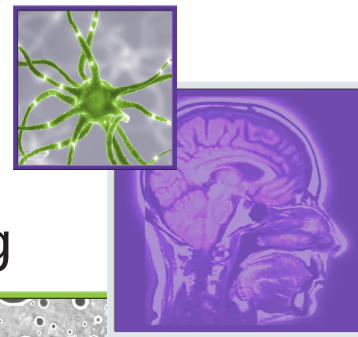
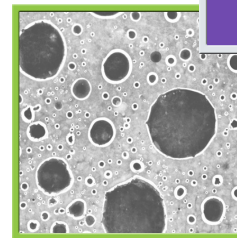


REVIEW

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Emerging methods for disease monitoring in malignant gliomas



Prakash Ambady^{1,2}, Chetan Bettegowda^{1,3} & Matthias Holdhoff^{*1,2}

Practice Points

- Contrast-enhanced MRI is suboptimal for assessment of disease burden and response to therapy in malignant gliomas as it cannot reliably identify tumor burden and differentiate between active tumor and treatment effect.
- Further developments of MRI (diffusion-tensor imaging, diffusion-weighted images, perfusion and magnetic resonance spectroscopy) appear promising; however, they need to be evaluated in larger prospective studies before their actual clinical value will be known.
- Blood-based biomarkers are being investigated as potential adjunct tests in clinical decision-making. The research to date is still at an early stage and a promising blood-based biomarker has yet to be identified and prospectively validated.
- The development of better minimally or non-invasive techniques to assess disease status and response to therapy should be one of the highest priorities in neuro-oncology as it would accelerate clinical development of new treatments that are urgently required for these devastating malignancies.

SUMMARY MRI remains the backbone of measuring disease burden and treatment response in individuals with malignant gliomas. Traditional radiographic approaches, however, are largely limited to depicting anatomic changes and are not a direct measure of disease burden. For example, contrast enhancement is related to blood–brain barrier integrity rather than actual tumor size. Without accurate measures of disease, common clinical dilemmas include ‘pseudo-progression’ (e.g., after chemoradiation) or ‘pseudo-response’ (e.g., with steroid treatment and antiangiogenic agents), which can lead to delays in therapy, premature discontinuation of successful treatments and to unnecessary surgical procedures. This overview focuses on novel, minimally invasive approaches in the area of imaging and blood-based biomarkers that aim to more accurately determine disease status and response to treatment in malignant brain tumors.

Assessment of disease burden and response to therapy is particularly challenging in patients with malignant gliomas owing to the infiltrative

growth and complex anatomy of these cancers. Currently, the most commonly used technique for detecting these tumors has remained

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contrast-enhanced MRI. In individuals unable to obtain MRI scans, contrast-enhanced computed tomography is often used. Traditional contrast-based imaging, however, is dependent on changes in blood–brain barrier (BBB) integrity that is frequently altered with the multimodality treatment that patients with these cancers commonly receive. Treatment-related changes often cannot be reliably differentiated from actual tumor growth, leading to the well-described phenomena of ‘pseudo-progression’ (e.g., after chemoradiation) and ‘pseudo-response’ (e.g., with steroids and bevacizumab) [1]. It has been described that pseudo-progression is more commonly found in glioblastomas with methylated than with unmethylated *MGMT* promoters [2]. Nevertheless, it frequently requires repeat surgery to provide clarity on disease status and to differentiate treatment effect from active disease, and pathology has remained the gold standard for assessment of disease activity. The need for improved methods to measure disease and assess response to treatment has been highlighted by the development of brain cancer-specific response criteria that integrate neurological status, steroid dose and noncontrast-enhancing disease in the assessment [3,4]. These criteria, although an improvement, highlight the need for the development of further minimally invasive or non-invasive approaches to accurately determine disease status and tumor burden. This review provides an overview of several emerging concepts that are aimed at more accurate measurement of disease and that, if validated, could help streamline treatment decisions and avoid unnecessary repeat resections in patients. This overview is focused on new approaches in imaging, the current status in the search for a blood-based biomarker for malignant gliomas and imaging techniques that can detect disease-specific molecular alterations within these cancers.

