# Circulating Biomarkers for Glioma: A Review

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Accurate circulating biomarkers have potential clinical applications in population screening, tumor subclassification, monitoring tumor status, and the delivery of individualized treatments resulting from tumor genotyping. Recently, significant progress has been made within this field in several cancer types, but despite the many potential benefits, currently there is no validated circulating biomarker test for patients with glioma. A number of circulating factors have been examined, including circulating tumor cells, cell-free DNA, microRNA, exosomes, and proteins from both peripheral blood and cerebrospinal fluid with variable results. In the following article, we provide a narrative review of the current evidence pertaining to circulating biomarkers in patients with glioma, including discussion of the advantages and challenges encountered with the current methods used for discovery. Additionally, the potential clinical applications are described with reference to the literature.

**KEY WORDS:** Glioma, Glioblastoma, Circulating biomarkers, Liquid biopsy

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The identification of accurate circulating biomarkers, otherwise termed "liquid biopsy," is a major goal in oncology research due to the potential clinical applications in population screening, tumor monitoring, and delivery of individualized treatments resulting from tumor genotyping. Recently, large datasets have been made available due to advances in genome sequencing and mass spectrometry; however, the difficulty remains in translating these findings to clinically meaningful applications. Gliomas are the most common primary brain cancer, and its most aggressive form, glioblastoma (GBM), is rapidly and uniformly lethal.[1] Circulating biomarkers have the potential to aid in a number of challenges that are faced in managing these patients. Despite increased efforts in biomarker discovery, currently there is no validated liquid biopsy for glioma. We discuss the strategies and hurdles encountered in this field and summarize the potential clinical applications of a circulating biomarker in patients with brain cancer.

**ABBREVIATIONS: AUC,** area under the curve; **ccfDNA,** circulating cell-free DNA; **CSF,** cerebrospinal fluid; **ctDNA,** circulating tumor DNA; **CTC,** circulating tumor cell; **ddPCR,** droplet digital polymerase chain reaction; **EGFR,** endothelial growth factor receptor; **EpCAM,** epithelial cell adhesion molecule; **EV,** extracellular vesicle; **GBM,** glioblastoma; **GFAP,** glial fibrillary acidic protein; **LDA,** low density array; **LGG,** low grade glioma; **miRNA,** microRNA; **MS-PCR,** methylation specific polymerase chain reaction; **NGS,** Next Generation Sequencing; **PCR,** polymerase chain reaction; **qRT,** quantitative reverse transcriptase; **SWATH,** sequential windowed acquisition of all theoretical fragment ion

## CIRCULATING TUMOR CELLS

Circulating tumor cells (CTCs) are shed into the bloodstream from a tumor during its formation or growth. They are considered fundamental in the development of metastasis and can circulate as individual cells or as clusters, with evidence suggesting increased survival and metastatic potential when clustered.[2,3] The detection of CTCs is technically challenging due to low circulating concentrations and the requirements for sophisticated isolation technologies. The majority of detection platforms capture CTCs by identifying tumor-specific surface antigens, most commonly the transmembrane glycoprotein epithelial cell adhesion molecule (EpCAM) that is highly expressed in proliferating carcinomas. In tumors that do not express EpCAM, including gliomas, capture is dependent on less specific microfluidic techniques that isolate cells based on unique properties such as density.[4]

Although metastatic spread of glioma is exceedingly rare, several groups have reported

**TABLE 1. Reported Studies Examining CTCs in Glioma**

| Study | Patients (n) | Technique | Sensitivity | Specificity |
|---|---|---|---|---|
| Sullivan[6] 2014 | 33 | CTC-iChip for CD45 and CD16<br>Immunostaining for EGFR, MET, CDH11 | 39.4% | 100% |
| Müller[5] 2014 | 141 | Immunostaining for GFAP | 20.6% | 96.6% |
| MacArthur[7] 2014 | 11 | Telomerase-responsive adenoviral probe | 72% preradiotherapy<br>8% postradiotherapy | 100% |
| Gao[97] 2016 | 31 | SE-iFISH (polyploidy chromosome 8) | 77% | 100% |
| Krol[9] 2018 | 13 | Parsortix microfluidic device<br>Immunostaining for EGFR, Ki67, EB1, SOX2 | 54% | 100% |

