. Progression free survival and overall survival was determined for the overall patient group, but not by radiation dose. It was concluded that the MTD was 75 Gy. The phase I nature of the report yields class III data
Beauchesne et al. (2010)	This is a phase I/II study of ultrafractionated radiotherapy that was conducted in 7 French centers looking at adults with newly diagnosed, supratentorial, unresectable but histologically confirmed glioblastoma, with a WHO performance status of 0–2 were eligible. Three daily doses of 0.75 Gy were delivered at least 4 h apart, 5 days per week over 6–7 consecutive weeks (90 fractions for a total of 67.5 Gy). The primary end points were safety, toxicity, and tolerability, and the secondary end points were overall survival and progression-free survival	III	Thirty-one patients were analyzed. No acute Grade III and/or IV CNS toxicity was observed. Median progression free survival and overall survival from initial diagnosis were 5.1 and 9.5 months, respectively. When comparing with a historical European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada trial, in both progression free survival and overall survival multivariate analysis, ultra-fractionation showed superiority over radiation therapy alone, but not over radiation therapy and temozolomide. The lack of a concurrent comparator group results in this being class III data
Tsien et al. (2009)	A prospective, multi-institutional, phase I study of feasibility and toxicity of dose-escalated three-dimensional conformal radiotherapy (3D-CRT) concurrent with chemotherapy in patients with newly diagnosed glioblastoma. Four radiation therapy dose levels were evaluated: 66, 72, 78, and 84 Gy. The chemotherapeutic was carmustine 80 mg/m^2, given during radiation therapy, then every 8 weeks for 6 cycles. Patients were stratified into two groups (Group 1: planning target volume < 75 cm3; Group 2: planning target volume ≥ 75 cm3)	III	There were no dose-limiting toxicities (acute Grade ≥ 3 irreversible central nervous system toxicities) observed on any dose level in either group. On the basis of the absence of dose-limiting toxicities, the dose was escalated to 84 Gy in both groups. Late radiation therapy necrosis was noted at 66 Gy (1 patient), 72 Gy (2 patients), 78 Gy (2 patients), and 84 Gy (3 patients) in Group 1. In Group 2, late radiation therapy necrosis was noted at 78 Gy (1 patient) and 84 Gy (2 patients) Median time to radiation necrosis was 8.8 months (range, 5.1–12.5 months). Median survival in Group 1 was 11.6–19.3 months. Median survival in Group 2 was 8.2–13.9 months The phase I nature of this study with no concurrent or reasonably parallel comparison group results in it providing class III evidence
Villavicencio et al. (2009)	A single institution, retrospective analysis of radiosurgery (Cyberknife) in the management of glioblastoma. Twenty patients were treated with this modality as a part of the treatment regimen at initial diagnosis	III	The median survival from diagnosis for the patients treated with radiosurgery as an initial clinical therapy was 11.5 months (range, 2–33 months). The median survival from radiosurgery was 9.5 months. By Cox proportional hazards regression analysis there was no apparent survival advantage in using radiosurgery in initial management of glioblastoma patients. As a retrospective analysis this paper provides class III data

Table 7 (continued)

Author/year	Study description	Data Class	Conclusion
Chen et al. (2007)	A single institution, prospective phase I study of newly diagnosed glioblastoma patients with planned gross total resection and immediate placement of permanent I-125 seeds, followed by postoperative hyperfractionated radiotherapy to a dose of 60 Gy at 100 cGy b.i.d., 5 days per week. Toxicity and survival were followed	III	18 patients completed the therapy. The median brachytherapy dose measured 5 mm radially outward from the resection cavity was 400 Gy (range, 200–600 Gy). Ten patients underwent 12 reoperations, with 11 of 12 reoperations demonstrating necrosis without evidence of tumor. Because of high toxicity, the study was terminated early. Comparison to a similar cohort of newly diagnosed glioblastoma patients treated on other protocol therapies retrospectively showed a slight survival benefit but it was not statistically significant The authors concluded this therapy results in high toxicity and reoperation rates, without demonstrated improvement in survival. The phase I nature of this study with no concurrent or reasonably parallel comparison group results in it providing class III evidence
Reardon et al. (2007)	A prospective, feasibility study of ^{131}I-labeled murine antitenascin monoclonal antibody 81C6 (^{131}I-81C6) into a surgically created resection cavity to achieve a patient-specific, 44-Gy boost to the 2-cm SCRC margin in individuals with newly diagnosed malignant gliomas. 16 patients with glioblastoma were enrolled. Thereafter they received conventional radiation and chemotherapy. Toxicities were recorded and survival followed	III	Median overall survival for glioblastoma patients was 90.6 weeks and 87% were alive at one year The authors note some patients received the therapy before standard radiation and some afterward. One individual did not receive standard radiation or chemotherapy Toxicities included reversible grade 3 hematologic toxicity, headaches, and seizures. The toxicities were not reported by histologic subtype Feasibility is demonstrated but the inconsistent nature of the patients entire treatment regimen yields class III data

Abbreviations: ^{131}I-81C6: ^{131}I-labeled murine antitenascin monoclonal antibody 81C6, 3D-CRT: three-dimensional conformal radiotherapy, BPA: boronophenylalanine, BNCT: boron neurtron capture therapy, BSH: sodium borcaptate, CI: confidence interval, CNS: central nervous system, RT: radiation therapy, Gy: Gray, KPS: HDRT: high dose radiation therapy, I-125: iodine-125, IMRT: intensity modulated radiation therapy, Karnofsky Performance Status, MRI: magnetic resonance imaging, MTD: maximum tolerated dose, OS: overall survival, PFS: progression free survival, RPA: recursive partitioning analysis, SDRT: standard dose radiation therapy, TMZ: temozolomide, WHO: World Health Organization, XRT: x-ray therapy

for patients with newly diagnosed GBM [125]. A total of 21 patients were enrolled, and 18 finished treatment. After gross total resection, patients had I-125 brachytherapy (> 250 Gy at 5 mm deep to resection margin). Patients subsequently also received hyperfractionated radiation treatment (60 Gy in 1 Gy BID). Grade 3–5 toxicities were observed in 11 – 18 patients, and study was terminated early due to toxicity. The study provided class III data due to small sample size, and phase I in design. Several other studies also reported cyberknife radiosurgery, brachytherapy with gliasite or mammosite, boron neutron capture therapy, and irradiation of cancer stem-cell niche in the subventricular zone in the management of GBM [128, 134, 137, 140]. However, all these were retrospective studies with small sample size (11 – 40 patients). They only yielded class III data. Additional information on the efficacy of emerging techniques in radiation therapy is available in Table 7.

Synthesis of results

Class III data regarding the suboptimal coverage of tumor defined by MET-PET resulting in a higher risk of tumor recurrence may signal the eventual value of combining metabolic imaging with anatomic imaging for radiation treatment planning. Currently, there is no high level evidence to support radiation dose escalation, altered fractionated, or new radiation techniques for patients with newly diagnosed GBM. Validation of the aforementioned approaches through randomized phase II/III trials is warranted for their future development.

Chemotherapy

Study selection and characteristics

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 12 were chosen as relevant to chemotherapy for full text review and assessment. Eleven of these publications met the criteria for inclusion regarding chemotherapy [154–164].

Data extraction included assessment of study design, primary treatment modality, total number of patients, and evaluation of toxicity and responses. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Of the eleven studies designed to assess chemotherapy for newly diagnosed glioblastoma, two studies were randomized clinical trials (RCT) while seven studies were prospective cohort studies, and three were retrospective studies. The random allocation of participants in the RCT decreases the likelihood of selection bias, however, the non-blinded design renders the study subject to potential ascertainment and selective reporting bias as well as variability due to random errors related to problems with unintentional data entry and oversight. Among the seven prospective cohort studies, six were single center studies while one study was performed at two centers. These studies were at risk of being influenced by selection bias, hidden agenda bias, and attrition bias. Additionally, outcomes in the seven prospective studies were compared to historical controls rendering these studies subject to comparison choice bias and change in methods over time bias.

Results of individual studies

The key results of the selected individual studies are outlined in Table 8.

Temozolomide

Three studies evaluated the administration of temozolomide (TMZ) beyond the conventional 6 cycle regimen. Bhandari et al. performed a non-blinded, prospective randomized clinical trial involving 40 patients with 20 patients receiving 6 cycles of TMZ and 20 patients receiving > 6 cycles [154]. An increase in median PFS (12.8mo vs 16.8mo) and median OS (15.4mo vs 23.8mo) was observed in the group receiving extended TMZ administration. Hematologic toxicity ≥ grade 3 was observed in 15% of the extended TMZ cohort compared to 5% in the conventional 6 cycle cohort. The small sample size of this randomized clinical trial subjects this study to significant randomization bias that limits meaningful conclusions. Two retrospective studies also evaluated extended use TMZ beyond 6 cycles with each study demonstrating an increase in median PFS with administration of TMZ beyond 6 cycles. However, in each study, multivariate analysis incorporating age, KPS, extent of resection, and MGMT status demonstrated no statistical difference in median OS for patients receiving extended regimen TMZ [155, 156].

Carmustine (BCNU)

The use of carmustine (BCNU) for the treatment of newly diagnosed glioblastoma was evaluated in four studies, including one RCT. In the multi-center RCT presented by Buckner et al., 451 patients were randomized to receive carmustine or carmustine + cisplatin, in addition to either a standard radiation treatment or accelerated radiation [157]. No statistical difference in median survival was observed for patients receiving the combination of carmustine and cisplatin compared to those receiving carmustine alone. Toxicity (nausea, vomiting, hearing loss) was statistically higher in

the group treated with cisplatin. In two of the studies, carmustine was administered at the time of surgical intervention. Roux et al. performed a single-institution retrospective study evaluating 340 patients with newly diagnosed glioblastoma, including 123 patients with carmustine wafer implantation at the time of surgical resection. They observed no increased risk of postoperative infection, hematoma, or seizures in group implanted with carmustine wafers and a statistically significant increase in event-free survival with carmustine wafer implantation [158]. Despite the large number of patients included in this study, the retrospective nature of the study renders this class III data. Salmaggi et al. reported their results using carmustine wafers implanted within the surgical resection cavity [159]. In this single-arm, single-institution prospective study, 35 patients were enrolled with all patients also receiving adjuvant temozolomide and radiation therapy. Overall, a 50% progression free survival at twelve months was observed along with a median survival time of 17.8 months. Using a historical comparison group, the authors concluded that the addition of carmustine wafers improved median survival. Jenkinson et al. reported their non-randomized, phase II trial evaluating direct intratumoral administration of DTI-015 (BCNU dissolved in ethanol) [160]. Eight newly diagnosed glioblastoma patients were treated with DTI-015 via stereotactic injection at the time of biopsy. Overall median survival was 47 weeks, however, mean tumor blood flow and volume were reduced compared to historical controls.

Lomustine (CCNU)

The combination of lomustine (CCNU) and temozolimide was evaluated in a prospective, multi-center (two centers) phase II trial enrolling 31 patients. Herrlinger et al. reported a median PFS of 6 months and overall median survival of 22.6 months [161]. MGMT promoter methylation was strongly associated with increased PFS and the authors concluded the addition of lomustine to temozolomide cannot overcome chemotherapy resistance in patients without MGMT methylation.

Other studies

In a single-institution prospective cohort study, Adair et al. investigated co-administration of O6-benzylguanine (O6BG) and temozolomide to 7 patients pretreated with gene therapy conferring O6BG resistance [162]. CD34 + hematopoietic stem cells were transformed with a retroviral vectors encoding PI40K cDNA. The authors concluded that gene therapy permitted a significant increase in the number of mean cycles of O6BG + temozolomide administered without dose limiting myelosuppression. The small sample size and lack of an appropriate control group renders this class III data. A more recently published report of a planned phase III study of radiation therapy and BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea: carmustine) with or without O6BG was stopped for futility at the first interim analysis. Beier et al. looked at pegylated doxorubicin combined with the extended use of postradiation temozolomide and found no tumor control or survival advantage over historic controls. Based on this they recommended the regimen not be pursed further [152, 163]. Blumenthal et al. concluded O6BG did not provide added clinical benefit to BCNU alone and in fact caused additional toxicity [164].

Synthesis of results

The well-designed RCT reported by Buckner et al. provides class I evidence that cisplatin does not increase survival for newly diagnosed glioblastoma when combined with carmustine but does increase toxicity [157]. The lack of an appropriate control group reduces the other prospective studies evaluating chemotherapy for newly diagnosed glioblastoma to class III data, however, no significant survival benefit was observed for MGMT-unmethylated patients treated with lomustine + temozolomide or carboplatin + etoposide compared to historical control groups of temozolomide alone. This is important information, but negative in nature, limiting the ability formulate new declarative recommendations for patient management. Thus, it is suggested that, when available, patients with newly diagnosed glioblastomas be enrolled in properly designed clinical trials of cytotoxic chemotherapy.

Molecular and targeted therapy

Study selection and characteristics

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 55 were chosen as relevant to molecular and targeted therapies for full text review and assessment. A total of 50 publications met the criteria for inclusion regarding molecular and targeted agents.

Data extraction included (state inclusion/exclusion criteria) study design, level of evidence, primary treatment modality, total number of patients, results and author's conclusions. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Of the 50 studies designed to assess the use of molecular and targeted agents for newly diagnosed glioblastoma, three studies was a randomized clinical trials (RCT) and 44 others were performed prospectively. Three studies were

Table 8 Evidence for chemotherapy

Author/year	Study description	Data class	Conclusion
Bhandari (2017)	Single institution, non-blinded, prospective randomized study involving 40 patients at the All India Institute of Medical Sciences evaluating 6 cycles versus twelve cycles (extended) of temozolomide following surgical resection of newly diagnosed glioblastoma. The median f/u for the entire cohort was 17.3 months with MRI every 3 months	II	Hematologic toxicity ≥ grade 3 was observed in 5% of patients treated with conventional 6 cycles of TMZ (C-TMZ) and 15% with 12 extended cycles (E-TMZ). At the time of randomization, gross total surgical resection was performed in 60% of those receiving C-TMZ compared to E-TMZ and 39/40 patients in the study completed initial RT (60 Gy) and induction TMZ. The median PFS was 12.8mo and 16.8mo in the C-TMZ and E-TMZ arms, respectively. Median OS was 15.4mo for the C-TMZ arm compared to 23.8mo in the E-TMZ arm. Although this study was randomized, meaningful conclusions are limited by the small sample size and non-blinded study design. MGMT methylation status was not determined
Skardelly (2017)	Retrospective single-center cohort study assessing the role of extended administration of temozolomide beyond 6 cycles for newly diagnosed glioblastoma. 169 patients were included in the study and the cohorts included patients who discontinued temozolomide prior to completing 6 cycles, those that received 6 cycles and were followed with serial MRI until time of progression, and those that received > 6 cycles. Exclusion criteria included patients who demonstrated radiographic progression within the first 6 cycles of TMZ	III	The median PFS for patients receiving 6 cycles of TMZ was 13.7mo vs 20.9mo in the group receiving > 6 cycles. Median OS was 25.2mo for the 6 cycle group compared to 28.6mo in the extended TMZ group. Multivariate Cox regression analysis incorporating age, Karnofsky Performance Status, MGMT status, and extent of surgical resection demonstrated no statistical difference in median OS between the conventional and extended TMZ groups
Gramatzki (2017)	Multi-institutional retrospective cohort study assessing the role of extended administration of temozolomide beyond 6 cycles for newly diagnosed glioblastoma. 142 patients were included in the study and the cohorts included patients who received 6 cycles of TMZ and those that received > 6 cycles	III	61/142 patients received > 6 cycles of TMZ. The median PFS for the extended TMZ group was 20.5mo compared to 17.2mo in the group receiving 6 cycles. No significant difference was noted in medican OS between the groups and multivariate analysis incorporating age, KPS, extent of resection, MGMT and IDH status showed no statistical difference between the groups for either PFS or OS
Roux (2017)	Single-center retrospective study evaluating carmustine wafer implantation at the time of surgical resection for newly diagnosed glioblastoma. 340 patients with a supratentorial glioblastoma were included in the study with 123 (36%) of patients receiving carmustine wafer implantation. Surgical complications including postoperative infections and hematomas requiring surgical evacuation were compared between the groups with and without the carmustine wafer implantation	III	No statistical differences was noted between the cohorts who had carmustine wafers implanted at the time of surgical resection and those that did not in regard to postoperative infection, postoperative hematoma requiring surgical evacuation, seizures, or new neurologic deficit. In multivariate analysis, carmustine wafer implantation was associated with a longer event-free survival
Blumenthal (2015)	A multi-institutional phase III study of O6-BG + BCNU (40 mg/m^2 BCNU six hours after the administration of 120 mg/m^2 O6-BG intravenously over one hour every six weeks) plus radiation therapy (RT) or BCNU (200 mg/m^2 intravenously over 1 h every six weeks.) plus RT	I	The trial was halted at first interim analysis per stopping guidelines due to futility (less than 40% improvement on O6BG + BCNU arm). Following adjustment for stratification factors, there was no significant difference in overall (OS) or progression-free survival (PFS) between the two groups (one sided $p = 0.94$ and $p = 0.88$ respectively). Median OS was 11 months (95% confidence interval 8 – 13 months) for patients on the O6BG + BCNU arm and 10 months (95% confidence interval 8 – 12 months) for the BCNU arm. PFS was 4 months for patients in each arm. Adverse events were reported in both arms, with significantly more grade 4 and 5 events in the experimental arm