Imaging biomarkers in gliomas

■ Gadolinium-enhanced MRI: the current state of the art of response assessment in malignant gliomas

Gadolinium-enhanced MRI is the current gold standard for diagnosis and monitoring of high-grade gliomas. Enhancement is specific to disruption in the BBB but not to active tumor. Contrast agents that extravasate into the brain tissue alter T_1 , T_2 and/or T_2^* relaxation rates of tissues that can be detected by MRI imaging as contrast enhancement [5]. Contrast MRI is

unable to differentiate active tumor from tumor necrosis or so-called treatment effect after radiation. Abnormal contrast enhancement is seen in up to 40% of the first scans after radiation therapy, and it is believed to be due to increased vessel permeability, a phenomenon known as pseudo-progression [1]. Alterations to the BBB due to the effects of steroids and antiangiogenic drugs (i.e., bevacizumab) can further complicate the picture. In order to bring uniformity to radiographic response assessment in these tumors, brain cancer-specific response criteria have been developed. The Macdonald criteria integrated clinical status as well as steroid dose as part of the assessment (Table 1) [3]. Limitations of these criteria included interobserver variability, inability to measure nonenhancing tumors and problems with measuring enhancing lesions in the walls of cysts and surgical cavities. This led to the development of the Revised Assessment in Neuro-oncology criteria that incorporate the nonenhancing T_2 W/fluid-attenuated inversion recovery portions of the MRI in order to overcome some of these drawbacks (Table 2) [4]. The utility of changes in areas of T_2 has been shown to be a potentially sensitive marker of tumor progression in patients treated with bevacizumab in whom measurement of enhancement is not reliable [6]. These criteria have not yet been prospectively validated.

Novel techniques in MRI

Contrast-enhanced MRI can detect changes in BBB integrity; however, the etiology of the changes often remains unclear. Several novel MRI-based approaches are being designed to add new dimensions to existing imaging protocols. These advances may provide more information on the underlying disease process itself. Some of these techniques are already clinically available and can be ordered as additional sequences to standard brain MRI. However, these approaches have not been formally evaluated in larger prospective studies. The following discussion provides an overview of some of the novel imaging-based methods for monitoring malignant gliomas and the current evidence for their application in clinical practice (Table 3).

■ Diffusion-weighted images

The apparent diffusion coefficient (ADC) is the rate of water movement measured in mm^2/s and is visualized using the diffusion-weighted image sequences with each voxel representing the rate

Table 1. Criteria for response assessment in malignant gliomas: Macdonald criteria.

Response	Criteria
Complete response	Complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; no corticosteroids; and stable or clinically improved
Partial response	50% decrease in enhancing lesion volume compared with baseline, sustained for at least 4 weeks; no new lesions with stable or reduced corticosteroid dose; and stable or clinical improvement
Stable disease	Does not qualify for complete response, partial response or progression; and clinically stable
Progression	25% increase in enhancing lesion volume or any new lesion or clinical deterioration

Data taken from [3].

of Brownian motion of water molecules or their diffusion rate in tissue. Increased cellularity is often associated with reduced ADC values. Although ADC values could not reliably predict tumor types in individual cases, lower ADCs suggest a higher grade [7]. Higher ADC values were also reported in 25 patients with *MGMT* promoter methylation and a linear correlation was demonstrated with progression-free and overall survival [8]. Small studies have suggested ADC values to be helpful in differentiating pseudo-progression from true progression [9–11], but this needs confirmation in larger prospective trials.

■ Diffusion-tensor imaging

Diffusion-tensor imaging (DTI) is a refined version of diffusion-weighted imaging that visualizes white matter tracts (trachography) utilizing the magnitude and directionality of water diffusion along a vector (eigenvectors) in 3D space. It can improve target volume delineation for image-guided biopsies and help predict the extent of resection, which in turn, can have prognostic value [12,13]. Fractional anisotropy values measured using DTI were shown to be predictors of cellularity in astrocytic tumors, such as glioblastoma, anaplastic astrocytoma and pilocytic astrocytoma [14]. With the incorporation of DTI into intraoperative neuronavigational

systems, some groups have demonstrated its utility during tumor resections in eloquent areas. DTI has been used for preoperatively identifying tract projections and association fibers, such as those associated with vision and motor function. A recent study suggested that differences in brain stem fiber tract response to radiation can be detected with DTI and may be a helpful tool in radiation planning [15]. Previous studies suggested that DTI may play a role in the differentiation of pseudo-progression and true tumor growth based on the assumption that recurrent tumors compress or displace the white matter tracts while radiation necrosis leads to destruction of these tracts [16,17]. However, more recent studies have failed to confirm these findings [18].