circulating cells with glioma characteristics (Table 1). Müller et al[5] identified glial fibrillary acidic protein (GFAP) positive cells in the blood of 29 out of 141 patients with glioma, confirming them to be of tumor origin by identifying shared mutations in MDS1 and EVI1 complex locus (MECOM), myosin 11 (MYH11), neurofibromin 1 (NF1), and platelet derived growth factor receptor Alpha as well as amplification of endothelial growth factor receptor (EGFR). Sullivan et al[6] found higher numbers of CTCs in more progressive disease and a tendency for CTCs to overexpress genes from the more aggressive mesenchymal subtype. MacArthur et al[7] found that CTCs significantly reduced following radiotherapy and reported a single patient in whom levels increased in the setting of tumor recurrence, suggesting the potential of CTCs to monitor for disease recurrence in GBM.

The clinical significance of CTCs and their contribution to tumor progression is unknown. Glioma mouse model data suggested that CTCs exhibit aggressive stem cell-like properties, are resistant to treatment, and can re-seed the primary tumor, promoting local recurrence.[8] Recently, CTCs were shown to cross the blood-brain barrier in metastatic clusters[9]; however, the lack of extracranial spread is likely due to the inability of glioma cells to adapt to local microenvironments. Low sensitivity is a common feature across current CTC detection platforms, and identification of CTCs in noncancerous pathologies has also been reported, questioning reliability.[10] Notwithstanding these challenges, CellSearch® (Menarini Silicon Biosystems), a CTC platform used in metastatic breast, prostate, and lung cancer, has been approved by the food and drug administration and the development of newer platforms with improved sensitivity may allow CTCs to be more accurately isolated in glioma. Additionally, the emergence of single-cell RNA sequencing allows for transcriptome profiling of individual CTCs to investigate mechanisms of metastasis, drug resistance, and cancer stemness.[11] Despite the potential, significant progress is still required prior to considering CTCs a viable biomarker target in glioma patients.

## CIRCULATING CELL-FREE DNA

Circulating cell-free DNA (ccfDNA) is a normal constituent of blood at low concentrations (1-10 ng/mL), and increased amounts are seen in many physiological and pathological processes including exercise, infection, trauma, and cancer.[12] Cancer-specific mutations found within the ccfDNA are termed circulating tumor DNA (ctDNA)[13] with release thought to occur from apoptosis and/or necrosis of tumor cells, or via direct secretion of extracellular vesicles (EVs) into the circulation.[14] There are 2 general methods used for detection of ctDNA: targeted mutation-specific assays or broad sequencing panels.

The total amount of ctDNA in the blood is small, comprising less than 0.1% to 5% of total ccfDNA depending on tumor type, grade, and burden.[15,16] The presence of the blood-brain barrier and the dilution of ctDNA within the cerebrospinal fluid (CSF) prior to reaching the systemic circulation further complicates the ability to detect ctDNA in brain tumor patients. An early report found that ctDNA was detected in less than 10% of gliomas, significantly less than other malignancies.[16] However, improvements in sequencing depth and polymerase chain reaction (PCR) techniques have led to others reporting greater detection (Table 2). Bagley et al[17] reported significantly higher concentrations of ccfDNA in patients with GBM compared to healthy controls and Boisselier et al[18] noted that higher concentrations of IDH1-R132H mutation were found in larger or enhancing tumors. Lavon et al[19] used a combination of genetic and/or epigenetic alterations to detect ctDNA in the plasma of 53% of 70 gliomas. In an alternative to targeting known mutations, multigene sequencing platforms have been used by Piccioni et al[20] and Zill et al[21] identifying ctDNA in 55% and 51% of GBM patients, respectively. Further techniques reported to improve the ability of ctDNA detection include isolating mitochondrial ctDNA,[22] analyzing epigenetic alterations,[23] or targeting ccfDNA of a certain fragment size to limit background interference.[24] A recent paper applied a methylated DNA immunoprecipitation approach to ccfDNA and showed that plasma methylomes could very accurately distinguish gliomas from both healthy controls and patients with intracranial metastasis, as well as differentiate histological types of primary brain tumors with only small inputs (1-10 ng) of ccfDNA.[25]

Due to the low nontumor background of ccfDNA and the anatomic proximity, CSF is an ideal biofluid for ctDNA.[26] Wang et al,[26] in 35 patients, detected ctDNA from CSF