Table 8 (continued)

Author/year	Study description	Data class	Conclusion
Adair (2014)	A single institution prospective study assessing whether coadminstration of O6-benzylguanine (O6BG) and TMZ to patients following gene therapy to confer O6BG resistance results in improved OS, PFS, and hematologic toxicity. 7 patients with newly diagnosed glioblastoma with overexpression of MGMT were enrolled. Patients underwent standard RT without concomitant TMZ. At the conclusion of RT, (HSC) were mobilized with G-CSF, collected by plasmapheresis, and transformed with a retroviral vector encoding the PI40K cDNA which provides protection against hematopoietic toxicity from O6BG/alkylator chemotherapy. The gene-modified HSC were then reinfused and patients subsequently treated with 06BG + TMZ (472 mg/m2) in 28-day cycles	III	Compared to historical controls, gene therapy permitted a significant increase in the number of mean cycles of O6BG/TMZ that were tolerated without dose limiting myelosuppression (4.4 cycles/patient, $P < 0.05$). A median PFS of 9 months and OS of 20 months was observed. The authors conclude that chemoprotective gene therapy may improve outcomes for patients with overexpression of MGMT. The small number of patients treated and use of historical controls renders this class III data
Salmaggi (2013)	A single institution, prospective study of carmustine wafers placed at the time of surgical resection in combination with metronomic temozolomide beginning 7–15 days after surgery and 60 Gy of radiation beginning up to 30 days after surgery in 35 newly diagnosed glioblastomas. The temozolomide was continued for up to 6 months. Progression free survival and overall survival were tracked	III	After a median follow-up of 15 months, the median time to tumor progression was 12.5 months and median survival was 17.8 months. Of the 32 patients with a follow-up longer than or equal to 12 months, 21 experienced tumor progression, with 50% PFS at 12 months. At univariate analysis, survival time in patients was significantly longer, at a statistically borderline level, only in patients with a methylated MGMT promoter ($p = 0.049$)
Jenkinson (2010)	Phase II trial of intratumoral BCNU injection and radiotherapy on untreated adult malignant glioma	III	This is a single arm, non-randomized, phase II trial to evaluate DTI-015 (BCNU dissolved in ethanol) and fractionated external beam radiotherapy on newly diagnosed, malignant gliomas investigated early changes in tumor physiology and metabolism, clinical outcome and safety. 12 patients were enrolled, including 8 GBM, and 4 anaplastic astrocytoma. DTI-015 was injected using Riechert stereotactic frame, along with biopsy. CT perfusion, SPECT, and perfusion MRI were performed before and after injection. Patients then received radiation treatment. Primary endpoint was radiographic response. Secondary endpoints were progression free (PFS) and overall survival (OS)
Beier (2009)	A two institution phase I and then phase II study of newly diagnosed glioblastoma patients ($n = 63$) treated with pegylated liposomal doxorubicin in addition to 12 months of temozolomide given after concurrent temozolomide/radiation therapy. The pegylated doxorubicin was given just before radiation and the twice a month with temozolomide after radiation was completed. Toxicity and then response was assessed and compared to historical temozolomide and radiation data	III	The toxicity of the combination of pegylated doxorubicin and prolonged administration of temozolomide, and radiotherapy was deemed tolerable by the investigators. The progression free survival after 12 months was 30.2%, and the median overall survival was 17.6 months in all patients including the ones from the phase I component of the study. None of the endpoints differed significantly from the historical combined radiation and temozolomide data utilized (EORTC26981/NCIC-CE.3) in a post-hoc statistical comparison The authors concluded from this result that further investigation of this regimen is not warranted The lack of concurrent controls and the combination of phase I and phase II information yield class III data

Table 8 (continued)

Author/year	Study description	Data class	Conclusion
Buckner (2006)	Phase III trial of carmustine and cisplatin compared with carmustine alone and standard radiation therapy or accelerated radiation therapy in patients with glioblastoma multiforme: North Central Cancer Treatment Group 93–72-52 and Southwest Oncology Group 9503 Trials	III	Randomized clinical trial involving 451 patients assessing whether cisplatin + carmustine (BCNU) improves survival compared to BCNU administered with radiation therapy, delivered as either standard RT (SRT) or accelerated RT(ART). Adults underwent surgical treatment (biopsy or resection) of newly diagnosed GBM were randomized to one of four treatment arms: (A) BCNU + SRT; (B) BCNU + ART; (C) cisplatin/BCNU + SRT; (D) cisplatin/BCNU + ART. SRT total dose was 65 Gy (36 fractions) while the ART dose was 48 Gy (15 fractions). RT was delivered concurrently with BCNU alone therapy and after the first cycle (8 weeks) for the cisplatin/BCNU arms
Herrlinger (2006)	Prospective phase II cohort study of newly diagnosed glioblastoma patients involving 31 patients (2 centers) treated with lomustine (100 mg/m2), temozolomide, and RT. Primary endpoints were PFS at 6 months and acute toxicity. Lomustine and temozolomide were administered in 6-week courses with dose adjustments made according to WBC and platelet counts	III	WHO grade 4 hematotoxicity was observed in five patients (16%) and one of these patients died as a result of septicemia. Nonhematologic toxicity included one patient with WHO grade 4 drug-induced hepatitis (leading to discontinuation of lomustine and temozolomide) and one patient with WHO grade 2 lung fibrosis (leading to discontinuation of lomustine). The progression-free survival rate at 6 months was 61.3%. The median progression-free survival was 9 months (95% CI, 5.3 to 11.7 months), the median overall survival time was 22.6 months (95% CI, 12.5 to not assessable), the 2-year survival rate was 44.7% The authors concluded the notable toxicity of the regimen was acceptable and the survival promising The lack of a meaningful comparison cohort results in this being class III data

Abbreviations: AA: anaplastic astrocytoma; BCNU: bis-chloroethylnitrosourea; cDNA: complimentary DNA; CI: confidence interval; CT: computerized tomography; DTI-015: BCNU dissolved in ethanol; GBM: glioblastoma; G-CSF: granulocyte colony stimulating factor; Gy: gray; HSC: CD34 positive hematopoietic stem cells; MGMT: methylguanine methyltransferase; O6BG: O6-benzylguanine; OS: overall survival; PFS: progression free survival; RT: radiation therapy; SPECT: single photon emission computerized tomography; TMZ: temozolomide;

retrospective in nature. Among the prospective cohort trials, 20 were considered phase 1 trials and 24 were phase II clinical trials. Among the phase II trials, with the exception of the Batchelor et al., outcomes were compared to historical controls rendering these studies class III in nature and subject to comparison choice bias and change in methods over time bias [165]. Additionally, these studies were at risk of being influenced by selection bias, hidden agenda bias, and attrition bias. The random allocation of participants in the CENTRIC study by Stupp et al. decreases the likelihood of selection bias, however, the non-blinded trial design renders the study subject to potential ascertainment and selective reporting bias as well as variability due to random errors related to problems with unintentional data entry and oversight [166]. Two retrospective studies were subject to case selection bias, bias due lack or loss of information over time, the biases of the interpreting investigator in regard to the study and publication bias [167, 168]. The use of biomarkers for outcome assessment was performed in nine studies with only the CENTRIC study using *MGMT* promoter methylation status as a component of its eligibility criteria [166].

Results of individual studies

The key results of individual studies are outlined in Table 9.

The identification of multiple genomic and epigenomic mutations in glioblastoma has led to a wide variety of clinical trials utilizing molecular and targeted agents. The most frequent targets of therapeutic inhibition have been the human epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGF), mechanistic target of rapamycin (mTOR), farnesyltransferase, and integrins.

Integrin inhibition

Integrins are heterodimeric, transmembrane receptors that regulate signaling pathways involved in tumor migration, invasion, and proliferation. Cilengitide, an integrin inhibitor, was evaluated in three studies for the treatment of newly diagnosed glioblastoma, including one RCT [166, 169, 170]. Two phase II trials demonstrated that cilengitide was well tolerated when combined with standard of care temozolomide (TMZ) and radiation therapy (RT) and suggested possible increase in progression free survival and overall survival compared to historical controls. Survival benefit was most significant in patients treated with higher doses of cilengitide (2000 mg twice weekly) and exhibiting MGMT promoter methylation. In a multi-center study administering cilengitide at a dose of 2000 m twice weekly beginning 1 week prior to TMZ/RT, Stupp et al. observed that PFS was increased in methylated MGMT patients (13.4mo vs 3.4mo) as was overall survival (23.2mo vs 13.1mo) [170] The use of historical control groups as a validation cohort in each of these studies results in class III data.

On the basis of these phase II studies, Stupp et al. subsequently performed a randomized, non-blinded, multi-institutional trial (CENTRIC) enrolling adult patients with newly diagnosed glioblastoma patients with methylated *MGMT* [166]. Overall, 3471 patients were screened with 926 patients having a methylated *MGMT* promoter and 545 patients randomly assigned in a 1:1 ratio to receive either TMZ/RT with cilengitide (2000 mg IV twice weekly) or TMZ/RT alone. Cilengitide was continued for up to 18 months or until disease progression. No significant difference in overall survival was observed with the addition of cilengitide to standard of care TMZ/RT compared to the control TMZ/RT treatment group with a median OS of 26.3mo observed in each group ($p = 0.86$). Additionally, two-year survival did not differ between the groups nor did the incidence of treatment-related adverse effects.

Farnesyltransferase inhibition

The farnesyltransferatse inhibitor tipifarnib has been evaluated in three clinical trials for newly diagnosed glioblastoma [171–173]. In 2008, Lustig performed a multi-institutional study in 28 patients who demonstrated the presence of residual tumor after having undergone surgical intervention [171]. Tipifarnib was administered prior to radiation. No objective tumor responses were observed and the median OS was 7.7 months, leading the authors to recommend that tipifarnib not be administered prior to radiation for newly diagnosed glioblastoma. Ducassou et al. treated 27 patients with tipifarnib concurrently with radiation therapy [173]. The median PFS and OS were similar to historical controls and overexpression of the biomarkers FGF receptor-1 and avb3 were associated with reduced survival.

Mammalian target of rapamycin (mTOR) inhibition

The mammalian target of Rapamycin (mTOR) is activated by the PI3K/AKT pathway. Loss of *PTEN* in glioblastoma may lead to increased mTOR activity. The mTOR inhibitors everolimus and temsirolimus have been evaluated in four phase I and two phase II trials for newly diagnosed glioblastoma [174–180]. Sarkaria et al. demonstrated severe immunosuppression in patients treated with temsirolimus with 25% of patients experiencing fatal infectious complications when combined with TMZ/RT [174]. Conversely, everolimus appears to be well tolerated in combination with standard of care therapy when administered either daily or weekly. Mason et al. demonstrated that everolimus clearance is significantly affected by enzyme-inducing anti-epileptic drugs (EIAED) [176]. Chinnaiyan et al. carried out a phase I analysis of everolimus and standard temozolomide and

radiation concluding it fairly safe [177] They subsequently reported the results of their phase II study of patients randomized to receive standard TMZ/RT or TMZ/RT + everolimus [178]. There was no statistical difference in median PFS between patients randomized to receive everolimus and the control group (8.2 vs 10.2 months, respectively). Additionally, patients receiving everolimus had an increase in grade 4 toxicities including lymphopenia and thrombocytopenia. Hainsworth et al. evaluated the combination of everolimus and bevacizumab as maintenance therapy in 57 patients [179]. Four fatal treatment-related events were observed during the study including a fatal gastrointestinal perforation, intracranial hemorrhage, myocardial infarction, and pulmonary embolism. The median PFS was 11.3 months and OS 13.9 which the authors concluded were similar to historical studies utilizing bevacizumab, suggesting that everolimus has limited activity.

Tyrosine kinase inhibition

Protein kinases are involved in cell signaling pathways regulating a multitude of cellular functions including metabolism, growth, and differentiation and are characterized by the amino acid they phosphorylate. A variety of tyrosine and serine/threonine kinase inhibitors have been investigated for the treatment newly diagnosed glioblastomas.

Epidermal growth factor receptor inhibition

EGFR activation results in signaling through the MAPK, PI3K, and STAT3 pathways. Seven studies have investigated the use of EGFR inhibitors for the treatment of newly diagnosed glioblastoma [181–186]. An initial phase I/II trial by Ramos et al. in 2006 evaluated h-R3, a humanized anti-EGFR antibody, in 27 patients with newly diagnosed malignant gliomas including 16 patients with glioblastoma [181]. Overall, following intravenous administration of h-R3, 38% of patients exhibited an objective radiographic response including 17% with a complete response. Erlotinib, an oral EFGR inhibitor, was evaluated in a multi-center phase I/II trial with erlotinib administered one week prior to radiation followed by standard TMZ/RT therapy concurrent with daily erlotinib [182]. No increase in survival was noted compared to historical controls and *EGFRvIII*, *p53*, *PTEN*, and *EGFR* amplification biomarkers were not predictive of survival. Nimotuzumab was investigated in a single-institution phase II trial for high-grade gliomas, enrolling 19 patients with glioblastoma and 16 patients with anaplastic astrocytoma [184]. No dose-limiting toxicities were observed with nimotuzumab and radiation therapy. The median OS for all patients was 12.4 months and data interpretation was limited by the mixed pathology enrollment.

Vascular endothelial growth factor receptor inhibition

Intravenous administration of bevacizumab failed to improve overall survival when combined with standard radiation therapy and temozolomide in two phase III trials, one phase II trial and one retrospective study [187–190]. Wirshing et al. evaluated the addition of bevacizumab to hypofractionated radiation therapy in elderly patients but observed no significant improval in OS with combination therapy [191]. Vatalanib (PTK787/ZK222584) is an oral molecular agent that targets VEGF receptors 1–3 as well as platelet derived growth factor-beta (PDGF-β) and c-kit. Two phase I trials demonstrated that vatalanib is well-tolerated at doses of 1000 mg/day and median PFS were 6.8mo and 7.2mo in the two studies investigating vatalanib in combination with TMZ/RT [192, 193]. Batchelor et al. performed a phase II trial evaluating cediranib, an oral pan-VEGFR inhibitor with a prolonged half-life allowing for once daily dosing [165]. Forty patients with newly diagnosed glioblastoma were treated with cediranib in addition to standard TMZ/RT and radiographic assessment of tumor perfusion was performed on a weekly basis. Additionally, the study included assessment of serum angiogenesis biomarkers. The authors determined that 39 patients experienced reduced volume of contrast enhancement and vasogenic edema in response to treatment and 50% of patients had MRI evidence of increased microvessel tumor perfusion. After stratification for KPS and MGMT status, the authors reported that patients with increased tumor perfusion had a statistically significant increase in median OS (26.3mo vs 17.0mo). Hainsworth et al. evaluated the oral tyrosine kinase inhibitor sorafenib which inhibits a wide variety of targets including VEGFR, PDGFR, and Raf family kinases [183]. No significant toxicity was observed with the addition of sorafenib to standard TMZ maintenance therapy, however, no increase in median PFS or OS was achieved with the combination therapy in comparison with historical controls. In this study, 21% of enrolled patients failed to receive sorafenib therapy due to early tumor progression or progressive neurologic impairment.