■ MRI perfusion

MRI perfusion provides information about tumor blood volume and vascular permeability by measuring the relative cerebral blood volume (rCBV). rCBV can be calculated using dynamic contrast enhancement and dynamic susceptibility contrast (DSC) methods. DSC is more commonly used clinically, and relies on T_2^* signal intensity changes that occur with passage of contrast agents through the tissue to generate rCBV maps [19]. Relationships between rCBV and tumor vascularity, VEGF expression, tumor

Table 2. Revised Assessment in Neuro-Oncology criteria.

Criterion	T_1 gadolinium-enhancing disease	T_2 /FLAIR	New lesion	Corticosteroids	Clinical status	Requirement for response
Complete response	None	Stable or improved	None	Off steroids or on physiological replacement doses only	Stable or improved	All
Partial response	≥50% decrease	Stable or improved	None	Stable or decreased	Stable or worse	All
Stable disease	<50% decrease or <25% increase	Stable or improved	None	Stable or decreased	Stable or worse	All
Progression	≥25% increase	Increased	Present	NA	Worse	Any

FLAIR: Fluid-attenuated inversion recovery; NA: Not available.
Data taken from [4].

Table 3. Frequently performed novel imaging techniques.

Novel imaging techniques	Concept	Ref.
DWI	Measures the rate of Brownian motion of water molecules in tissue. Identification of areas of high cellularity/active tumor growth	[7–11]
DTI	As a refined form of DWI imaging, DTI helps to visualize white matter tracts. May be helpful in preoperative planning and differentiating tumor growth from pseudo-progression	[12–18]
MRI perfusion imaging	Measures tumor vascular permeability. Active tumors tend to have higher relative cerebral blood volumes	[19–21,23–26]
Magnetic resonance spectroscopy	Measures tissue metabolic activity. May be helpful in preoperative grading as well as differentiating tumor growth from pseudo-progression	[26–31]
PET imaging	Measures biochemical activity of tumor. May be helpful in preoperative grading as well as for assessment of disease progression	[32–37]

DTI: Diffusion-tensor imaging; DWI: Diffusion-weighted imaging.

grade cellular density and proliferation, as well as time to progression, have been demonstrated [12,20,21]. In addition, a study of 65 patients with high-grade gliomas showed that mitotic activity and endothelial proliferation rates correlated with the rCBV ($p < 0.01$) [22]. Another study found that rCBV of 1.5 or higher was associated with a higher grade and also reduced survival in a cohort of 46 consecutive patients [23]. It is thought that the relationship between rCBV and survival could be explained by higher tumor grade [24]. New evidence suggests that quantitative parameters such as 10% maximum values of the transfer coefficient (also called K_{trans} -T₂*:10%) derived from pretreatment T₂*-weighted dynamic perfusion MR scans may be a potential predictive biomarker. A small study with 39 patients demonstrated that high maximum transfer coefficient values had an independent statistical relationship with low survival in high-grade glioma patients [25]. Larger studies are required to further validate these associations and to define the role of MRI perfusion in high-grade gliomas.