**TABLE 2. Studies Reporting ctDNA in Glioma**

| Study | Patients (n) | Fluid | Method | Marker | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| Lavon[19] 2010 | 70 | Serum | RT-PCR<br>Methylation specific polymerase chain reaction (MS-PCR) | LOH 1p, LOH19q, LOH 10q, MGMT methylation, PTEN | 51% astrocytoma<br>55% oligodendroglioma | 100% |
| Boisselier[18] 2012 | 25 | Plasma | Cold-PCR | IDH1 R132H | 60% | 100% |
| Bettegowda[16] 2014 | 27 | Plasma, serum | RT-PCR, SafeSeq | IDH1, TP53, EGFR, PIK3A, PTEN | <10% | ns |
| Majchrzak-Celinska[98] 2013 | 17 | Serum | MS-PCR | MGMT, RASSF1A, p15INK4B, p14ARF | 70% | 100% |
| Piccioni[20] 2017 | 211 | Plasma | Next Generation Sequencing (NGS) | Multigene (Guardant360) | 55% | ns |
| Zill[21] 2019 | 107 | Plasma | NGS | Multigene (Guardant360) | 51% | ns |
| Bagley[17] 2020 | 42 | Plasma | NGS | Multigene (Guardant360) | 55% | ns |
| Wang[26] 2015 | 21 | CSF | Whole exome sequencing (WES) | Multigene (Illumina) | 71% | ns |
| Mouliere[27] 2018 | 13 | CSF | Whole genome sequencing | SCNA's and DNA fragmentation | 39% | ns |
| Martínez-Ricarte[99] 2018 | 20 | CSF | Droplet digital polymerase chain reaction (ddPCR) | IDH1/2, TP53, ATRX, TERT, H3F3A, HIST1H3B | 85% | ns |
| Pan[29] 2019 | 57 | CSF | NGS | Multigene (Genetron) | 75% | ns |
| Miller[28] 2019 | 85 | CSF | NGS | Multigene (MSK-IMPACT) | 49.4% | ns |

collected at the time of surgery in 74%, which improved to 100% when the tumor abutted a CSF space, whereas Mouliere et al[27] were able to detect single-copy number alterations in the CSF of glioma patients using shallow whole genome sequencing (WGS), suggesting the concentration of ctDNA is significantly higher in CSF than plasma. Miller et al[28] found an association between higher levels of ctDNA in progressive disease, spread towards CSF spaces, and a larger tumor burden. They noted a high concordance of mutations between tumor tissue and CSF ctDNA and reported 5 patients with multiple longitudinal CSF samples demonstrating genomic evolution consistent with sequential tumor biopsies. Pan et al[29] found at least one tumor-specific mutation in the CSF in 47 of 57 patients with brainstem gliomas and could accurately identify molecular profiles that reflected known survival trends, suggesting CSF analysis as an alternative to tissue biopsy in eloquent or deep tumor locations.

ctDNA is highly specific and provides important genomic information about a tumor.[30-32] Studies comparing matched tumor tissue and ctDNA have shown good concordance of mutational profile, even identifying cancer-specific mutations in the blood that were not identified on tissue biopsy.[33-35] ctDNA is hypothesized to represent cells throughout a tumor, and thereby avoids the intratumoral heterogeneity that affects tissue biopsy. However, the underlying mechanisms that result in ctDNA release are not known, including whether specific tumor cell subpopulations are primarily responsible.[35-37] In gliomas, a major limitation is low concentrations of ctDNA in the plasma, thus requiring highly sensitive detection methods. CSF appears to be a better alternative than plasma; however, it has practical limitations for routine use in the neuro-oncology clinic. Improvements in detection techniques for rare ctDNA mutations have been demonstrated in other cancers types, and although promising, are yet to be validated in glioma.