Serine/threonine kinase inhibition

Enzostaurin is an oral serine/threonine kinase inhibitor that suppresses protein kinase C and protein kinase B/AK signaling. Two phase II trials investigating enzostaurin have been performed with Butowski et al. combining enzostaurin (250 mg/d) with standard TMZ/RT [194–196]. A median PFS of 9mo and OS of 18.5mo was observed with *MGMT* promoter methylation status positively correlating with survival [109]. No correlation of PFS or OS was observed with the molecular markers EGFR, PTEN, VEGF, or MAPK. Wick et al. subsequently administered

Table 9 Evidence for molecular and targeted therapy

Author/year	Study description	Data class	Conclusion
Reyes-Botero (2018)	Multi-center, open-label, nonrandomized Phase II study investigating TMZ + bevacizumab in elderly (age ≥ 70 years) patients with KPS < 70. 66 patients received TMZ + bevacizumab (10 mg/kg every 2 weeks)	III	The median OS was 23.9 weeks. 22/66 (33%) of patients had improvement of their KPS to > 70 during the treatment. No control group was included
Wirsching (2018)	Bevacizumab plus hypofractionated radiotherapy versus radiotherapy alone in elderly patients with glioblastoma: the randomized, open-label, phase II ARTE trial	II	75 patients were randomized and no statistical difference in median OS was observed between with two groups with patients receiving hypofractionated RT + bevacizumab experiencing a median OS of 12.1 months vs 12.2 months for patients treated solely with hypofractionated RT
Chinnaiyan (2017)	Randomized, open-label, multi-center trial evaluating the addition of everolimus (mTOR inhibitor) to standard chemoradiation for newly diagnosed glioblastoma. 171 patients were randomized to receive standard chemoradiation with or without daily everolimus (10 mg/day). The primary outcome was PFS	II	171 patients were randomized. There was no statistical difference in median PFS between patients randomized to receive everolimus and the control group (8.2 vs 10.2 months, respectively). Additionally, patients receiving everolimus had an increase in grade 4 toxicities including lymphopenia and thrombocytopenia
Darmon (2017)	Multi-center, retrospective study evaluating the use of TMZ + bevacizumab in patients with severe neurologic impairment (MRC neurologic status ≥ 3 and/or KPS < 70) or with rapid tumor progression following surgical resection. A cohort of 39 patients was investigated with study outcomes including median PFS and OS, KPS improvement, and subsequent treatment with RT	III	39 patient received a median of 3 months of induction TMZ + bevacizumab treatment. The median PFS and OS were 8.4 months and 11.0 months, respectively. KPS improvement was observed in 38% of patients and 62% of patients were deemed candidates for radiation therapy. This retrospective study lacks a control group for comparison is subject to selection bias., thus providing class III data
Galanis (2017)	Phase I/II multi-center, nonrandomized trial evaluating the addition of vorinostat (histone deacetylase inhibitor) to standard chemoradiation for newly diagnosed glioblastoma. RNA sequencing was also performed to evaluate possible vorinostat response signatures	III	15 patients were enrolled in the Phase I trial component and the maximally tolerated dose of vorinostat in combination with temozolomide was determined to be 300 mg/day. 107 patients were enrolled in the Phase II component and received vorinostat (300 mg/day) + standard chemoradiation. The primary study endpoint was OS at 15 months with 55% of patients surviving at 15 months. The overall median OS was 16 months and median PFS was 8 months. The authors concluded that the addition of vorinostat to standard chemoradiation did not significantly increase survival at 15 month. RNA sequencing analysis demonstrated that vorinostat response signatures may predict sensitivity and resistance
Yu (2017)	Single center, open-label, single arm Phase II trial evaluating the safety of high-dose pulse adminstration of lapatinib (EGFR inhibitor) in combination with standard chemoradiation	III	12 patients with newly diagnosed glioblastoma received lapatinib 2500 mg twice daily for two consecutive days per week throughout concomitant and adjuvant standard chemoradiation with temozolomide. The authors concluded that this is a tolerable and safe regimen, although increased rates of lymphopenia were observed
Reardon (2016)	Phase I multi-center trial investigating the safety and toxicity of ABT-414, an antibody–drug conjugate consisting of a humanized recombinant antibody targeting aberrant (amplified, overexpressed, mutated) EGFR linked to a microtubule toxin (MMAF). 45 patients received 0.5–3.2 mg/kg ABT-414 every 2 weeks + standard RT/TMZ	III	The most common toxicty with ABT-414 was ocular with 40/45 patients experiencing any grade ocular toxicity (blurred vision, dry eye, keratitis, photophobia, eye pain). 12/45 patients experienced ≥ 3 ocular toxicity
Wick (2016)	Randomized, non-blinded, multi-institutional study investigating the efficacy of temsirolimus (mTOR inhibitor) in combination with RT for patients with newly diagnosed GBM and unmethylated MGMT. 111 patients with unmethylated MGMT newly diagnosed glioblastoma were randomized t receive either standard RT/TMZ or RT + temsirolimus. The primary study enpoint was overall survival at 12 months	II	No statistically signficant difference in survival at 12 months was observed between the TMZ and temsirolums groups (72% vs 69%, respectively). In multivariate analysis, phosphorylation of mTORSer2448 may predict improved response to temsirolimus in patients with an unmethlyated MGMT promoter

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Krauze (2015)	A single institution, phase II study of concomitant radiation and temozolomide therapy and relatively high-dose valproic acid, as a histone acetylase inhibitor with possible radiation sensitizing abilities, followed by adjuvant temozolomide in patients (n = 37) with newly diagnosed glioblastoma	III	81% of patients took valproic according to protocol. Median OS was 29.6 months and median PFS was 10.5 months OS at 6, 12, and 24 months was 97%, 86%, and 56%, respectively. PFS at 6, 12, and 24 months was 70%, 43%, and 38% respectively. Valproate levels were not correlated with grade 3 or 4 toxicity levels. The authors concluded the results were superior to the historic experience of the EORTC/NCIC with radiation and temozolomide. Thus this is class III data
Stupp (2014)	Randomized, non-blinded, multi-institutional study investigating the efficacy of cilengitide (integrin inhibitor) in combination with standard TMZ/RT for patients with newly diagnosed GBM and methylated MGMT. The study was performed at 146 sites in 25 countries with randomization occurring based on RTOG recursive partitioning analysis class and geographic region. Treatment cohorts included standard TMZ/RT vs standard TMZ/RT + cilengitide 2000 mg IV administered twice weekly for up to 18 months or until time of progression	I	3,471 patients were screened and 545 were randomized. No significant difference in OS was noted between the treatment groups with a median OS of 26.3 in each cohort. Subgroup analysis evaluating age, RPA class, steroid use, extent of resection, and enzyme-inducing antiepileptic therapy failed to identify a group in which cilengitide conferred additional benefit. The authors concluded that cilengitide does not improve outcomes when combined with standard TMZ/RT
Solomon (2014)	Phase II single-institution trial investigating the addition of nimotuzumab (EGFRi) to RT in patient with high-grade gliomas. 35 patients were included in the study including 19 patients with GBM and 16 with AA. Two patients had previously undergone surgery for AA prior to the diagnosis of GBM. 25 patients underwent fractionated radiation therapy of at least 50 Gy. Nimotuzumab was adminstered weekly at doses of 200 mg during RT and then on a 21-day cycle	III	The median OS was 12.4 months for patients with high-grade glioma. No DLT were observed with the most common treatment-related toxicities including increased LFTs, fever, nausea, anorexia, tremors, and dizziness. The authors concluded that the OS of 12.4 months compared favorably to their institutional control population of RT alone therapy. The data interpretation of survival is limited by the small sample size, mixed pathologies, inconsistent RT dose but demonstrates that nimotuzumab is well tolerated. The use of historical controls for outcome comparison renders this study class III data
Alonso-Basanta (2014)	This is a phase I trial to examine the maximally tolerated dose (MTD) of the oral protease inhibitor nelfinavir (NFV) in combination with temozolomide and concurrent radiotherapy in patients with glioblastoma and to gather preliminary data for response. A total of 21 pts with newly diagnosed GBM were enrolled. All received standard radiation with temodar, and together with daily oral NFV starting 7–10 days prior to chemoradiotherapy continuing for the duration of chemoradiation for 6 weeks. There were two dose levels of NFV, 625 mg twice daily and 1250 mg twice daily. The primary endpoint was the determination of MTD. Secondary endpoints were efficacy outcomes and time to progression and overall survival	III	At the maximum tolerated dose, 18 subjects were enrolled to further evaluate toxicity and for preliminary estimate of efficacy for further phase II study. No dose-limiting toxicity was noted at 625 mg bid. At 1,250 mg bid, 3 dose-limiting episodes of hepatotoxicity were noted and one dose-limiting episode of diarrhea. The median OS is 13.7 m. The median PFS is 7.2 m. The author concluded The MTD for this study was 1,250 mg bid. NFV (1,250 mg bid) concurrent with temozolomide and radiotherapy is tolerated in most patients with glioblastoma. This is a single arm dose escalating phase I trial to evaluate the MTD of NFV. It provided class III data
Alexander (2013)	A single-arm, multi-institutional phase II study sponsored by the Radiation Therapy Oncology Group to determine the safety and efficacy of concurrent and adjuvant daily thalidomide (with escalation by 100–200 mg/day every 1–2 weeks) with radiation therapy in patients (n = 128) with newly diagnosed glioblastoma	III	The median survival time of the 89 evaluable patients was 10 months. When compared with a historical database stratified by recursive partitioning analysis (RPA) class, this end point was not different [P = 0.93] The authors concluded there was no benefit to the use of the combination of thalidomide with radiation in the initial management of newly diagnosed glioblastoma The phase II nature of the study with no concurrent control cohort results in this study providing class III data

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Ducassou (2013)	Phase I/II trial evaluating tipifarnib (farnesyltransferase inhibitor) with radiotherapy in patients with newly diagnosed GBM. In addition to determination of PFS and OS, biomarker analysis was performed	III	27 patients were treated in the Phase II trial with tipifarnib 200 mg/daily concurrent with radiotherapy. The median PFS was 23.1 weeks (5.8 months) and OS 80.3 weeks (20.1 months). Overexpression of FGFR1 and alpha-v-beta-3 integrin were associated with reduced OS. The authors concluded that tipifarnib + radiotherapy does not increase OS compared to historical controls. The use of historical controls for outcome comparison renders this study class III data
Batchelor (2013)	Phase II study (NCT00662506) investigating the addition of cediranib (pan-VEGF receptor tyrosine kinase inhibitor) to standard TMZ/RT. 40 patients with newly diagnosed GBM underwent treatment with standard RT/TMZ + cediranib (30 mg/d). Inclusion criteria required at least 1 cm diameter of residual tumor. All patients underwent weekly MRI scanning during chemoradiation followed by monthly MRI until 14 months with blood perfusion maps and vessel architecture imaging (VAI) generated using dynamic susceptibility contrast (DSC) imaging. Circulating blood biomarker evaluation for VEGF, plasma growth factor (PIGF), sVEGFR1, basic FGF, soluble VEGFR2, stromal cell-derived factor 1α carbonic anhydrase IX, and Ang-2 was also performed. Imaging and biomarker results were compared to a parallel prospective cohort of 14 pts who had identical inclusion criteria and monitoring protocol but did not receive cediranib	II	The median OS was 20.2 months and PFS 15.6 months. All but one patient experienced response to therapy with reduced volume of contrast enhancement and vasogenic edema as well as tumor vascular permeability and tumor vessel size. 26/30 patients were able to reduce or discontinue corticosteroid use during chemoradiation. 50% of patients had an increase in microvessel tumor perfusion as opposed to only 1/14 patients in the non-cediranib parallel study. After stratification for MGMT status and KPS, patients with increased perfusion had a statistically significant increase in median OS (26.3 months vs 17.0 months, $P < 0.05$). A statistically significant decrease in plasma PlGF and sVEGFR2 was observed in the group receiving cediranib, however, no biomarker correlated with OS. The authors concluded that cediranib increases tumor blood perfusion in a subset of patients and this increased perfusion is correlated with improved survival. This study includes a well-matched control group, although derived from a parallel study. No randomization or blinding was performed, rendering this class II evidence
Chinnaiyan (2013)	Phase I multi-center trial investigating the addition of everolimus (mTORi) to concurrent TMZ/RT therapy followed by TMZ + everolimus maintenance therapy. During concurrent TMZ/RT, the everolimus dose was escalated from 2.5 mg/d (dose level 1) to 5 mg/d (dose level 2) to 10 mg/d (dose level 3) followed by maintenance therapy at a dose of 10 mg/d	III	25 patients were treated with everolimus and evaluable for toxicity. The most common toxicities were grade 1/2 hypercholesterolemia and hypertriglyceridemia. DLT were observed in 2/8 patients at dose level 1 (gait disturbance and febrile neutropenia), 3/9 patients at dose level 2 (rash, fatigue/thrombocytopenia, hypoxia), and 2/8 patients at dose level 3 (ear pain, mucositis). The authors concluded that daily oral everolimus (10 mg) combined with RT and TMZ and adjuvant TMZ if fairly well tolerated
Wick (2013)	Phase II multi-center trial investigating the addition of enzastaurin (serine/threonine kinase inhibitor) to RT in patients with newly diagnosed GBM and without MGMT promoter methylation. Patients were treated with a loading dose (1125 mg) of enzostaurin 1 week prior to RT and then daily with 500 mg (either 500 mg once daily or 250 mg twice daily) until RT was completed. Enzastaurin was then adminstered 250 mg twice daily throughout the maintenance period	III	The median PFS was 6.6 months and OS was 15 months. 54% of patients were PFS at 6 months. Extent of resection correlated significantly with OS with biopsy alone patients having an OS of 3.9 months compared to 18.9 months in patients with gross total resection. The combination of enzastaurin and RT was well tolerated and the authors conclude that the OS compares favorably to those seen for all patients with TMZ/RT. The use of historical controls for outcome comparison renders this study class III data

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Hainsworth (2012)	Phase II multi-center trial investigating concurrent TMZ/RT therapy with bevacizumab followed by bevacizumab (VEGFi)/everolimus (mTORi) maintenance therapy for newly diagnosed GBM patients. 68 patients were enrolled and standard TMZ/RT therapy along with bevacizumab (10 mg/kg) during RT every 2 weeks. Following combined modality therapy, bevacizumab (10 mg/kg every 2 weeks) and everolimus (10 mg/d) were administered. 64 patients completed initial combined modality therapy and 57 patients began maintenance therapy with bevacizumab/everolimus	III	The median PFS using combination therapy with bevacizumab/everolimus was 11.3 months and OS 13.9. Four fatal treatment-related events were observed during the study period including a fatal GI perforation, CNS hemorrhage, myocardial infarction, and pulmonary embolism. Other DLT observed were hypertension, fatigue, pneumonitis, and mucositis. The authors concluded that the PFS results were similar to other first-line bevacizumab trials and compared favorably to historical controls of standard TMZ/RT regimens. The use of historical controls for outcome comparison renders this study class III data
Nabors (2012)	Phase II multi-center open-label, randomized trial investigating the addition of cilengitide (integrin inhibitor) to TM/RT and maintenance TMZ. 94 patients received conventional RT/TMZ + cilengitide at either a dose of 500 mg or 2000 mg twice weekly. MGMT methylation status was assessed for 79% of tumor samples	III	No DLTs were directly attributed to cilengitide in either dose cohort. The estimated median OS for all patients was 19.7mo (17.4mo for 500 mg cohort & 20.8 months for 2000 mg cohort). The median PFS was 10mo for all patients (9.5 months for 500 mg cohort & 9.3 months for 2000 mg cohort). The median OS for patients with methylated MGMT was 30 months vs 19.1 months in unmethylated patients. The authors concluded that cilengitide is well tolerated and improves survival compared to standard TMZ/RT based on historical controls. The use of historical controls for outcome comparison renders this study class III data
Mason (2012)	Phase I multi-center trial investigating the addition of everolimus (mTORi) to TMZ in patients with newly diagnosed GBM or at time of first recurrence. Everolimus was adminstered daily at a starting dose of 2.5 mg/d and escalated to 10 mg/d. Pharmacokinetic assessment of TMZ and everolimus whole blood concentrations were performed and patients receiving enzyme-inducing antiepileptic agents were compared to those not receiving these agents	III	32 patients (13 receiving EIAEDs, 19 not receiving EIAEDs) were assessed. No DLT was observed in the patients receiving EIAEDs and decreased everolimus concentrations were detected. In the non-EIAED cohort, 5 grade 3 toxicities were observed (2 lymphopenia, 2 granulocytopenia, 2 thrombocytopenia, and 1 cardiac dysfunction). The authors concluded that everolimus clearance is increased by EIAEDs and the recommended everolimus dose for future trials with concomitant TMZ is 10 mg per day
Lee (2012)	Phase I dose-finding study evaluating vorinostat (histone deacetylase inhibitor) + temozolomide in 59 patients with high-grade glioma	III	The maximal tolerated dose (MTD) of vorinostat was 500 mg/day in combination with a TMZ dose of 150 mg/m2/day (given on days 1–7 & 15–21 of an every 28-day cycle). Escalation of the TMZ dose to 200 mg/m2/day resulted in a vorinostat MTD of 400 mg/day. The combination of vorinostat and TMZ was well tolerated with dose escalation limited by thrombocytenia. Dose limiting toxicities included grade 3 anorexia, grade 3 ALT elevation, and grade 5 hemorrhage
Sarkaria (2011)	Phase I multi-center trial investigating the addition of everolimus (mTORi) to concurrent TMZ/RT therapy followed by TMZ + everolimus maintenance therapy. Everolimus was adminstered orally 1 week prior to TMZ/RT and then continued weekly through the duration of RT. During adjuvant TMZ therapy, everolimus was delivered weekly until disease progression or toxicity. The dose was escalated from 30 mg/week to 70 mg/week	III	18 patients were enrolled and the authors concluded that everolimus delivered weekly in combination with TMZ/RT and adjuvant TMZ was well tolerated. DLTs were observed in 3 patients including grade 3 fatigue, grade 4 hematologic toxicity, and grade 4 liver dysfunction