■ **Magnetic resonance spectroscopy**

Magnetic resonance spectroscopy (MRS) adds to the information provided by conventional MRI by measuring the metabolic activity in the imaging area of interest. Apart from providing presurgical guidance regarding tumor grade, MRS is increasingly used as an adjunct to try to differentiate true progression from treatment effect, however, this has not yet been validated prospectively. Spectral patterns or specific metabolite intensities can be compared with traditional MRI to identify changes in spectra from adjacent voxels or to obtain a distribution pattern of a particular metabolite within the tumor. Standard brain proton MRS uses proton and measures choline (assessment of membrane turnover and

proliferation), creatine (energy homeostasis), *N*-acetyl aspartate (glioneural structures) and lactate or lipids (necrosis) [26]. A direct correlation between choline and cellular proliferation has been shown [27–29]. In a blinded prospective study with 14 patients, detection of new choline accumulation was shown to suggest residual or recurrent tumor after treatment, especially if a pretreatment choline level was available [30]. Glutamine peaks are detected more frequently in tumors than in controls, and a rise in glutamine peak may relate to a role of glutamate as an excitotoxin in accelerated cell proliferation of malignant brain tumors. Lactate is normally not found in the brain, and its presence indicates anaerobic or nonoxidative metabolism, which is expected to be elevated in areas of recurrent tumor, but it can also be elevated in areas of necrosis and abscess [31]. Detection of new choline accumulation may indicate residual or recurrent tumor after treatment. Necrotic areas are devoid of any spectroscopic activity and a decrease in the level of choline and modest increases in *N*-acetyl aspartate indicate decrease in tumor burden [26]. Even though multiple metabolites and their ratios have been studied, to date none have been demonstrated to have sufficient diagnostic specificity to differentiate active tumor from treatment effect. MRS is a promising candidate marker for treatment response assessment, follow-up and early detection of tumor recurrence; however, larger studies are still required to define its role in clinical practice and in clinical trials.

■ **PET**

PET scans measure biochemical activity of short-lived positron-emitting radionucleotides (radio-tracers). ¹⁸F-fluorodeoxyglucose PET (¹⁸F-FDG-PET) scans have been used in the evaluation of gliomas since the 1980s and are still frequently

performed. Higher uptake of tracer is thought to be present in tumors as compared with surrounding normal tissue or necrosis due to its higher metabolic activity [32]. The ability of FDG-PET scans to grade and prognosticate survival in patients with brain tumors was demonstrated in a retrospective study of 331 patients with brain tumors where over 90% of patients with high uptake were found to have a high-grade glioma and approximately 30% of those patients survived over 12 months and none survived over 5 years [33]. FDG-PET was one of the first tracers that has been studied regarding its ability to differentiate recurrent tumor from radiation necrosis, however, the sensitivity and specificity of this test in this setting was 75 and 81%, respectively [34]. It has been argued that the sensitivity of FDG-PET is lower in the brain than in other organs due to the physiologically higher uptake of ^{18}F -FDG in the brain. Furthermore, it may have limited ability to distinguish residual tumor after therapy owing to its uptake into macrophages in areas with inflammation after radiation [35,36]. Amino acid tracers, such as ^{11}C -methionine, and newer agents, such as ^{18}F -fluoroethyltyrosine and ^{18}F -fluorothymidine, are promising agents owing to improved specificity due to their low physiological uptake in normal brain tissue. In a small study with 24 patients (five patients with WHO grade III and 19 with WHO grade II gliomas), ^{11}C -methionine PET scans were reported to detect tumor progression with a sensitivity and specificity of over 90% [37]. Another study with 45 patients using ^{18}F -fluoro-ethyl-tyrosine-PET in a similar population was able to achieve a sensitivity of over 90% and specificity of 100% in patients with suspected disease progression based on contrast-enhanced MRI [38]. The specificity of MRI was only approximately 50% in this study.

PET agents can be tailored for specific applications. For example, PET imaging with ^{18}F -arginine-glycine-aspartic acid was used as a surrogate imaging biomarker to detect $\alpha\text{v}\beta\text{3}$ integrin expression in a randomized clinical trial using the targeted agent cilengitide (integrin-targeted therapy) in patients with newly diagnosed glioblastoma [39–41]. Other potential uses of this technique that are being explored include presurgical tumor grading, biopsy and margin delineation, as well as radiation planning [42–49]. Current studies are clustered around diagnosis at time of presentation, tumor grading, particularly at recurrence. Unfortunately, limited information is available to recommend

using it for guiding tumor biopsy or planning local therapy [50]. Differential uptake within the tumor is the key parameter with any radioactive tracer and the selection of an appropriate cutoff value for background activity is crucial to the interpretation of studies utilizing PET scans. At this time small sample sizes and heterogeneous study designs limit our ability to derive straightforward conclusions for adoption of PET scans into routine clinical practice.