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Wakabayashi (2011)	Phase I multi-center trial investigation the addition of interferon-β to TMZ/RT and maintenance TMZ. IFN-β was administered at a dose of 3 MIU/body intravenously on alternate days during induction TMZ/RT followed by monthly administration. Maintenance TMZ dose was escalated from 150 mg/m2/d to 200 mg/m2/d based on hematologic toxicity. The study population included 16 patients with newly diagnosed GBM and 7 patients with recurrent high-grade gliomas	III	Grade 3–4 DLT included lymphocytopenia and neutropenia in 7 & 13% of patients, respectively. The most common adverse event was grade 1 anorexia. An objective CR or PR was observed in 40% of patients. In patients with newly diagnosed GBM, the median OS was 17.1 months. The authors concluded that IFN-b administered with standard TMZ/RT is well tolerated and may prolong survival. The use of historical controls for outcome comparison renders this study class III data
Gerstner (2011)	Phase I dose-finding study evaluating vatalanib (VEGFR, PDGFR, c-kit inhibitor) in 19 patients with newly diagnosed GBM treated with TMZ, radiation, and an enzyme inducing antiepileptic drug. Toxicity, radiographic response, and survival were assessed along with circulating biomarker evaluations, pharmacokinetic assessments, and single-nucleotide polymorphism (SNP) analysis from peripheral blood	III	Vatalanib was well tolerated and the MTD was not reached during the study period. The maximal dose given was 1000 mg twice daily. The median PFS was 7.2 months and OS 16.2 months. Pharmokinetic analysis demonstrated that vatalanib is rapidly metabolized with peak concentration within 1–3 h
Butowski (2011)	Phase II single-institution trial investigating the addition of enzastaurin (serine/threonine kinase inhibitor) to TMZ/RT in patients with newly diagnosed GBM and gliosarcoma. Enzastaurin was adminstered daily (250 mg) during RT and adjuvant period. 66 patients were enrolled and molecular markers including MGMT methylation, EGFR, PTEN, VEGF, and MAPK were evaluated	III	The median PFS was 9 months and OS 18.5 months. MGMT promoter methylation status positively correlated with survival but no other molecular markers were identified that predicted an improved survival outcome with enzastaurin treatment
Nghiemphu (2011)	Phase I trial investigating tipifarnib (farnesyltransferase inhibitor) in combination with radiation therapy. The MTD was evaluated in 3 cohorts based on concurrent use of enzyme inducing antiepileptic drugs and temozolomide	III	The authors determined that tipifarnib is well tolerated at doses of 300 mg twice daily with DLTs including rash, fatigue, and intracranial hemorrhage. No conclusions regarding PFS or OS could be determined from this study
Stupp (2010)	Phase I/II multi-center trial investigating the addition of cilengitide (integrin inhibitor) to TMZ/RT + maintenance TMZ. Cilengitide was administered at a dose of 500 mg twice weekly beginning 1 week prior to TMZ/RT and continuing until disease progression or conclusion of maintenance TMZ. 52 patients were enrolled and MGMT methylation status determined in 45 patients (87%)	III	The median PFS was 8mo and OS 16.1mo. PFS was increased in patients with methylated MGMT (13.4 months vs 3.4 months) as was OS (23.2 months vs 13.1 months). No DLT attributable to cilengtide was observed. The authors concluded that cilengitide administered intravenously to standard therapy does not significantly increase toxicity and may provide a survival benefit
Sarkaria (2010)	Phase I trial investigating the mTOR inhibitor temsirolimus with TMZ and radiation for newly diagnosed GBM. In the first 12 patients, temsirolimus was administered concurrently with TMZ/radiation and then weekly along with adjuvant TMZ for up to 6 cycles. An additional 13 patients only received temsirolimus during initial TMZ/radiation	III	In the initial 12 patients, the authors observed fatal infectious complications in 25% of patients. Limiting temsirolimus treatment to the radiation/TMZ phase along with administration of prophylactic antibiotics reduced the rate of infectious complications although exacerbation of pre-existing infections was observed. Immune suppression with reduced T, B, and NK cell populations was observed with concurrent temsirolimus/TMZ/radiation therapy
Hainsworth (2010)	Phase II multi-center trial investigating the addition of sorafenib (oral EGFRi) to adjuvant TMZ therapy for newly diagnosed GBM patients. 47 patients were enrolled for treatment with concurrent TMZ/RT followed by maintenance therapy with TMZ and sorafenib (400 mg twice daily) with 19 patients withdrawing prior to initiation of maintenance therapy	III	The median PFS was 6 months and OS 12 months. So significant toxicity was reported with TMZ + sorafenib maintenance therapy. The authors concluded that addition of sorafenib to TMZ during the maintenance phase of treatment does not provide significant benefit, although a large number of patients (40%) did not receive sorafenib due to early tumor progression or progressive neurologic impairment. The use of historical controls for outcome comparison renders this study class III data

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Motomura (2011)	Retrospective review of 68 consecutive patient who had undergone surgical treatment for a newly diagnosed GBM followed by TMZ/RT therapy including 39 patients who received interferon-b therapy in combination with TMZ. IFN-β was adminstered concurrently with TMZ/RT and then continued along with maintenance TMZ. Prognostic variables were assessed with multivariate analysis including genetic and epigenetic analysis on fresh frozen tissue. The most common alterations observed were: *EGFR* amplification (52%), *TP53* mutation (34%), *CDKN2A* loss (32%), *TP53* loss (16%), MGMT promoter methylation (34%), and *IDH1* mutation (6%)	III	Multivariate analysis revealed that MGMT promoter methylation and combination therapy with TMZ + IFN-b were independent prognostic markers of improved survival. The median OS in patients receiving TMZ + IFN-b was 19.9 months compared to 12.7 months with TMZ alone. In patients without *MGMT* promoter methylation, combination TMZ + IFN-b had a median OS of 17.2 months. The authors concluded that addition of IFN-b to standard RT + TMZ improves survival for newly diagnosed GBM patients. The study is limited by its retrospective nature and relatively small sample size
Butowski (2010)	Phase 1 single-institution investigating the addition of enzastaurin (serine/threonine kinase inhibitor) to TMZ/RT in patients with newly diagnosed GBM. In patients not receiving enzyme-inducing antiepileptic agents, enzastaurin was adminstered at a starting dose of 250 mg daily during initial TMZ/RT and during the TMZ maintenance period with escalation to 500 mg daily	III	There were no DLT at the enzastaurin dose of 250 mg. DLT were experienced in 2/6 patients at the 500 mg dose including grade 3 & 4 thrombocytopenia. The authors concluded that the recommended dose for phase II trials with enzastaurin is 250 mg daily
Drappatz, (2010)	This is a phase I study to evaluate the safety of of vandetanib, an inhibitor of vascular endothelial growth factor receptor 2 and epidermal growth factor receptor, in patients with newly diagnosed GBM combined with RT and temozolomide (TMZ). A total of 13 pts were treated with vandetanib, radiotherapy, and concurrent and adjuvant TMZ, using a standard ''3 + 3'' dose escalation	III	Of the 13 patients, 6 were treated with vandetanib at a dose of 200 mg daily. Of the 6 patients, 3 developed dose-limiting toxicities within the first 12 weeks, including gastrointestinal hemorrhage and thrombocytopenia in 1 patient, neutropenia in 1 patient, and diverticulitis with gastrointestinal perforation in 1 patient. The other 7 patients were treated with 100 mg daily, with no dose-limiting toxicities observed, establishing this dose as the maximal tolerated dose combined with TMZ and RT. The authors concluded vandetanib can be safely combined with RT and TMZ in GBM patients. It represents class III data since it is a phase I trial
Nabors (2010)	Phase I single-institution trial investigating the addition of ABT-510 (thrombospondin-1 mimetic agent) to concurrent TMZ/RT + maintenance TMZ therapy. 23 patients with newly diagnosed GBM were enrolled and treated with induction TMZ + RT + ABT-510 followed by ABT-510 alone for 4 weeks. Maintenance ABT-510 + TMZ was then administered until time of disease progression or toxicity. Dose level cohorts for ABT-510 were 20 mg, 50 mg, 100 mg, and 200 mg/d	III	No DLT were observed in any of the treatment cohorts. The median OS was 16.1 months. The authors concluded that ABT-510 is well tolerated at a dose of 200 mg/d and may show activity against GBM. The use of historical controls for outcome comparison renders this study class III data
Gilbert (2010)	Phase I multi-center trial investigating the addition of thalidomide (400 mg/d), isotretinoin (100 mg/m2), and/or celecoxib (400 mg bid) to TMZ following induction TMZ/RT. Patients with newly diagnosed GBM with stable disease following TMZ/RT were randomized to adjuvant treatment with TMZ alone or with TMZ + some combination of additional agents to create 8 treatment arms. 54 patients were enrolled with 42 receiving adjuvant therapy	III	The authors concluded that all combination were well tolerated. 1 patient treated with TMZ + isoretinoin + thalidomide experienced grade 3 fatigue. 1 patient receiving all 4 agents had grade 4 neutropenia. The median OS was 20mo with a 40% 2-year survival rate. The authors concluded that multiple cytostatic agents may be added to adjuvant TMZ without signficant increase in toxity
Brandes (2010)	Phase I/II study evaluating the multiple VEGF inhibtor PTK787/ZK222584 (PTK/ZK) in 20 patients with newly diagnosed GBM. PTK/ZK was adminstered at doses of 500 mg, 1000 mg, and 1250 mg in combination with radiotherapy and TMZ	III	The authors concluded that PTK/ZK is well tolerated at doses of 1000 mg/day. Increased dosing resulted in DLTs of diarrhea, ALT increase, and myelosuppression. The median PFS was 6.8 months and OS 17.3 months. The planned Phase II trial was discontinued due to loss of industry support

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Grossman (2009)	Phase II multi-center trial investigating concurrent treatment of newly diagnosed GBM patients with radiation, TMZ, and talampanel (AMPA receptor inhibitor). 72 patients were enrolled and the dose of talampanel was escalated to 50-75 mg thrice daily depending upon whether the patient was taking an enzyme inducing antiepileptic agent and the agent continued until the time of toxicity or tumor progression	III	The authors concluded that adding talampanel to standard TMZ/RT does not add significant toxicity. The median OS was 18.3 months. Among patients ages 18–70, the median OS was 20.3 months and 41.7% of patients were alive at 2 years which compares favorably to historical controls, suggesting that AMPA inhibitors may be beneficial in the treatment of newly diagnosed GBM. The use of historical controls for outcome comparison renders this study class III data
Butowski (2009)	Phase II multi-center trial investing the addition of poly-ICLC (double-stranded RNA immune modulating agent) to RT followed by single agent poly-ICLC maintenance therapy. Poly-ICLC was adminstered at a dose of 20mcg/kg thrice weekly via intramuscular injection. The primary endpoint was overall survival	III	30 patients underwent treatment with poly-ICLC with a median PFS of 4.5 months and survival of 16.3 months. The combination was well tolerated and the authors concluded that the addition of poly-ICLC improves survival compared to historical control of radiation therapy alone but does not provide a survival advantage compared to adjuvant TMZ. No DLT related to poly-ICLC were observed. The use of historical controls for outcome comparison renders this study class III data
Kubicek (2009)	Phase I single-institution trial investigating the addition of bortezomib (proteasome inhibitor) to concurrent TMZ/RT. 27 patients were enrolled in the trial including 13 newly diagnosed high-grade gliomas. Bortezomib was adminstered on Days 1, 4, 8, and 11 of a 21-day cycle during TMZ/RT at 3 treatment doses: 0.7, 1.0, and 1.3 mg/m^2/dose	III	No DLT were observed in any of the treatment cohorts. The median OS for the newly diagnosed high-grade glioma patients was 16.9 months. The authors concluded that bortezomib is well tolerated at a dose of 1.3 mg/m^2/dose
Drappatz (2009)	Phase I multi-center trial investigating the MTD of lenalidomide when administered concurrently with RT and then as maintenance therapy. 23 patients diagnosed GBM were administered lenalidomide on a 4 week cycle (single daily dose for 3 weeks with 1-week rest) with dose de-escalation from 20 mg/m2/d based on toxicity during the first 12 weeks of therapy. Aspirin was co-administered to decrease thrombotic complications	III	The authors concluded that the MTD for lenalidomide adminstered concurrent with RT is 15 mg/m2/d. DLT were eosinophilic pneumonitis and LFT abnormalities. Common toxicities included venous thromboembolic events, fatigue, and nausea. The median PFS was 5 months and OS 11 months and the authors concluded that there is little benefit in adding lenalidomide to RT alone. The use of historical controls for outcome comparison renders this study class III data
Lustig (2008)	Multi-center, open label Phase II study investigating the farnesyltransferase inhibitor RII5777(tipifarnib) in 28 patients with newly diagnosed GBM. R115777 was administered prior to radiation in patients who had undergone surgery with residual enhancing tumor. 1–3 monthly cycles (300–600 mg twice per day) were administered with radiation initiated at time of progression or after 3 cycles	III	No tumor responses were observed and the median OS was 7.7 months. The authors concluded that R115777 administered prior to radiation is not recommended for newly diagnosed GBM patients. The use of historical controls for outcome comparison renders this study class III data
Phuphanich (2008)	Phase I multi-center trial investigating atrasentan (endothelin-A receptor inhibitor) for the treatment of recurrent high-grade glioma. 25 patients were treated with oral atrosentan at doses of 10–150 mg/day	III	The MTD for atrasentin was determined to by 70 mg/d. The only DLT was a single patient with grade 3 hypoxia at the dose of 90 mg/d. PR were observed in 2 patients (8%) and the estimated median OS was 6mo

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Kesari (2008)	Phase II multi-center trial investing the addition of thalidomide (angiogenesis inhibitor) and celecoxib (angiogenesis inhibitor) to adjuvant TMZ for newly diagnosed GBM patients. Patients eligible for enrollment had stable disease following completion of standard RT and the primary outcome was 4 month PFS from time of study enrollment. 50 patients were treated with escalating dosing of thalidomide (200-1200 mg/daily) and celecoxib (200-400 mg twice daily). Additionally, reponse was correlated with MGMT promoter methlylation status and serum levels of angiogenic peptides (VEGF, bFGF, endostatin, and thrombospondin-1)	III	The median PFS was 5.9 months from study enrollment (after completion of RT) and median OS 12.6 months. 20% of evaluable patients had a radiographic partial or minor response (11% partial, 9% minor). The authors concluded that the addition of thalidomide + celecoxib to adjuvant TMZ is well tolerated (1 treatment-related death due to neutropenia and fever) but does not significantly improve PFS compared to historical controls of newly diagnosed GBM treatment. No correlation between treatment response and angiogenic peptides was identified. The use of historical controls for outcome comparison renders this study class III data
Fadul (2008)	Phase II trial investigating the combination of thalidomide (dose escalation to target of 400 mg/day) and irinotecan (350–700 mg/m^2) in 26 patients with newly diagnosed (n = 10) and recurrent (n = 16) GBM	III	Among newly diagnosed GBM patients, the 6 month PFS rate was 40%. Only a single partial response was observed among evaluable patients (7%) and 6 patients (23%) experienced venous thromboembolic complications. The authors concluded that the combination of thalidomide and irinotecan has limited activity against GBM and thromboembolic complications were common. The use of historical controls for outcome comparison renders this study class III data
Brown (2008)	Phase I/II multi-center trial investigating the addition of erlotinib (EFGRi) to standard TMZ/RT and maintenance TMZ therapy. Adults with newly diagnosed GBM not taking enzyme-inducing antiepileptics were treated with a single oral dose of erlotinib (150 mg) one week prior to RT followed be standard TMZ/RT therapy concurrent with daily erlotinib	III	97 patients underwent treatment with erlotinib. The median OS was 15.3 months. The presence of diarrhea or rash were not predictive of survival. The *EGFRvIII*, *p53, PTEN*, and *EGFR* amplication status were not predictive of survival. The authors concluded that erlotinib does not increase survival compared to historical TMZ-era controls and no analysis of molecular subsets predicted sensitivity to erlotinib. The use of historical controls for outcome comparison renders this study class III data
Mikkelsen (2007)	Phase II multi-center trial investigating the addition of carboxyamido-triazole (CAI), a calcium channel inhibitor, to standard RT. 55 patients were enrolled and treated with 250 mg/d CAI beginning with RT and continued until progression	III	The median OS was 10.3 months. Grade 3 or greater toxicity was observed in 16/55 patients including 2 patients with irreversible vision loss and plasma concentrations of CAI were significantly affected by enzyme inducing antiepileptic agents. The median OS was less than those of historical controls and the authors concluded that no significant benefit was observed with CAI. The use of historical controls for outcome comparison renders this study class III data
Vogelbaum (2007)	Phase I multi-center trial investigating cintredekin besudotox (CB), a recombinant cytotoxin consisting of IL-13 and truncated Pseudomonas exotoxin, administered via convection-enhanced delivery prior to RT. Eligible patient had undergone gross-total resection of a newly diagnosed high-grade glioma. Within 14 days of surgery, 2–4 intraparenchymal catheters were stereotactically placed and CB was infused (0.25–0.5 ug/ml) for 96 h. Standard RT was then administered 10–14 days later either with or without concurrent TMZ	III	22 patients underwent treatment with CB. No DLT were observed at the low-dose of CB in either RT alone or RT + TMZ cohorts. A single DLT was observed in both the high-dose CB + RT (grade 4 seizure) and high-dose CB + RT + TMZ cohorts (grade 3 aphasia). However, adverse events were observed in all patients with 33% of neurologic or psychiatric. No meaningful data regarding PFS or survival could be determined due to the small sample size, mixed pathologies, and short follow up period
Farray (2006)	Phase II trial investigating the activity of 9-Amino camptothecin (9-AC), a topoisomerase inhibitor, in 14 patients with newly diagnosed GBM and residual disease following surgery. 9-AC was infused at 1100 μg/m^2/24 h every 2 weeks for up to 6 cycles prior to RT	III	The median OS was 7.5 months and progressive disease was observed in all patients after at least 2 cycles. The most common adverse event was transient lymphopenia. In comparison to historical controls of newly diagnosed GBM, no significant improvement in OS was observed. The authors concluded that 9-AC lacks activity against GBM. The use of historical controls for outcome comparison renders this study class III data