■ Multimodality imaging

Coregistration of images from MRI or CT scans with the metabolic signature of target tissue using PET scans have shown potential in the diagnosis and monitoring of brain tumors [51]. There is an emerging trend towards incorporating MRI and PET scanners to achieve better morphologic as well as metabolic images in brain tumors by avoiding sequential scanning [52,53]. This may be of particular benefit if these techniques are used for high precision biopsies or to monitor very small lesions. Most standard MRI scanners have the ability to incorporate diverse imaging sequences and even MRS with the addition of appropriate software. Hybrid MRI/PET scanners incorporate a specially designed PET detector with good results [53].

Another approach uses currently available MRI voxel-based techniques using serial ADC maps (functional diffusion maps), and rCBV derived from DSC-MRI parametric response map have been described as potential early indicators of survival [54–57]. A strong correlation was demonstrated in the 1-year and overall survival rates with 3-week midtreatment scans that used a composite of ADC and rCBV as analyzed by parametric response map [56]. Such early biomarkers may help clinicians to reassess treatment options in a timely manner, but these techniques are still in the early stages of development.

■ Selected other novel imaging concepts for malignant gliomas

Studies on the MRI contrast agent ferumoxytol, a novel superparamagnetic iron oxide nanoparticle, pointed towards a potential role in differentiation between tumor progression and pseudo-progression in a group of 19 patients with glioblastoma [58]. Ferumoxytol is a blood pool agent that does not require contrast agent leakage correction for calculation of rCBV. A survival advantage was observed in patients with rCBV less than or equal to 1.75 using ferumoxytol,

suggesting pseudo-progression rather than active tumor in these patients. Recent advancements have also enabled development of nanoparticles with engineered physical and chemical properties, including shape, size and surface properties, which can reduce the T_2 relaxation time of tumor cells. For example, encouraging results were shown in animal studies with iron-based single-domain antibodies against tumor blood vessel-specific antibodies [59]. Another novel magnetic resonance probe utilizes superparamagnetic iron oxide nanoparticles linked to a short DNA sequence, complementary to the cerebral mRNA of GFAP found in glial cells and astrocytes [60,61]. In addition, studies are under way to image transcription-related events in tumors cells using MRI scans. ‘Transcription MRI’ techniques use mRNA-linked radiotracers that bind to intracellular mRNA in tumor cells with high specificity. A recent report of self-assembling nano-complexes by combining ferumoxytol, heparin and protamine in rat models also shows promise in tracking as few as 1000 neural stem cells [62]. These small and hypothesis-generating studies will certainly require further validation in larger prospective studies.

Amide proton transfer scans are another novel MRI-based imaging technique that detects endogenous mobile proteins and peptides in tissue via saturation of the amide protons in their peptide bonds. Tumor cells proliferate rapidly and have higher content of proteins and peptides than radiation necrosis as demonstrated in rat glioma models that had been treated with radiation [63,64]. A study in humans is currently ongoing [65].

In summary, there are several novel imaging techniques that are being developed to provide more disease-specific assessment of disease status and tumor burden in malignant gliomas. However, contrast-enhanced MRI still remains the current standard imaging technique in clinical practice and in therapeutic clinical trials. Carefully designed larger, prospective trials are required to identify the actual value of these individual novel imaging techniques.

Blood-based tumor markers in malignant gliomas

Over the past years there has been an increasing interest in the search for blood-based tumor markers for malignant gliomas, which is in part a result of the limitations of the currently available imaging studies as detailed above. A valid blood-based tumor marker would serve as an adjunct

in clinical decision-making if it was sufficiently sensitive and disease specific and if it accurately reflected changes in tumor burden. As an example, it could function as a ‘tie breaker’ when the patient’s disease status is unclear based on ambiguity of the imaging findings (e.g., pseudo-progression). If imaging showed findings concerning for progression versus treatment effect, an increase in the marker levels (compared with baseline) would suggest progression, whereas a decrease of the marker level would be more consistent with treatment-related changes. A complicating factor in the search for a blood-based biomarker for malignant gliomas is certainly the BBB. As evidenced by contrast enhancement on MRI, however, the BBB is commonly altered and more permeable in high-grade gliomas which leads to shedding of cellular components of the tumor (Figure 1). As blood-based tumor markers in other diseases, the marker may not need to be ‘perfect’, i.e., have 100% sensitivity and specificity, in order to be clinically meaningful. Examples of such clinically relevant, but ‘sub-optimal’ markers that are currently in use are PSA in prostate cancer, CEA in colorectal and CA-125 in ovarian cancer.

The following overview focuses on three tumor-derived blood-based biomarker classes that are evaluated in malignant gliomas: circulating tumor cells, circulating nucleic acids and circulating proteins. Other circulating markers that are not directly derived from the malignant cells themselves, including circulating hematogenous progenitor cells and circulating endothelial cells, have been investigated and may also be useful for response to certain treatments, however, they are not part of this review that focuses on markers that are comprised of components of the tumor itself.

■ Circulating tumor cells

CTCs have been detected and are under clinical development in a variety of solid tumors, including lung, breast and prostate cancer [66]. CTCs have not yet been successfully isolated in patients with malignant gliomas, although there is biological evidence that CTCs exist in these cancers. Although rarely seen in clinical practice, there are numerous reports in the literature of metastatic glioblastomas and gliosarcomas, indicating that hematogenous tumor dissemination of these cancers does occur, reportedly in approximately 0.4–0.5% of cases [67–71]. In addition, to date there have been 17 reports of

organ transplant-related glioblastoma multiforme (GBM) transmission in patients [72,73]. Recipients of organs from donors with GBM grew metastatic GBM, proving that GBM cells must have been present in the distant organs at time of transplant and that they presumably made their way through the bloodstream as CTCs.

■ Circulating nucleic acids

Circulating tumor DNA is a particularly attractive tumor marker with a significant amount of promising data in other solid tumors including colorectal and breast cancer [74–76]. Tumor-specific mutations can be detected even in minimal concentrations in plasma using novel ‘digital’ PCR-based methods that differentiate mutant tumor-derived DNA from wild-type DNA in the blood of patients. Techniques including BEAMing and personalized analysis of rearranged ends can detect and quantify tumor-derived DNA with a sensitivity of up to one in 10,000 and 100,000, respectively [77,78]. Two pilot studies described that DNA with tumor-specific mutations can be detected in patients with gliomas. One study showed detection of mutated IDH-1 DNA in plasma of patients with IDH-1-positive gliomas [79]. The other recently published report demonstrated detectability of EGFRvIII-mutated DNA in plasma of patients with glioblastomas using personalized analysis of rearranged ends analysis. Presence or absence of EGFRvIII in plasma matched that in tissue [80]. In addition to circulating genetic alterations, there have been several reports on the detectability of methylated DNA in blood of patients with gliomas with reported sensitivities of 67–83% and specificity of 75–100% [81–83]. The results were, however, not quantitative. In addition to DNA, there have been some early data on miRNA that was detected in blood of patients with GBM. In a screening study of 20 patients with GBM compared with 20 age-matched controls, there were two miRNAs that appeared significantly altered in GBM, miR-128 that was upregulated and miR-342-3p that was downregulated [84]. Another study identified miRNA-21 as a potentially promising marker for GBM [85].

■ Circulating proteins

The third group of tumor-derived candidate markers for malignant gliomas are circulating proteins. Many of the proteins that have been investigated as potential glioma biomarkers were initially found as potential markers of hypoxic or