Table 9 (continued)

Author/year	Study description	Data class	Conclusion
Crombet Ramos (2006)	Phase I/II multi-center trial investigating treatment of newly diagnosed high-grade gliomas with RT and a humanized EFGR antibody (h-R3). 29 patients were enrolled (16 GBM, 12 AA, 1 anaplastic oligodendroglioma) and received 6 weekly infusions of h-R3 at a dose of 200 mg in combination with RT (total dose 5–60 Gy). Radiographic response was determined via monthly CT or MRI	III	No grade 3 or 4 DLT were observed. The median OS for GBM patients was 17.5 months and the median OS for AA was not determined during the study period. Objective radiographic responses were detected in 38% of patients (17% CR, 21%PR). The authors concluded that h-R3 is well tolerated and active against GBM with improved survival compared to RT alone. The use of historical controls for outcome comparison renders this study class III data
Kuan (2006)	Retrospective analysis of 50 GBM samples assessing mRNA and protein expression levels of human transmembrane glycoprotein nonmetastatic melanoma protein B (GPNMB) in both its wildtype form (GPNMB-WT) and a splice variant form (GPNMB-SV). These levels were then correlated with retrospective patient survival data. 50 newly diagnosed GBM biopsy specimens were assessed for GPNMB RNA transcript levels by RT-PCR and protein expression by immunohistochemistry. Survival analyses were then performed on these two variables	III	The authors previously identified GPNMB as a potential glioma tumor-specific target based on gene expression analysis revealing preferential expression in GBM samples relative to normal brain. In this study, the authors determined that GPNMB transcripts were present in 70% GBM samples while protein expression was detected by immunohistochemistry in 66% of samples. Normal brain samples expressed little or no GPNMB mRNA. Using survival data from 39 patients, univariate and multivariate analysis demonstrated that high levels of GPNMB mRNA transcript levels and positive immunohistochemistry correlated with higher risk of death (hazard ratios 3.0 and 2.8, respectively). No other known survival variables for newly diagnosed GBM were included in this study and no data regarding clinical treatment was provided. The authors conclude that GPNMB may represent a glioblastoma-specific tumor target The retrospective and selective nature of the patient population leaves this as class III data
Tuettenberg (2006)	Phase 1 single-institution study investigating the addition of rofecoxib (COX-2 inhibitor) to RT + continuous low-dose TMZ in patients with subtotally resected newly diagnosed GBM. 13 patients who had subtotal resection of a GBM and completed RT (60 Gy) were enrolled and divided into 3 cohorts: (A) TMZ 10 mg/m^2 every 3rd day + rofecoxib 25 mg/d; (B) TMZ 10 mg/m^2 daily + rofecoxib 25 mg/d; (C) TMZ 5 mg/m^2 twice daily + 12.5 mg twice daily. Immunohistochemistry was performed on tumor tissue was for COX-2 and VEGF as well as blood vessel density	III	No DLT was observed in any treatment cohort. One PR and 2 minor responses were observed. The median PFS was 8 months, 7 months, and 9.5 months for cohorts A, B, and C, respectively with an overall PFS of 8 months. The estimated median OS was 15 months. Patients with high blood vessel density had prolonged PFS. The authors concluded that highly vascular tumors may benefit from the anti-angiogenic activity of low dose TMZ + rofecoxib. The phase I nature of this study yields class III data

Abbreviations: 9-AC: 9-Amino camptothecin; AA: anaplastic astrocytoma; ABT-510: A synthetic peptide that mimics the anti-angiogenic activity of the endogenous protein thrombospondin-1 (TSP-1); ALT: alanine aminotransferase; AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; bFGF: basic fibroblast growth factor; CAI: carboxyamido-triazole; CB: cintredekin besudotox; CDKN2A: cyclin-dependent kinase inhibitor 2A gene; COX-2: cyclooxygenase 2; DLT: dose limiting toxicity; DSC: dynamic susceptibility contrast imaging; *EGFR*: epidermal growth factor gene; EGFRi: epidermal growth factor receptor inhibitor; *EGFRvIII*: EGFRvIII mutation; EIAED: enzyme-inducing anti-epileptic drug; FGF: fibroblast growth factor; FRFR1: fibroblast growth factor receptor 1; GBM: glioblastoma; GI: gastrointestinal; GPNMB: glycoprotein nonmetastatic melanoma protein B; GPNMB-WT: glycoprotein nonmetastatic melanoma protein B-wild type; GPNMB-SV: glycoprotein nonmetastatic melanoma protein B-splice variant; Gy: gray; h-R3: humanized EFGR antibody; ifn: interferon; IV: intravenous; IL-13: interleukin-13; LFT: liver function tests; MAPK: mitogen activated protein kinase; MGMT: O6-methylguanine methyltransferase; MIU: million international units; MRI: magnetic resonance imaging; mRNA: messenger ribonucleic acid; MTD: maximal tolerated dose; mTORi: mammalian target of rapamycin inhibitor; NFV: nelfinavir; NK: natural killer; OS: overall survival; *p53*: protein p53 gene; PDGFR: platelet derived growth factor receptor; PIGF: plasma growth factor; PFS: progression free survival; poly-ICLC: polyinosinic-polycytidylic acid; PTEN: phosphatase and tensin homolog; RNA: ribonucleic acid; RPA: recursive partitioning analysis; RT: radiation therpy; RT-PCR: reverse transcriptase polymerase chain reaction; RTOG: Radiation Therapy Oncology Group; SNP: single nucleotide polymorphism; sVEGFR1: soluble vascular endothelial growth factor receptor 1; TMZ: temozolomide; *TP53*: tumor protein p53 gene; VAI: vascular architecture imaging; VEGF: vascular endothelial growth factor; VEGFi: vascular endothelial growth factor inhibitor; VEGFR2: vascular endothelial growth factor receptor 2

enzostaurin in combination with RT to newly diagnosed glioblastoma patients without *MGMT* promoter methylation [196]. A loading dose (1125 mg) of enzostaurin was administered one week prior to RT and then daily (500 mg) as maintenance therapy. The median OS was 15mo and the authors concluded that the OS compares favorably to historical controls of patients treated with TMZ/RT. Dose limiting thrombocytopenia may be observed with enzostaurin.

Additional molecular and targeted agents

A wide spectrum of additional molecular and target agents have been evaluated for the treatment of newly diagnosed glioblastoma with the results detailed in Table 9. Seven additional phase II trials were performed including two studies investigating thalidomide, an inhibitor of angiogenesis [197–204]. Kesari et al. combined thalidomide with celecoxib, a selective COX-2 inhibitor, and TMZ [201]. Fifty patients were enrolled and the combination therapy was well-tolerated, however, no improvement in the study's primary endpoint (4-month PFS) was observed compared to historical controls. A nonsignificant trend ($p = 0.07$) toward correlation of higher circulating VEGF levels and decreased OS was observed. Mikkelsen et al. studied the addition of carboxyamido-triazole to standard radiation therapy alone and similarly found no benefit to overall survival and encountered two patients with irreversible visual loss [198]. In another study showing no obvious therapeutic benefit polyinosinic-polycytidylic acid was added to radiation therapy by Butwoski et al. [203]. Fadul et al. investigated the combination of thalidomide and irinotecan, a topoisomerase 1 inhibitor [199]. Among 40 patients enrolled with high-grade gliomas, including 10 patients with newly diagnosed glioblastoma, only a single partial response was observed and 23% of patients experienced thromboembolic complications. The RTOG studied the use of thalidomide and radiation alone in a phase II fashion in newly diagnosed glioblastoma patients noting it yielded a median survival of 10 months and concluded it was a feasible regimen but provided no therapeutic benefit compared to historic controls [78, 200]. In an investigation of another topoisomerase inhibitor, 9-Amino camptothecin (9-AC), yielded no improvement in OS compared to historical controls [197]. Grossman et al. performed a multi-institutional phase II trial investigating talampanel, an oral alpha-amino-3-hydroxy-5-methyl-4-isocaxazolepropionic acid (AMPA) receptor blocker, in combination with standard TMZ/RT [202]. Seventy-two patients were enrolled and a median OS of 18.3mo was observed which compared favorably to historical controls. Talampanel was well-tolerated in combination with temozolomide with no additional hematologic toxicity observed. Krauze et al. reported on the use of valproic acid as a histone deacetylase inhibitor with hopes of it acting as a radiation sensitizer with temozolomide and radiation in standard fashion. Of the patients that completed the treatment regimen, the 1 year overall survival rate was 86% and the 6-month progression free survival rate was70%. The authors concluded that provides a level of promise warranting a phase III study [204]. Galanis et al. evaluated the addition of vorinostat, a histone deacetylase inhibitor, to standard chemoradiation and observed no increase in survival at 15 months, the study's primary endpoint. They did identify several RNA signatures that may predict vorinostat sensitivity and resistance that may warrant additional investigation [205].

Among the additional phase I trials, several studies have suggested potential activity against newly diagnosed glioblastoma [206–213]. Gilbert et al. performed a multi-institutional phase I trial assessing various combinations of thalidomide, isotretinoin, and celecoxib as maintenance therapy for patients with stable disease after induction TMZ/RT. Fifty-four patients were enrolled into 8 treatment arms and the authors concluded that multiple cytostatic agents may be combined with adjuvant TMZ without significant increase in toxicity 220].

Synthesis of results

The use of molecular and targeted agents has shown great promise in a variety of cancer types and our understanding of the molecular pathways disrupted in glioblastoma is rapidly advancing. A comprehensive assessment of the published studies investigating these agents for newly diagnosed glioblastoma, however, demonstrates a relative lack of success in extending survival rates beyond those achieved using standard of care temozolomide and radiation therapy. Despite phase II trials suggesting activity of integrin inhibitors against glioblastoma with objective patient responses in a dose-dependent manner, the multi-institutional CENTRIC randomized clinical trial by Stupp et al. investigating cilengitide in combination with TMZ/RT resulted in no improvement in median or two-year survival compared to standard of care controls [166]. The negative outcome of this phase III study demonstrates the limitations of early phase clinical trials and the importance of appropriate control groups in assessing efficacy of novel treatments. Additional studies and reviews have concluded that, though of scientific merit, the use of cilengitide provides no clear clinical benefit, no matter what subgroup analysis is used [215, 216]. Class III data from phase II studies investigating talampanel and enzostaurin have suggested potential efficacy for newly diagnosed glioblastoma including patients without *MGMT* methylation, a subgroup for which advancement in therapy is desperately needed.

Glioblastoma represents a heterogeneous disease with genetic, epigenetic, and metabolomics factors influencing diagnosis, prognosis, and treatment. While the CENTRIC

trial incorporated *MGMT* promoter methylation status as a component of the enrollment criteria, the majority of clinical trials to date have either failed to correlate these molecularly-defined subgroups with treatment response or done so in a limited and inconsistent manner potentially preventing the identification of subsets of patients for whom targeted therapies may prove effective. Encouragingly, the available data demonstrates that the majority of molecular and targeted agents are safe and well-tolerated in combination with standard of care therapies. Future treatment protocols will most likely investigate multiple agents in combination with defined target inhibition. Inclusion of molecularly-defined patient subgroups and valid control groups will be essential for the future critical assessment of targeted agents for the treatment of newly diagnosed glioblastoma.

Immunotherapy

Study selection and characteristics

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 19 were chosen as relevant to immunotherapy for full text review and assessment. A total of 17 publications met the criteria for inclusion regarding immunotherapy [217–233].

Data extraction included assessment of study design, primary treatment modality, total number of patients, and evaluation of toxicity and responses. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Of the 17 studies designed to assess the use of immunotherapy for newly diagnosed glioblastoma, 4 of the studies were performed as randomized clinical trials (RCT). Two of the RCT were double-blinded, with the study by Kong et al. lacking sufficient stratification and sample size to generate class I data [229]. Eleven studies included a phase II component in the design and two were purely phase I studies. In all studies assessing progression free survival and overall survival, outcomes were compared to historical controls rendering the data class III in nature and subject to comparison choice bias and change in methods over time bias. The open-label design of all of these studies renders them at risk of being influenced by selection bias, hidden agenda bias, and attrition bias.

Results of individual studies

The key results of individual studies are outlined in evidence Table 10.

Immune system modulation represents an emerging treatment for newly diagnosed glioblastoma. Treatment strategies have included a dendritic cell vaccination, tumor- and peptide-based vaccination, and oncolytic viral-based immunotherapy.

Sixteen studies investigating the use of vaccine-based immunotherapy for newly diagnosed glioblastoma were reported. Nine studies utilized a treatment schema involving autologous dendritic cells pulsed with either whole tumor cell lysates or tumor-associated peptides. In all of these studies, the authors concluded that DC-based vaccination was safe and well-tolerated. Inclusion criteria for nearly all studies required gross total or near-gross total tumor resection and minimal corticosteroid requirements. Chang et al. performed a single-center study evaluating vaccination utilizing dendritic cells pulsed with whole tumor cell lysates in 16 patients with newly diagnosed glioblastoma [217]. The authors reported a median overall survival of 525 days which they stated was significantly increased compared to a historical control group from their institution. Fadul et al. investigated the safety and efficacy of intranodal tumor-lysate DC vaccination [218]. Following combined TMZ/RT treatment, pulsed DC were inoculated into the bilateral cervical lymph nodes every two weeks for a total of three inoculations. A median OS of 28 months was reported and an increase in tumor-specific IFN-gamma production was observed. No tumor-specific delayed type hypersensitivity (DTH) reaction was observed in any of the patients. Prins et al. investigated vaccination with tumor cell lysate pulsed DC in combination with the toll-like receptor (TLR) immunomodulatory agents imiquimod and poly-ICLC [219]. No dose-limiting toxicity was observed with the combination of DC vaccination and TLR agonists. The authors reported a median OS of 31.4 months. The use of historical control groups as a validation cohort in each of these studies results in class III data. Buchroithner et al. performed a prospective randomized trial evaluating vaccination with tumor-lysate pulsed DC in addition to standard chemoradiation. No statistically significant increase in PFS at 12 months was observed compared to standard of care therapy [220]. Liau et al. presented their initial survival data combining autologous tumor-lysate pulsed DC vaccination (DCVax-L) with standard chemoradiation [221]. Patients were randomized 2:1 to receive DCVax-L and the studies cross-over design resulted in approximately 90% of patients receiving the vaccination. An overall median survival of 23.1 months for the entire study population was observed but these preliminary findings obtained prior to unblinding limits meaningful assessment of this therapy at this time.

Two studies investigated the use of DC vaccines pulsed with tumor-specific peptides. Sampson et al. performed a phase I study evaluating the safety and immunogenicity

of DC pulsed with the EGFRvIII antigen in patients who had undergone gross total tumor resection and radiation therapy [222]. No dose-limiting toxicity was observed and EGFRvIII-specific immune responses were evident in most patients. Similarly, Phuphanich et al. performed a phase I study evaluating the immunogenicity of ICT-107, a vaccine consisting of autologous DC pulsed with six synthetic tumor-associated peptides [223]. The authors determined that among the 16 patients with newly diagnosed glioblastoma included in their study, all patient tumor samples expressed at least 3 of the peptide antigens. Progression free survival and overall survival were significantly associated with expression of the AIM-2 and MAGE1 antigens.

Two studies utilized a whole tumor -based vaccination strategy. Muragaki et al. and Ishikawa et al. administered autologous formalin-fixed tumor vaccines in combination with radiotherapy [224, 225]. The authors reported a median OS of 20 and 22 months, respectively. No statistically significant association between development of a tumor-specific DTH response and OS was observed in either study.

Rindopepimut, a vaccine consisting of EGFRvIII conjugated to keyhole limpet hemocyanin, has been demonstrated in an open-label, single-arm phase II study (ACT-III) of 65 patients with newly diagnosed glioblastoma to significantly increase anti-EGFRvIII antibody production and potentially extend survival in patients with EGFRvIII-expressing tumors. Despite these promising phase II results, a phase III randomized clinical trial (ACT -IV) investigating rindopepimut and GM-CSF vaccination in patients with glioblastoma and minimal residual disease was stopped after a preplanned interim analysis determined the study could not reach statistical significance for its primary endpoint of increased overall survival compared to standard-of-care treatment [226, 227].

Synthesis of results

Immune-based therapy represents one of the most active areas of investigation for the treatment of glioblastoma. The advances achieved in other tumor types combined with the promising results observed in preliminary studies for recurrent glioblastoma has led investigators to utilize both passive and active immunotherapy strategies for the treatment of newly diagnosed glioblastoma. Dendritic cell vaccines pulsed with either whole tumor cell lysates or tumor-associated peptides have been evaluated in multiple small studies without matched control groups. While these agents have been shown to be safe, well-tolerated, and potentially immunogenic, the strict inclusion criteria for these studies, including the need for gross total tumor resection, renders them highly subject to potential selection bias. Several DC-based vaccines, including ICT-107 and DCVax-L, are currently being investigated in phase III trials for patients with newly diagnosed glioblastoma.

These discouraging preliminary results of some of these well designed and thought out studies underscore the necessity of utilizing appropriate control groups in evaluating novel therapies and point to the myriad challenges that remain for immune-based therapies including overcoming the immunosuppressive effects of the tumor and its microenvironment. Additionally, as immunomodulatory agents such as checkpoint inhibitors are increasingly combined with other immunotherapies in an attempt to overcome these effects, the need for appropriate evaluation of potentially immune-related adverse events will likely become more important.

Novel therapies

Study selection and characteristics

A total of 1840 citations were screened and assessed for eligibility based on the criteria noted above. Of these, 18 were chosen as relevant to novel therapies for full text review and assessment. A total of 13 publications representing twelve studies met the criteria for inclusion regarding the therapeutic value of novel therapies [148, 234–245]. Data extraction included study design, level of evidence, primary treatment modality, total number of patients, etc. The screening is summarized in Fig. 1.

Assessment for risk of bias and study limitations

Among the studies investigating the use of novel therapies for newly diagnosed glioblastoma, only two were designed as a randomized phase III trials [234–236]. The study performed by Westphal et al. this study using adenovirus gene therapy was non-blinded and utilized a non-standard primary end point of composite time to death or re-intervention [234]. This design is subject to bias of data interpretation particularly related to placebo effect. The EF-14 trial preliminary and final analyses presented by Stupp et al. was performed as an open-label trial without a sham control and inadequate blinding may introduce bias [235, 236]. The remainder of the publications were retrospective or non-randomized phase I or II studies and possessed a substantial risk of bias. Stylli et al. performed a retrospective study investigating the use of photodynamic therapy [237]. Retrospective studies are subject to case selection bias, bias due lack or loss of information over time, the biases of the interpreting investigator in regard to the study and publication bias. The other eight studies were prospective, single arm phase I or I/II trials [238–245]. All of these were subject to bias related

Table 10 Evidence for immunologic-based therapies

Author/year	Study description	Data class	Conclusions
Buchroithner (2018)	Randomized, open-label phase II trial evaluating the addition of Audencel, an autologous tumor lysate pulsed dendritic cell vaccine, to standard chemoradiation for newly diagnosed glioblastoma. 76 patients who had undergone surgical resection of ≥ 70% were randomized 1:1 to receive Audencel. The vaccine was adminstered by injection into an inguinal lymph node by ultrasound guidance	II	76 patients were randomized to receive Audencel vaccination + chemoradiation. Overall, the investigators observed no statistically significant difference in the study's primary endpoint of PFS at 12 months (28.4% vs 24.5%, respectively). No severe toxicity was attributed to the vaccine
Ji (2018)	Prospective, single-arm, single-center study evaluating the addition of heat shock protein complex-96 vaccine to standard chemoradiation. Patients with KPS ≥ 70 who had undergone surgical resection ≥ 80% of the enhancing newly diagnosed supratentorial glioblastoma were eligible for treatment. Autologous tumor-derived heat shock protein complexes were generated from fresh frozen tumor tissue and following induction RT/TMZ, the patients underwent 6 weekly intradermal vaccinations with 25 µg vaccine doses. Maintenance temozolomide was then continued and the study's primary endpoint was PFS at 6 months. Tumor-specific immune responses were evaluated by IFN-gamma ELISPOT assay	III	20 patients received the autologous heat shock protein peptide vaccination and the median overall survival was 31.4 months, median PFS 11 months, and PFS at 6 months of 89.5%. Patients with high tumor-specific immune response as measured by IFN-gamma ELISPOT experienced prolonged survival compared to those with low response. No grade 3 or 4 toxicity was attributed to the vaccine. The lack of a control group renders this class III data
Liau (2018)	Randomized, double-blinded, multi-center trial evaluating the addition of an autologous tumor lysate-pulsed dendritic cell vaccine (DCVax-L) to standard chemoradiation. Patients with newly diagnosed glioblastoma, KPS > 70, and completion of induction chemoradiation without imaging evidence of early progression were randomized (2:1) to receive the vaccine. All patients were allowed to receive DCVax-L following tumor progression/recurrence as well as other therapies per the treating physician recommendations. For the intention-to-treat (ITT) population, an interim analysis of survival is presented prior to unblinding	III	The authors present their interim results of the ITT population. While these interim results remain blinded and unable to compare outcomes between the groups initially randomized to receive initial DCVax-L + RT/TMZ vs RT/TMZ alone, due to the cross-over trial design approximately 90% of patients total received DCVax-L. The median OS was 23.1 months from the time of surgery
Wakabayashi (2018)	Randomized, open-label phase II trial evaluating the addition of Audencel, an autologous tumor lysate pulsed dendritic cell vaccine, to standard chemoradiation for newly diagnosed glioblastoma. 76 patients who had undergone surgical resection of ≥ 70% were randomized 1:1 to receive Audencel. The vaccine was adminstered by injection into an inguinal lymph node by ultrasound guidance	II	76 patients were randomized to receive Audencel vaccination + chemoradiation. Overall, the investigators observed no statistically significant difference in the study's primary endpoint of PFS at 12 months (28.4% vs 24.5%, respectively). No severe toxicity was attributed to the vaccine
Bloch (2017)	Prospective, single-arm, multi-center study evaluating the addition of heat shock protein complex-96 vaccine to standard chemoradiation. Patients with KPS > 70 who had undergone surgical resection > 90% of the enhancing newly diagnosed glioblastoma were eligible for treatment. Autologous tumor-derived heat shock protein complexes were generated from fresh frozen tumor tissue and following induction RT/TMZ, the patients underwent 4 weekly intradermal vaccinations with 25 µg vaccine doses. Maintenance temozolomide was then continued and the study's primary endpoint was overall survival. Expression of PD-L1 on peripheral blood leukocytes was assessed at the time of surgical resection	III	46 patients received the autologous heat shock protein peptide vaccination and the median overall survival was 23.8 months. High expression of PD-L1 on peripheral blood myeloid cells was associated with decreased overall survival. No grade 3 or 4 toxicity was attributed to the vaccine. The lack of a control group renders this class III data

Table 10 (continued)

Author/year	Study description	Data class	Conclusions
Inoges (2017)	Single-center, nonrandomized phase II trial investigating the addition of an autologous tumor lysate pulsed dendritic cell vaccine administered prior to standard chemoradiation. Eligible patients had undergone fluorescence-guided surgical resection with post-resection imaging demonstrating < 1 cc residual tumor volume. Initial intradermal vaccination was performed prior to chemoradiation with temozolomide and continued at maximum every 2 weeks until all available vaccine doses had been adminstered. Tumor-specific immune responses were assessed by T cell proliferation assay and IFN-gamma cytokine production	III	32 patients received DC vaccination following surgical resection and prior to standard chemoradiation. The median OS was 23.4 months and an increase in post-vaccination tumor-specific immune response was detected in 11/27 evaluated patients. No correlation between immune response and survival was observed. The lack of a control group renders this class III data
Kong (2017)	Randomized, nonblinded, multi-center trial evaluating the addition of cytokine-induced killer cell (CIK) adoptive immunotherapy to standard chemoradiation. 180 patients were randomized 1:1 to receive CIK + RT/TMZ vs RT/TMZ alone. CIK were generated from peripheral blood-derived mononuclear cells cultured with IL-2 and monoclonal antibody to CD3 for 12–21 days. The CIK group received intravenous administration of the vaccine on a schedule of at least weekly for a total of 14 vaccinations	II	A statistically significant increase in median PFS was observed between the CIK and control groups (8.1 months vs 5.8 months, respectively), although no overall increase in median survival was evidenced. No increase in treatment-related toxicity was observed in the CIK cohort. This study was nonblinded and lacked information on molecular biomarkers including MGMT and IDH-1 status that are heavily associated with outcome. In this relatively small RCT, the lack of inclusion of these markers may have resulted in inadequate randomization and insufficient sample size rendering this class II data
Ursu (2017)	Randomized, single-blinded (patients unaware of randomization cohort), multi-center trial investigating the addition of local intracerebral administration of immunostimulating oligodeoxynucleotides containing unmethylated cystosine-guanine motifs (CpG-ODN). Patients with imaging-presumed newly diagnosed glioblastoma with expectation of gross total or near gross total surgical resection were randomized. Following resection, 10 mg total CpG-ODN was infused locally to a depth of 1 cm (5–10 injection) using a 22G needle over 2 min. Following randomization, all patients were subsequently treated with standard chemoradiation and the primary study endpoint was 2-year OS	II	82 patients were randomized in 7 centers. No statistically significant increase in 2-year overall survival was observed between the groups receiving CpG-ODN + RT/TMZ vs RT/TMZ alone (31% vs 26%, respectively). No difference in median PFS between the groups was observed, either. Fever and postoperative hematoma were more frequent in the CpG-ODN cohort
Weller (2017)	Randomized, double-blinded, multi-center trial evaluating the addition of rindopepimut, a vaccine consisting of an EGFRvIII specific peptide conjugated to keyhole limpet haemocyanin. 745 patients with newly diagnosed, EGFRvIII-expressing glioblastoma were randomized 1:1 to receive either rindopepimut + temozolomide vs temozolomide alone (+keyhole limpet haemocyanin control)	I	745 patients were enrolled and randomized. The study was terminated at interim analysis for futility with no stastically significant difference in median OS between the rindopepimut and temozolomide cohorts (20.1 months vs 20.0 months, respectively). This well-designed randomized trial provided class I data showing a lack of efficacy of rindopepimut for newly diagnosed, EGFRvIII-expressing glioblastoma

Table 10 (continued)

Author/year	Study description	Data class	Conclusions
Schuster (2015)	An open-label, multicenter, phase II, single-arm of a unique 13 amino acid sequence created by the in-frame deletion of EGFRvIII, chemically conjugated to keyhole limpet hemocyanin (rindopepimut). Vaccinations consisted of 500 mg rindopepimut admixed with 150 mg granulocyte–macrophage colony stimulating factor in 0.8 mL volume, administered as 4–8 separate intradermal injections within an area of 3–5 cm in diameter in the groin. Study treatment began 14–20 days after completion of standard chemoradiation The primary objective of the study was to evaluate PFS status at 5.5 months from study day 0, which coincided with the third disease assessment. Secondary study objectives were to assess OS, safety, and immune responses to rindopepimut vaccinations	III	Progression-free survival at 5.5 months (about8.5 months from diagnosis) was 66%. Relative to study entry, median OS was 21.8 months, and 36-month OS was 26%. Extended rindopepimut vaccination (up to 3.5 + years) was well tolerated. Grades 1–2 injection site reactions were frequent. Anti-EGFRvIII antibody titers increased ≥ fourfold in 85% of patients, and increased with duration of treatment. EGFRvIII was eliminated in 4/6 (67%) tumor samples obtained after > 3 months of therapy The authors concluded this study confirms the preliminary survival, immunologic response and safety results seen in previous phase II trials of rindopepimut and that a phase III trial is indicated As there is no report of a concurrent or selective historical cohort treated without rindopepimut this represents class III data
Ishikawa (2014)	Phase I/IIa trial investigating the efficacy of an autologous formalin-fixed tumor vaccine (AFTV) in combination with RT (60 Gy) and TMZ. 24 patients with newly diagnosed GBM who underwent surgical resection were treated with the whole tumor cell lysate vaccine after completion of TMZ/RT. Three weekly inoculations of AFTV were administered 4 weeks after completion of RT, concomitant with initiation of adjuvant TMZ. Delayed-type hypersensitivity tests were performed 2 days prior to the first vaccination and 2 weeks after the 3rd vaccination. Patients receiving steroids were excluded from the study. MIB-1, p53, and MGMT status were also investigated	III	The authors concluded that the treatment regimen was well tolerated. The median OS was 22.2mo and PFS 8.2mo. Patients with a DTH response > 10 mm experienced great PFS but no statistically significant difference in OS was observed
Phuphanich (2013)	Open-label, single-arm Phase I study evaluating the immunogenicity of ICT-107, an autologous vaccine consisting of patient dendritic cells (DC) pulsed with six synthetic peptides from AIM-2, MAGE1, TRP-2, gp100, HER2/neu, and IL-13Ralpha2. Sixteen patients with newly diagnosed glioblastoma who had undergone GTR were enrolled in the study. Additional inclusion criteria included a Karnofsky score of at least 60% and daily corticosteroid requirement of 4 mg dexamethasone or less, and absence of any imaging signs of tumor recurrence after completion of concurrent TMZ/RT therapy. After completion of concurrent TMZ/RT, patients received intradermal vaccinations every 2 weeks for 3 consecutive doses	III	The authors concluded that multi-epitope DC vaccination is well tolerated with only grade 1 or 2 adverse events observed. All patient tumor samples expressed at least 3 of the antigents with 74% expressing all 6 antigens. Expression of AIM-2 and MAGE1 was significantly associated with increased PFS and OS. The overall PFS was 16.9mo and median OS 38.4mo
Chang (2011)	Single-center Phase I/II study investigating the safety and efficacy of whole tumor cell loaded dendritic cell (DC) vaccination in 17 patients with newly diagnosed malignant glioma (16 GBM, 1 AA). Patients who had underone maximal surgical resection with Karnofsky score > 70 and requiring ≤ 20 mg/d corticosteroids were eligible for inclusion. Following standard RT (60 Gy), vaccination was performed using monocyte-derived DC that were loaded with whole tumor cell lysate. The vaccination protocol involved 10 vaccinations (1–6 × 10^7DC) delivered over a 6-month period	III	The study was performed from 2003–2005 and the median OS was 525 days. The authors noted that compared to a historical control group from their institution, survival was increased from a median of 380 days with the largest increases in survival observed for patients with recurrent GBM rather than newly diagnosed GBM. No significant toxicity related to vaccination was noted, although 8/17 patients had transient serum ALT/AST elevations during the vaccination period. The authors conclude that autologous DC-based vaccinations increase survival, however, their small number of patients and use of historical controls renders this class III data

Table 10 (continued)

Author/year	Study description	Data class	Conclusions
Fadul (2011)	Single-center study evaluating safety and efficacy of intranodal dendritic cell (DC) vaccination in following TMZ/RT in 10 patients with newly diagnosed GBM. Following surgical resection, monocyte-derived DC were loaded with autologous whole cell tumor lysate. Standard TMZ/RT was prescribed and DC vaccination performed3-7 weeks after completion of RT. 1 × 10^7 DC were inoculated into the bilateral cervical lymph nodes every 2 weeks for a total of 3 vaccinations. Immune response was monitored via tumor-specific INF-gamma ELISPOT assay, DTH, a T-cell phenotypic determination using whole blood pre- and post-vaccination. Patients with gross total or near gross total resection, good Karnofsky performance status, and off corticosteroids were selected for evaluation in this study	III	The median OS was 28 months and PFS 9.1 months. No serious adverse events related to vaccination were observed. A non-significant trend toward increased proportion of IFN-gamma producing CD4 + and CD8 + T cells tumor-specific CD8 + T cells was observed. ELISPOT assays demonstrated an increase in tumor-specific IFN-gamma production in 4 patients. No DTH response was observed in any patient. Clustering of immunologic response parameters revealed that patients with the lowest combined response had shorter survival. The authors concluded that intranodal DC vaccination in combination with standard TMZ/RT is safe and capable of generating a tumor-specific immune response. This immune activation response is heterogeneous multiple immunologic parameters may be necessary for assessment of response. Selection bias and lack of control group renders this study class III data
Muragaki (2011)	Phase I/IIa trial investigating the efficacy of an autologous formalin-fixed tumor vaccine (AFTV) in combination with RT (60 Gy). 24 patients with newly diagnosed GBM who underwent surgical resection were treated with the whole tumor cell lysate vaccine with 3 weekly inoculations administered during the last weeks of RT. Delayed-type hypersensitivity tests were performed 2 days prior to the first vaccination and 2 weeks after the 3rd vaccination	III	The authors determined that AFTV can be safely administered with concomitant RT with no significant treatment-related adverse events observed. The median OS was 19.8mo and PFS 7.6mo. All patients underwent gross total or near gross total resection. A non-statistically significant correlation between the size of the DTH response after the 3rd vaccination and OS was noted
Prins (2010)	Single-institution Phase I/II study evaluating toxicity of autologous whole tumor cell lysate dendritic cell (DC) vaccination in combination with toll-like receptor (TLR) agonists in patients with newly diagnosed and recurrent GBM. A total of 23 patients, including 15 with newly diagnosed GBM, were enrolled. Inclusion criteria including surgical resection of tumor, no corticosteroid use within 10 days of vaccination, and Karnofsky score ≥ 60. Newly diagnosed GBM patients underwent surgical resection followed by standard TMZ/RT. They then received 3 biweekly DC vaccinations that had been loaded with autologous tumor lysate. Patients who did not develop any toxic effects from the vaccinations for more than 3 months were boosted with booster vaccinations at 3-month intervals supplemented with either 5% imiquimod cream (TLR-7 agonist) or poly-ICLC (TLR-3 agonist) given intramuscularly (20ug/ml). Immune monitoring was performed by assessing the proportion of CD4 + T cells and CD4 + /CD25 + T regulatory cells. Additionally, cytokine analysis was performed using Th1/Th2 Capture Beads. Gene expression profiling and immunohistochemistry were also performed	III	The median OS for newly diagnosed GBM patients was 31.4 months. No dose-limiting toxicity was observed with the combination of DC vaccination and TLR agonists. No change in the frequency of CD4 + or CD4 + /CD25 + cells was observed between the pre-and post-vaccination samples. Variable increases in TNFa and IL-6, however, the magnitude of increase did not correlate with clinical outcome. Increasing dosage of administered DC (1, 5, & 10 × 10^6) did not affect clinical outcome