traumatic brain injury. The major limitation of proteins as candidate markers for gliomas is their lack of specificity, and to date no truly glioma-specific protein marker has been identified. Protein-based markers that have previously been studied in malignant gliomas can be grouped into three categories: glial- or neuronal-specific markers (e.g., GFAP), proangiogenic proteins (e.g., EGFR, VEGF and β -FGF) and inflammatory markers (e.g., TGF- β and MMP-9). The most extensively studied protein as a potential circulating marker for malignant gliomas is GFAP. Several studies showed that GFAP is detectable in most glioblastomas and to lesser degree in lower grade gliomas [86–89]. A study that showed a significant increase in plasma GFAP levels within 1–2 days after resection, irrespective of tumor grade, illustrates that GFAP is not a truly specific marker for gliomas but rather a marker of brain injury [88]. An exploratory investigation of eight circulating proteins (GFAP, BDNF, GDNF, PGF, S100B, secretagogin, IL-8 and Neuropeptide Y) in 105 patients with various brain tumors assessed, concluded that none of the investigated markers was suitable to substitute histological diagnosis, but that there was a statistically significant association of GFAP with a diagnosis of GBM [89].

In addition to analyzing plasma, there has been a growing interest in the potential utility of circulating microvesicles in patients with malignant gliomas. Microvesicles are small, membrane-bound compartments that are shed in large quantities from glioblastomas into the circulation. These vesicles contain, in part, tumor-derived molecules and may give indirect information on tumor biology, providing a potentially noninvasive way to assess for presence or absence of certain molecular changes in the tumor that may be important as molecular targets or a prerequisite for enrollment in certain clinical trials [90,91].

It appears likely that the BBB (or the altered blood–tumor barrier) limits the amount of the respective molecules that are shed into the bloodstream. This raises the question of whether, instead of blood, cerebrospinal fluid may be the more appropriate compartment to test for tumor-specific biomarkers and for changes in their levels over time. This work clearly needs to be carried out; however, a major barrier is the need for non-therapeutic lumbar punctures that could provide an additional burden for patients.

In summary, these preliminary observations show that glioma-specific blood-based markers