Table 10 (continued)

Author/year	Study description	Data class	Conclusions
Sampson (2009)	This is a phase I study to evaluate the safety and immunogenicity of a dendritic cell (DC)–based vaccine targeting the EGFRvIII antigen. Adults with newly diagnosed GBM, who had undergone gross-total resection and standard conformal external beam radiotherapy, received three consecutive intradermal vaccinations with autologous mature DCs pulsed with an EGFRvIII-specific peptide conjugated to keyhole limpet hemocyanin. The dose of DCs was escalated in cohorts of three patients. Patients were monitored for toxicity, immune response, radiographic and clinical progression, and death	III	A total of 15 pts were enrolled in the study. No allergic reactions or serious adverse events were seen. Adverse events were limited to grade 2 toxicities. The maximum feasible dose of antigen-pulsed mature DCs was reached at 5.7×107 without dose-limiting toxicity. EGFRvIII specific immune responses were evident in most patients. The mean time from histologic diagnosis to vaccination was 3.6 months. Median time to progression from vaccination was 6.8 months, and median survival time from vaccination was 18.7 months. Overall median survival from time of histologic diagnosis was 22.8 months. The authors concluded EGFRvIII mutation is a safe and immunogenic tumor specific target for immunotherapy. This is a phase I study. There is no validation data set. It is class III data

Abbreviations: AA: anaplastic astrocytoma; AFTV: autologous formalin-fixed tumor vaccine; AIM-2: interferon-inducible protein AIM2 also known as absent in melanoma 2; ALT: alanine transaminase; AST: aspartate transaminase; CD4 +: cluster of differentiation 4 positive; CD8 +: cluster of differentiation 8 positive; CD25 +: cluster of differentiation 25 positive; DC: dendritic cells; DTH: delayed type hypersensitivity; EGFRvIII: epidermal growth factor variant III; ELISPOT: enzyme-linked immune spot assay: GBM: glioblastoma; gp100: glycoprotein 100; GTR: gross total resection; Gy: Gray; HER2/Neu: human epidermal growth factor receptor 2/proto-oncogene Neu; IL-6: Interleukin 6; IL-13Ralpha2: Interleukin-13 receptor subunit alpha-2; INF: interferon; MABE1: melanoma-associated antigen 1; MGMT: methylguanine methyltransferase; MIB-1: monoclonal antibody directed toward the Ki-67 protein; OS: overall survival; p53: tumor protein p53; PFS: progression free survival; poly-ICLC: polyinosinic-polycytidylic acid; RT: radiation therapy; Th1: t-helper cell type 1; Th2: t-helper cell type 2; TLR: toll-like receptor; TLR-3: toll-like receptor 3; TLR-4: toll-like receptor 4; TLR-7: toll-like receptor 7; TMZ: temozolomide; TNFa: tumor necrosis factor alpha; TRP-2: tyrosine-related protein 2

to patient selection, loss or lack of information collection, hidden agenda bias, and variability due to random error related to problems with unintentional data entry oversight and neglect, data interpretation, and choice to publish.

Results of individual studies

The key results of individual studies are outlined in evidence Table 11.

A variety of novel strategies have been investigated for the treatment of newly diagnosed glioblastoma.

Gene therapy

Three studies evaluated a gene therapy approach for the treatment of newly diagnosed glioblastoma [162, 234, 241]. Chiocca et al. performed a phase I trial of AdV-tk (adenoviral vector containing herpes simplex thymidine kinase gene) and prodrug (valcyclovir) in combination with radiation and temozolomide [241]. The authors reported no dose-limiting or AdV-tk specific toxicities. However, due to the phase I design, the study provided only class III evidence. Westphal et al. performed an international, open-label, randomized, phase III trial assessing the efficacy and safety of a locally applied adenovirus-mediated gene therapy with prodrug converting enzyme, followed by intravenous ganciclovir in patients with newly diagnosed glioblastoma [234]. Two-hundred and fifty patients were enrolled and the authors reported the median time to the primary study endpoint of death or re-intervention was longer in the experimental group. However, there was no difference in overall survival. Although this study was designed as a phase III randomized trial, the investigators were not blinded and a non-standard primary end point of composite time to death or re-intervention was assessed. This study design provides class II evidence. Adair et al. performed a phase I/II trial evaluating autologous gene-modified hematopoietic stem cell transplant in patients with overexpression of MGMT, allowing for improved tolerance to chemotherapy [162]. The authors reported a favorable OS as compared to historical controls. However, due to the phase I design and use of a historical control cohort, the study provided class III evidence.

Tumor-targeted antibody approach

Four studies utilized an antibody-based therapy approach consisting of a tumor-specific antibody coupled to a radioactive isotope. Three phase II studies were performed involving radioactive iodine coupled to antibodies against histone H1, tenascin, and EGFR. Patel et al. investigated convection-enhanced delivery of Cotara, a I^{131}-labeled Ab against histone H1 [239]. The agent was administered via stereotactic catheter implantation within the tumor at a constant

infusion rate to 51 patients with malignant gliomas including 8 patients with newly diagnosed glioblastoma. Treatment-related adversed neurologic events included brain edema in 20%, hemiparesis in 35%, seizures in 31%, and headache in 61% of patients. Only 1 partial treatment response was observed. Li et al. utilized an I^{125}-labelled anti-epidermal growth factor receptor murine monoclonal antibody in 192 patients with newly diagnosed glioblastoma following surgical resection and radiotherapy [245]. The overall median survival in this study was 15.7 months which the authors concluded was favorable to a historical control group of 81 patients. Reardon, et al. performed a phase I trial of a novel human IgG2b/Murine chimeric antitenascin monoclonal antibody construct radiolabeled with ^{131}I administered into the resection cavity of patients with malignant glioma [240]. The MTD was established at 2.96 GBq for all patients, and encouraging overall survival was observed. However, due to the single arm, non-randomized phase I design, this study provides only class III evidence.

Photodynamic therapy

Two studies evaluated photodynamic therapy (PDT) [237, 242]. Stylli, et al., reported on a retrospective series of patients treated with adjuvant PDT following surgical resection [237]. The median survival of GBM patients was 13.5 months. However, this is a retrospective study and represents class III data. Muragaki et al. performed a single arm, non-randomized phase II trial evaluating the efficacy of intraoperative PDT [242]. The authors reported that among the newly diagnosed glioblastoma patients included in the trial, the 12-month OS, 6-month PFS, and 6-month local PFS rates were all 100%. Although these results are encouraging, this study was performed as a single arm, non-randomized trial and yields only class III evidence.

Additional novel therapies

There are other studies exploring other unique novel therapies [238, 243, 244]. Brem et al. performed a phase II multi-center, single arm, non-randomized trial of copper depletion in combination with radiation in patients with newly diagnosed glioblastoma [238]. The median OS was 11.3 months and PFS was 7.1 months. The authors concluded that this approach does not increase survival or PFS for patients with newly diagnosed glioblastoma. The study provides class III evidence due to its single arm, non-randomized phase II design. Rosenfeld et al. performed a phase I/II trial of hydroxychloroquine in combination with radiation and temozolomide in patients with newly diagnosed glioblastoma [243]. The authors conclude that the MTD for hydroxychloroquine in combination with TMZ/RT was 600 mg daily. The median OS was 15.6 months which the authors reported provided no increased survival compared to historical controls. The single arm, non-randomized design provided class III evidence.

One particularly noteworthy approach is the investigation of tumor-treating fields. The preliminary and final reports on a phase III randomized trial by Stupp et al., on the addition of tumor-treating fields to temozolomide demonstrated significant prolongation of PFS and OS compared to the matched temozolomide alone control group [235, 236]. These studies provide evidence that incorporation of tumor-treating fields into conventional chemoradiation treatment may be of value for patients with newly diagnosed glioblastoma. Significant attention is being paid to the evolution of this therapy for newly diagnosed glioblastoma in view of concerns over it's FDA approval based on a negative trial, lack of understanding of it's mechanism in complex tissue such as the central nervous system, lack of a true sham controls in any of the clinical studies, testing in a study design that allowed therapy after the primary endpoint and that favored patients with more indolent course as randomization occurred an average of almost four months after diagnosis, and the interim nature of the analysis available thus far [246]. Given these concerns regarding the RCT, a Level II recommendation is provided that the use of tumor-treating fields should be considered for patients with newly diagnosed glioblastoma who have undergone surgical debulking and completed concurrent chemoradiation without progression of disease at the time of device initiation.

Synthesis of results

A variety of novel therapeutic approaches have been evaluated in the treatment of newly diagnosed glioblastoma. Gene therapy represents a potentially attractive approach and may be used to improve tolerance to chemotherapy or render cytotoxic effect with the presence of prodrug [162, 234, 241]. Most of the studies were early phase I and phase I/II trials and provide only class III evidence [234, 241]. Westphal et al. performed a phase III randomized trial evaluating the efficacy of adenovirus-mediated gene therapy of a prodrug converting enzyme [234]. Unfortunately, the open label study design and incorporation of a non-conventional endpoint yields only class II evidence. Furthermore, analysis of median OS, a more conventional endpoint, demonstrated not statistically significant difference compared to the control group.

The additional studies evaluating novel therapies for newly diagnosed glioblastoma also provide only class III level evidence due to their early stages of development. The majority of these studies were single arm phase I or phase I/II studies [237–240, 242–244]. These approaches included PDT, ^{131}I-labeled antibody, hydroxychloroquine, and copper depletion. Unfortunately, the outcomes of these approaches

Table 11 Evidence for novel therapies

Author/year	Study Description	Data Class	Conclusion
Huang (2018)	Prospective, single-center phase I trial evaluating addition of disulfiram ± copper to standard chemoradiation. Following induction RT/TMZ, 7 patients received 500 mg disulfiram, 5 patients received 1000 mg disulfiram, and 6 patients received 500 mg disulfiram + copper (2 mg three times per day) in addition to maintenance temozolomide. Proteasome inhibition as a marker of disulfiram effect was evaluated using fluorometric 20S proteasome assay on peripheral blood	III	18 patients were treated with disulfiram ± copper. The maximally tolerated dose of disulfiram in combination with maintenance TMZ was determined to be 500 mg daily. Addition of copper did not increase proteasome activity and the overall median survival was 14.0 months which was felt to be similar to historical controls
Stupp (2017)	Prospective, randomized, non-blinded, multi-center phase 3 trial investigating the addition of maintenance therapy with tumor-treating fields (TTFields) to standard chemoradiation. Patients were randomized 2:1 following completion of concurrent chemoradiation to receive either TTFields + temozolomide or temozolomide alone. The primary study endpoint was PFS. Eligible patients had completed concurrent chemoradiation with temozolomide and were progression-free at the time of TTFields initiation. Treatment with TTFields was initiated within 4–7 weeks of last dose of concurrent TMZ and radiotherapy. Randomization was stratified based on extent of resection and MGMT methylation status. Carmustine wafer implantation was allowed at the time of surgical resection. Patients treated with TTFields had 4 transducer arrays placed on shaved scalp with delivery of 200-kHz alternating electric fields. Transducer arrays were replaced by the patient, a caregiver, or a device technician twice per week. Continued treatment with TTFields was permitted beyond the primary study endpoint of PFS	II	The final analysis included 695 total patients (466 in the TTFields + TMZ cohort, 229 in the TMZ alone cohort). Median PFS was signficantly improved in the intent-to-treat population for the TTFields + TMZ cohort compared to TMZ alone (6.7 months vs 4.0 months, respectively) with a hazard ratio of 0.63 ($P < 0.001$). Median OS was also signficantly extended in the per-protocol population (20.9 months vs 16.0 months). The addition of TTFields to temozolomide was not associated with any increase in systemic toxicity. Mild to moderate localized skin toxicity was observed in 52% of patients treated with TTFields. Concerns regarding indequate blinding with the lack of a sham control and a study design protocol that enabled continued therapy with TTFields beyond the primary endpoint renders this RCT class II data
Stupp (2015)	Prospective, randomized, non-blinded, multi-center phase 3 trial investigating the addition of maintenance therapy with tumor-treating fields (TTFields) to standard chemoradiation. Patients were randomized 2:1 following completion of concurrent chemoradiation to receive either TTFields + temozolomide or temozolomide alone. The primary study endpoint was PFS and the study was terminated following enrollment of 695 or the planned 700 patients based on positive results at the pre-planned interim analysis. Eligible patients had completed concurrent chemoradiation with temozolomide and were progression-free at the time of TTFields initiation. Treatment with TTFields was initiated within 4–7 weeks of last dose of concurrent TMZ and radiotherapy. Randomization was stratified based on extent of resection and MGMT methylation status. Carmustine wafer implantation was allowed at the time of surgical resection. Patients treated with TTFields had 4 transducer arrays placed on shaved scalp with delivery of 200-kHz alternating electric fields. Transducer arrays were replaced by the patient, a caregiver, or a device technician twice per week. Continued treatment with TTFields was permitted beyond the primary study endpoint of PFS	II	The interim analysis included 315 total patients (210 in the TTFields + TMZ cohort, 105 in the TMZ alone cohort) and was prematurely terminated for success at the pre-specified interim analysis timepoint. Median PFS was signficantly improved in the intent-to-treat population for the TTFields + TMZ cohort compared to TMZ alone (7.1 months vs 4.0 months, respectively) with a hazard ratio of 0.62 ($P = 0.001$). Median OS was also signficantly extended in the per-protocol population (20.5 monts vs 15.4 months). The addition of TTFields to temozolomide was not associated with any increase in systemic toxicity. Mild to moderate localized skin toxicity was observed in 43% of patients treated with TTFields, with severe skin reactions (grade 3 toxicity) observed in 2%. Concerns regarding indequate blinding with the lack of a sham control, a study design protocol that enabled continued therapy with TTFields beyond the primary endpoint, and the interim nature of the analysis renders this RCT class II data

Table 11 (continued)

Author/year	Study Description	Data Class	Conclusion
Adair (2014)	This is phase I/II trial. 7 patients with newly diagnosed GBM with overexpression of MGMT received autologous gene-modified hematopoietic stem cells (HSCs) that conferred resistance to O6-benzylguanine (O6BG) prior to chemotherapy treatment with O6BG + TMZ in 28-day cycles. The extent of myelosuppression and PFS and OS were determined	III	Autologous transplantation of gene therapy-modified HSC allowed for improved tolerance of 06BG/TMZ chemotherapy with a mean number of tolerated cycles of 4.4 which is higher than historical controls of 1.7 cycles/patient. A median PFS of 9 months and OS of 20 months was observed. The authors concluded that chemoprotective gene therapy allows for improved tolerance to O6BG/TMZ chemotherapy. The use of historical controls for outcome comparison, and single arm phase I/II design renders this study class III data
Rosenfeld (2014)	Phase I/II multi-center study investigating the addition of hydroxychloroquine (proposed mechanism: autophagy inhibition) to standard TMZ/RT. Escalating dosages of hydroxychloroquine (HCQ) were adminstered (200-800 mg oral daily) during concurrent TMZ/RT and adjuvant TMZ in 16 patients with newly diagnosed GBM followed by Phase II trial initiation using HCG at a dose of 600 mg. Quantitative electron microscopy and immunoblotting were used to assess changes in autophagic vacuoles (AVs) in peripheral blood mononuclear cells	III	At a dose of 800 mg HCQ/d, 3 out of 3 patients experienced dose limiting toxicity in the form of neutropenia and thrombocytopenia. 76 patients were then treated using the MTD of HCG at 600 mg/day. The median OS was 15.6 months. A statistically significant increase in AVs was noted with HCQ therapy and correlated with higher HCQ exposure, however, no significant increase in survival was observed in patients with increased AVs. The authors concluded that no improvement in survival is achieved with addition of HCQ at the maximally tolerated dose of 600 mg/d in combination with standard TMZ/RT compared to standard treatment alone (based on historical controls from EORTC Phase III trial) The use of historical controls for outcome comparison, and single arm phase I/II design renders this study class III data
Westphal (2013)	This is an international, open label, randomized, parallel multicenter phase III trial assessing the efficacy and safety of a locally applied adenovirus-mediated gene therapy with a prodrug converting enzyme (herpes-simplex-virus thymidine kinase; sitimagene ceradenovec) followed by intravenous ganciclovir in patients with newly diagnosed resectable glioblastoma. 250 pts were recruited. 124 patients were randomized to experimental group, and 126 pts into control group. In the experimental group, pts had surgical resection of the tumour and intraoperative perilesional injection of sitimagene ceradenovec (1×10^{12} viral particles) followed by ganciclovir (postoperatively, 5 mg/kg intravenously twice a day) in addition to standard care or resection and standard care alone. The primary endpoint was a composite of time to death or re-intervention, adjusted for temozolomide use, assessed by intention-to-treat (ITT) analysis	II	Median time to death or re-intervention was longer in the experimental group (308 days, 95% CI 283–373) than in the control group (268 days, 210–313; hazard ratio [HR] 1·53, 95% CI 1·13–2·07; p = 0·006). In a subgroup of patients with non-methylated MGMT, the HR was 1·72 (95% CI 1·15–2·56; p = 0·008). However, there was no difference between groups in terms of overall survival (median 497 days, 95% CI 369–574 for the experimental group vs 452 days, 95% CI 437–558 for the control group; HR 1·18, 95% CI 0·86–1·61, p = 0·31). More patients in the experimental group had one or more treatment-related adverse events those in the control group (88 [71%] vs 51 [43%]). Despite it is a phase III randomized trial, it is not blinded, and using non-standard primary end point of composite time to death or re-intervention. Thus this is class II data

Table 11 (continued)

Author/year	Study Description	Data Class	Conclusion
Muragaki (2013)	This is a single arm, non-randomized, phase II trial to perform a prospective evaluation of the potential efficacy and safety of intraoperative photodynamic therapy (PDT) using talaporfin sodium and irradiation using a 664-nm semiconductor laser in patients with primary malignant parenchymal brain tumors. 27 pts with newly diagnosed and recurrent malignant brain tumors enrolled in the trial. All patients received a single intravenous injection of talaporfin sodium (40 mg/m2) administered 1 day before resection of the neoplasm. The next day after completion of the tumor removal, the residual lesion and/or resection cavity were irradiated using a 664-nm semiconductor laser with a radiation power density of 150 mW/cm2 and a radiation energy density of 27 J/cm2. The procedure was performed 22–27 h after drug administration. The study cohort included 22 patients with primary malignant parenchymal brain tumor. Thirteen of these neoplasms (59.1%) were newly diagnosed GBM. Separate analysis of the treatment efficacy was also done in the subgroup of patients with newly diagnosed GBMs. Survival was assessed using the Kaplan–Meier method. Analysis of the treatment safety was done in all patients initially enrolled into the study who received talaporfin sodium	III	Among all 22 patients included in the study cohort, the 12-month OS, 6-month PFS, and 6-month local PFS rates after surgery and PDT were 95.5%, 91%, and 91%, respectively. Among patients with newly diagnosed GBMs, all these parameters were 100%. Side effects on the skin, which could be attributable to the administration of talaporfin sodium, were noted in 7.4% of patients and included rash (2 cases), blister (1 case), and erythema (1 case). Skin photosensitivity test results were relatively mild and fully disappeared within 15 days after administration of photosensitizer in all patients. The authors concluded intraoperative PDT using talaporfin sodium and a semiconductor laser may be considered as a potentially effective and sufficiently safe option for adjuvant management of primary malignant parenchymal brain tumors. The inclusion of intraoperative PDT in a combined treatment strategy may have a positive impact on OS and local tumor control, particularly in patients with newly diagnosed GBMs. This is a single arm, non-randomized trial. Therefore it is class III data
Chiocca (2011)	Phase I study evaluating the addition of AdV-tk (adenoviral vector containing herpes simplex thymidine kinase gene) + prodrug (valcyclovir) to RT and TMZ. 12 patients completed therapy consisting of tumor bed injection of AdV-tk at the time of initial surgical resection followed by 14 days of valcyclovir. RT was initiated 9 days after viral injection and TMZ started after completion of valcyclovir treatment. 3 viral dose cohorts were evaluated: 3×10^10, 1×10^11, 3×10^11. Quality of life (QOL) was determined using the Functional Assessment of Cancer Therapy-Brain (FACT-Br)	III	13 patients were enrolled with 12 completing treatment (3 at dose levels I and II, 6 at dose level III). No dose limiting or significant AdV-tk specific toxicities were observed, although one patient developed fever and confusion 1 week after surgery thought related to the viral agent. The median OS was 12.4mo and PFS 9.1mo. QOL was stable or improved in all patients. Four tumors underwent histologic evaluation after treatment with all 4 showing a significant T-cell infiltrate. It is a phase I, single arm trial, it provides class III evidence
Li (2010)	A single-institution Phase II study tests the efficacy of adjuvant radioimmunotherapy with ^{125}I-labeled anti–epidermal growth factor receptor 425 murine monoclonal antibody (125I-mAb 425) in patients with newly diagnosed glioblastoma multiforme. 192 patients with GBM were treated with ^{125}I-mAb 425 over a course of 3 weekly intravenous injections of 1.8 GBq following surgery and radiation therapy. 60 individuals also received temozolomide		The overall median survival was 15.7 months (95% CI 13.6–17.8 months). The 1- and 2-year survivals were 62.5 and 25.5%, respectively. No Grade 3 or 4 toxicity was seen with the administration of ^{125}I-mAb 425 The investigators used and 81 patient historical control group. Additionally nearly one-third of cases received temozolomide, leading to the need for subgroup analysis and adding bias to the treatment choices. These factors account for this study yielding class III data
Reardon (2006)	This is a phase I trial to evaluate the effect of these differences on the maximum tolerated dose (MTD), pharmacokinetics, dosimetry, and antitumor activity of 131I-ch81C6 administered into the surgically created resection cavity (SCRC) of malignant glioma patients. A total of 47 pts (35 with newly diagnosed and 12 with recurrent GBM) were included in trial. A single injection of 131I-ch81C6 was administrated. Pts were stratified into untreated, after EBRT, or recurrent. Dose escalation was performed independently in each stratum	III	MTD is 2.96 GBq (80 mCi) for all patients, regardless of treatment strata. Neurologic dose-limiting toxicity developed in 3 patients; however, none required further surgery to debulk radiation necrosis. Median survival was 88.6 weeks and 65.0 weeks for newly diagnosed and recurrent patients, respectively. The OS was compared to historical result. This is single arm, non-randomized, phase I trial. Thus this study represents class III data

Table 11 (continued)

Author/year	Study Description	Data Class	Conclusion
Patel (2005)	Phase II multi-institutional study investigating the safety of convection-enhanced delivery of Cotara, a 131I-labelled antibody against histone H1. 51 patients with malignant gliomas, including 8 patients with newly diagnosed glioblastoma,were included in the study. One or two catheters were stereotactically placed into the enhancing tumor volume and Cotara was in infused using a motorized microinfusion pump delivering the agent at a constant rate of 0.18 ml/hr. Single-photon emission computed tomography (SPECT) was performed 14 days after Cotara infusion to quantify distribution of the agent	III	51 patients underwent treatment and a total of 73 catheters were placed. Three patients required redirection of the catheters for proper placement and technical complications related to the catheters were experienced in an additional 3 patients. The treatment infusion volume ranged from 4.5—18 ml. The prescribed activity escalated from 20 to 96 mCi with 78% of the patients receiving 100% of the presribed administered activity to the targeted region. Treatment related adverse neurologic events included brain edema in 20% of patients, hemiparesis in 35%, seizures in 31%, and headache in 61% of patients. Of the 11 patients reported with a evaluable radiographic response, 1 had a partial response. No data on survival for the newly diagnosed GBM cohort was provided It is single arm, non-randomized, non-blinded trial. It provides class III evidence
Brem (2005)	Phase II multi-center trial investigating the effects of copper depletion in combination with RT (60 Gy) in 40 patients with newly diagnosed GBM. The authors posited that copper functions as an angiogenesis regulator and that copper depletion achieved through a combination of a low-copper diet and penicillamine. Baseline serum and urine copper levels were determined, a penicillamine was initiated on the start date of RT beginning at a dose of 250 mg/day and escalating over 5 weeks to a final dose of 2 g. A diet with a limit of 0.5 mg of copper/day was also initiated. Median OS and PFS were determined and compared against historical controls from the NABTT database	III	The median OS was 11.3 months and PFS 7.1 months. Serum copper levels decreased from a median of 130ug/dl (range 50–227 ug/dl) at baseline to 42 ug/dl (range 12–118 ug/dl) after 2 months. Short-term hypocupremia was well tolerated with no grade 3 or 4 anemia, sepsis, or osteoporosis noted. The authors concluded that copper depletion in combination with RT does not increase survival or PFS for newly diagnosed GBM patients The use of historical controls for outcome comparison, and single arm phase II design renders this study class III data
Stylli (2005)	This is a retrospective analysis of the survival of patients at the Royal Melbourne Hospital with residences in the State of Victoria, utilizing the Victorian Cancer Registry database for patients treated with adjuvant PDT following surgical resection of the tumor. Primary and recurrent groups were analyzed separately when calculating Kaplan Meier (KM) survival estimates. The effect of tumour grade was assessed by the Wilcoxon (Breslow) test for equality of survivor functions. Other prognostic variables were tested using a Cox proportional hazard model which included both primary and recurrent tumour patients, and incorporated primary/recurrent and tumour grade (AA/GBM) as stratification variables	III	For newly diagnosed tumors, median survival from initial diagnosis was 76.5 months for anaplastic astrocytoma (AA) and 14.3 months for GBM. 73% of patients with AA and 25% with GBM survived longer than 36 months. For recurrent tumor, median survival from the time of surgery was 66.6 months for AA and 13.5 months for GBM. Fifty-seven percent of patients with recurrent AA and 41% of patients with recurrent GBM survived longer than 36 months. Older age at the time of diagnosis was associated with poorer prognosis. Laser light doses above the sample median of 230 J/cm2 were associated with better prognosis in the 136 patients studied; recurrent tumor patients. There was no mortality directly associated with the therapy, three patients had increased cerebral edema thought to be related to photodynamic therapy that was controlled with conventional therapies. This is retrospective study and represents class III data

ADV-tk adenoviral vector containing herpes simplex thymidine kinase gene; *AV* autophagic vacuoles; *CI* confidence interval; *EORTC* European Organisation for Research and Treatment of Cancer; *FACT-Br* Functional Assessment of Cancer Therapy-Brain; *GBM* glioblastoma; *GBq* gigabecquerel; *HCQ* hydroxychloroquine; *HSC* hematopoietic stem cell; *HR* hazard ratio; *ITT* intention to treat; *mCI* millicurie; *KM* Kaplan–Meier; *MGMT* methylguanine methyl transferase; *MTD* maximum tolerated dose; *NABTT* New Approaches to Brain Tumor Therapy; *O6BG* O6-benzylguanine; *OS* overall survival; *PDT* photodynamic therapy; *PFS* progression free survival; *QOL* quality of life; *SPECT* Single-photon emission computed tomography; *TMZ* temozolomide

were similar to historical control and without clear signal of efficacy. Though studies of some novel therapies have not been fruitful, enrollment of patients with newly diagnosed glioblastomas in studies of novel therapies, when available, should be considered to assist in moving treatment of this difficult disease forward.

Overall conclusions

As was implied in the introduction, one would not expect a review of emerging therapies to provide high level recommendations that would be paradigm changing. The most common recommendation resulting from review of each of the topics was that patients be enrolled in properly designed clinical studies within those areas of interest. However, some topics have been the subject of considerable effort yielding higher level recommendations. Use of fluorescent guidance appears to provide a useful adjunct to surgery as measured by improved extent of resection and survival and warranted level 2 recommendations. Dissemination and use of various measurable quantities within tumor specimens has increased. Some have proven more to be of more value than others resulting in recommendation of use *MGMT* promoter methylation to predict progression free survival and overall survival and neuron-glia-2, neurofilament protein, and glutamine synthetaseto predict overall survival. Adjuncts to planning radiation therapy treatments are continually being sought. The literature suggests that pretreatment positron emission tomography with labeled amino acids may identify regions at the most risk of for tumor recurrence. This information allows the radiation oncologist to create a treatment plan that guarantees those regions are covered with proper doses of radiation. There is no shortage of studies underway looking at cytotoxic agents, molecular and targeted agents and immunologically. No specific recommendations could be arrived at for these treatments for the purposes of this guideline and therefore enrollment in properly designed clinical trials remains important. The steady improvement in tumor therapies based on vector containing herpes simplex thymidine kinase gene and prodrugs is very promising and continued enrollment in these studies are important to defining its role in newly diagnosed glioblastomas.

Key issues for future investigation

Extraction of prognostic data from imaging information has been a fairly natural extenstion of PET and MRI modalities. Truly diagnostic imaging, correlating with histology and grade has been more difficult to accomplish. Further refinement of magnetic resonance spectroscopic imaging has the promise to do this with improving resolution and metabolite analyses only limited by the investigators imagination [247].

Surgical adjuncts such as fluorescence are recognized as useful. Execution of truly randomized prospective studies of this modality are limited by a number of study design challenges. However completion of additional studies, especially with 5-ALA, in a manner that addresses the ongoing concerns of the Food and Drug Administration that have precluded approval are important to complete.

Though the status of *IDH-1* mutation in determining prognosis is reasonably well established, other less studied molecular markers, such as neuron-glia-2, neurofilament protein, glutamine synthetase and phosphorylated STAT3, offer promise based on well done studies and warrant confirmatory studies. There is no reason to believe we have come to the end of the potential catalog of prognostic markers and patients should be encouraged to participate in studies of new and hopefully better markers.

Validation of radiation techniques that alter fraction and dose and use local delivery of radiation sources as mentioned above through randomized phase II/III trials is warranted for their future development. Until that is done and reproduced across more than a single institution widespread adoption will not occur.

Though cytotoxic therapy in the form of temozolomide remains a mainstay in the management of glioblastoma, new agents, alternative administration regimens, sensitizing agents and promising combinations have not been published in a manner that allows inclusion in this guideline. However, the role of standard chemotherapeutic agents combined with targeted and novel agents is likely to remain an important one and maximization of their efficacy and safety in these regimens should be explored.

With faster, less expensive and more comprehensive genetic and expression data available on glioblastomas allowing better understanding of the underpinnings of their origin and subsequent proliferation molecular and targeted therapies are in the forefront of glioblastoma therapy. By profiling each tumor, the catalog of abnormalities in a given tumor can be identified and it becomes the challenge of the oncologic research community to find agents to alter those abnormalities in manner that can stop tumor growth and infiltration without substantial systemic toxicity. To state exactly what pathways or mechanisms of tumor growth would be most important addressed first would seem short sighted. Rather there should be encouragement of legitimate progress by scientists in any area that can be translated to patient therapy in the near or long term.

As noted above, studies of therapy based on vaccination with dendritic cells sensitized with tumor cell lysates or standardized tumor-associated peptides or even whole tumor-based strategies are becoming more commonly available for enrollment of newly diagnosed glioblastoma

patients. Almost all of these strategies seem to be developed in a manner that appears positive in highly selected patient population and combined with standard therapies. Proof of independent value of vaccine strategies in the broader patient population of glioblastomas remains to be accomplished and warrants proper funding and dedication of the neuro-oncology community to enrolling patient in studies that will accomplish those ends. Progress on non-vaccine based immunologic therapy such immune checkpoint inhibitors is clearly accelerating and identification of patients with new glioblastomas that may be candidates for these promising agents will be important [248].

Repeated early phase study success with adenovirus-mediated gene therapy followed by prodrug therapy has been reported as noted above. These studies are challenging and further development hinges on making them available to more patients and centers so as to show the value of this concept in the broader population of newly diagnosed glioblastoma.

There is a growing literature on the use of laser interstitial thermal therapy for a variety of central nervous system pathologies [249, 250]. Because of it's focused, accurate and immediate nature, it appears to be suited to the management of intracranial metastatic disease. It is being adopted at selected centers and ideally will be incorporated into properly designed studies comparing it to standard craniotomy for resection and stereotactic radiosurgery to determine if this technology has a real place in the management of metastatic brain tumors.

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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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Compliance with ethical standards

Conflict of interest The Newly Diagnosed Glioblastoma Guidelines Task Force members were required to report all possible COIs prior to beginning work on the guideline, using the COI disclosure form of the AANS/CNS Joint Guidelines Committee, including potential COIs that are unrelated to the topic of the guideline. The CNS Guidelines Committee and Guideline Task Force Chair reviewed the disclosures and either approved or disapproved the nomination. The CNS Guidelines Committee and Guideline Task Force Chair are given latitude to approve nominations of Task Force Members with possible conflicts and address this by restricting the writing and reviewing privileges of that person to topics unrelated to the possible COIs. Christopher Farrell: None. Wenyin Shi: Research Funding: Regeneron, Novocure, Brainlab Consulting: Novocure, Brainlab, Varian. Alexa Bodman: None. Jeffrey J. Olson:American Cancer Society, Editorial Consultant.

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