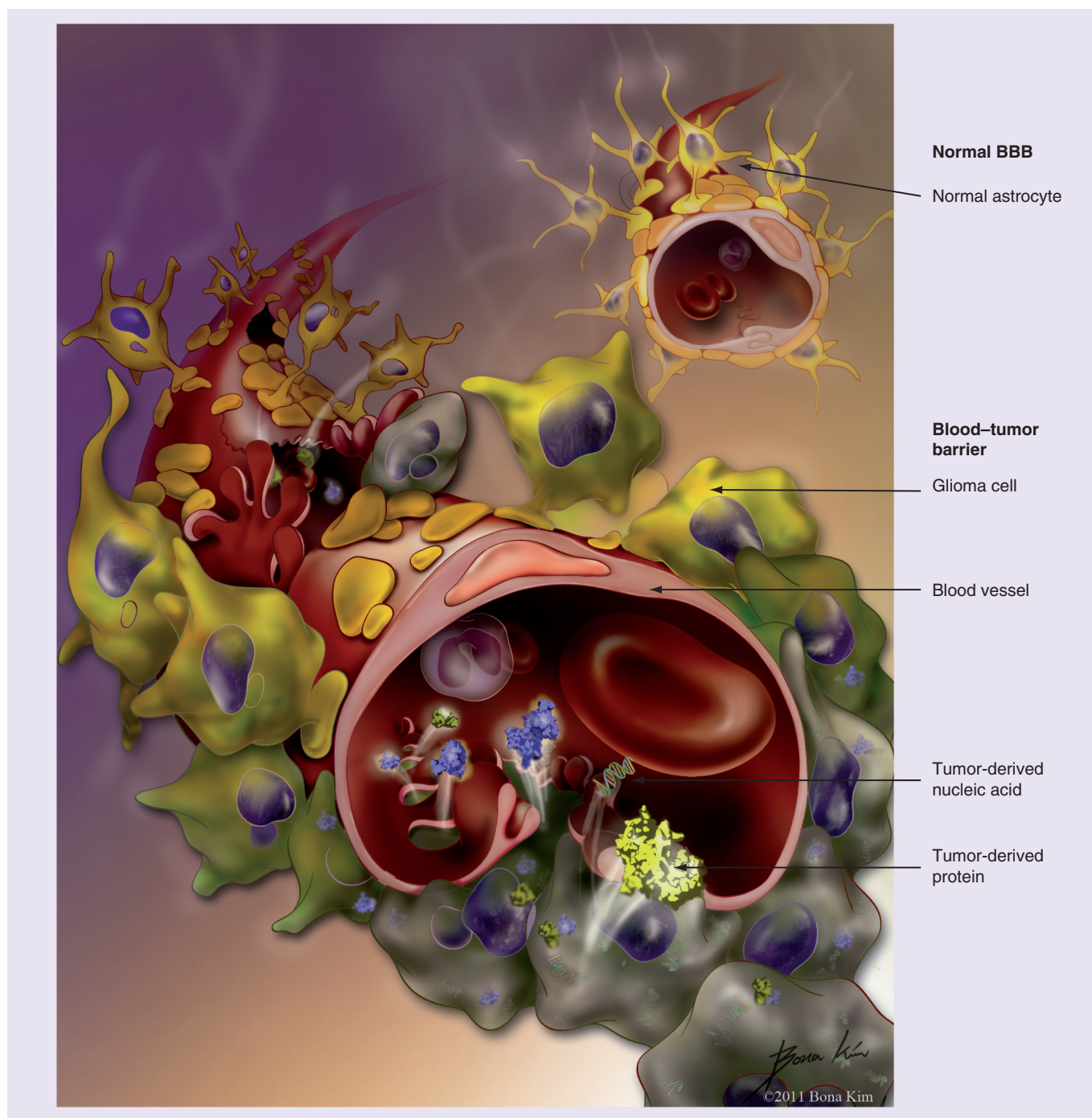


Figure 1. Blood–brain barrier versus blood–tumor barrier. Schematic illustration of the differences between the normal BBB (upper right) and the altered BBB in high-grade gliomas (blood–tumor barrier; lower left). It is hypothesized that cells, proteins and nucleic acids ‘leak’ through gaps in the blood–tumor barrier into the blood stream. BBB: Blood–brain barrier.

do exist and they can in part be detected and quantified with novel analytical methods. However, to date there has been no candidate marker that appears clearly superior and ready for further development and clinical use. Further work needs

to be performed to more systematically screen for candidate markers that deserve further validation in larger data sets and eventually in prospective clinical trials. Further technological advances are required to enhance sensitivity and additional

comprehensive, high-throughput screening studies will be necessary to identify disease-specific molecular alterations that can be exploited as cancer-specific biomarkers.

Conclusion

Despite recent advances and the introduction of novel therapies, survival of patients with malignant gliomas has remained poor and these cancers have remained incurable. Contrast MRI, the current standard imaging modality for these tumors, cannot reliably determine tumor burden or differentiate active tumor from treatment effect. More robust markers of disease burden and response to therapy are urgently required, both for standard therapy and for clinical trials. This is of ever greater importance due to the increased pace at which novel treatment approaches are introduced into and tested in clinical practice. Interesting and hypothesis-generating data exist for novel imaging techniques and blood-based markers for assessment of disease burden; however, the stage of development of these markers is still early. To date, there has been no imaging technique for malignant gliomas that proved to be superior to contrast MRI. However, several promising novel imaging techniques are actively being tested in trials to determine their clinical utility. Research on blood-based biomarkers is still in its infancy and to date there has been no marker or marker class identified that appears to be the most promising candidate for further development.

In summary, there exist several promising approaches for disease monitoring in malignant gliomas; however, a more comprehensive evaluation of these techniques within larger prospective studies is urgently required. We argue that

development of novel biomarkers of disease burden and treatment response should be among the highest priorities in neuro-oncology. Superior methods to measure disease would accelerate the development of new treatment paradigms and directly impact patient care.

Future perspective

It is anticipated that some of the imaging techniques mentioned in this article will at least, in part, be validated in larger clinical trials and a fraction of these will find their place in standard clinical practice. There will be more clarity on which circulating tumor markers deserve further evaluation, and it is likely that multimodality assessment of disease burden (standard plus novel imaging plus tumor biomarkers) will enhance our current standard of care that largely relies on MRI. This would lead to more prudent clinical decision-making, improved patient outcomes and more rapid implementation of new treatment paradigms.

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