## EXOSOMES

Exosomes are small phospholipid membrane-bound EVs that are highly regulated critical mediators of intercellular communication and play a role in tumorigenesis.[38,39] They contain a concentrated mix of RNA, microRNA (miRNA), proteins, and lipids representative of their parental cell; exist stably within the circulation; and protect their contents from enzyme degradation.[40] Importantly, they are able to cross the blood-brain barrier,[41] and Osti et al[42] demonstrated that total concentrations of circulating exosomes were higher in glioma patients compared to healthy controls. Skog et al[43] showed glioma-derived exosomes by identifying EGFRvIII mRNA in 7 out of 25 GBM patients but not in healthy controls. They found that patients had no detectable EGFRvIII 2 wk following resection, suggesting a direct relationship with tumor burden. Exosomal-derived mRNA has also been suggested to provide a more comprehensive picture of the genomic landscape,[43,44] as well as reflect resistance to therapy with temozolomide.[45] Other exosome contents, including noncoding RNA and proteins, have reported benefits in aiding diagnosis or prognostic predictions.[46-48]

The relative abundance of nontumor exosomes within the circulation is a challenge when attempting to identify exosomal biomarkers in cancer patients. Fraser et al[49] demonstrated that less than 10% of all circulating exosomes are tumor specific in glioma patients and were highly heterogeneous. CSF has been considered as an alternative to blood; however, the clinical practicalities in obtaining CSF remain.[50,51]

Enrichment strategies for glioma-derived exosomes are still being investigated,[52] and despite single-cell EV analysis[53] or widespread proteomic and genetic profiling,[46] there is no standard method to discriminating tumor and nontumor exosomes. Additionally, methods of bulk EV isolation are not standardized and are time consuming, limiting their use in the clinical setting.[39,54]

## MicroRNA

miRNA are small noncoding RNAs that account for approximately 1% of the human genome but regulate the translation and stability of 50% to 60% of mRNA.[55] There are over 2000 known miRNAs, with evidence suggesting important biological roles in tumor growth, angiogenesis, and immune evasion.[55,56] MiRNAs are able to cross the blood-brain barrier and exist stably in the blood either within exosomes or freely circulating.

There are a number of reports of circulating miRNAs being investigated as diagnostic biomarkers,[57-59] and a recent meta-analysis found that serum miRNA could distinguish between glioma patients and healthy controls with an area under the curve (AUC) of 0.93.[60] Alterations in miRNA expression have also been used to assist in prognostication,[61-63] and changes after surgery suggest a relationship to tumor burden.[64-66] A recent study reported that serum miRNA levels correlated with GBM and low grade glioma (LGG) tumor volume across the postoperative monitoring period but did not increase during pseudoprogression.[67]

The literature on circulating miRNAs in glioma is limited by small cohorts that typically focus on a selection of miRNA resulting in significant heterogeneity and no consistent expression variation across cohorts (Table 3).[68-71] Additionally, there is a lack of standard methods for blood collection, RNA extraction, RNA sequencing, and statistical analysis, including normalization methods, with no available endogenous controls due to circulating RNAses.[72] Another difficulty with circulating miRNA is that it does not have the innate specificity of ctDNA and is diluted by normal physiological changes found during circadian rhythm, food intake, medications, exercise, and other disease states. Nevertheless, miRNA remains a promising candidate biomarker.

## PROTEOMICS

One of the features that make proteomics an intriguing prospect as a biomarker is that it evaluates functional products, which may more accurately reflect disease status.[73] Additionally, protein isolation is relatively inexpensive and is historically the most commonly studied biomarker. However, there is no glioma-specific protein; therefore, studies rely on detecting altered levels of the many known circulating proteins that are derived from tumor cells or from other sites as a response to malignancy.

GFAP is the most widely studied protein with increased serum levels reported in glioma patients.[74,75] Higher concentrations have been associated with larger and higher grade tumors; however, these were not altered at the time of recurrence or predictive of prognosis.[76,77] Alterations in other aspects of the proteome have been reported, including increased levels of acute-phase proteins, haptoglobin alpha-2, which was associated with tumor growth, and serum YKL-40, which was found to reduce following surgery but was not altered in response to tumor recurrence.[78-80] Unbiased amino-acid analysis by mass spectrometry or electrophoresis has also been used in an effort to identify undiscovered biomarkers.[81-83] Miyauchi et al[84] utilized Sequential Windowed Acquisition of All Theoretical Fragment Ion (SWATH) mass spectrometry to identify short-chain amino-acid peaks in the plasma of 14 patients with GBM when compared to healthy controls. Using a spectral library created from the UniProt Human Protease Database, they identified 8 protein biomarker candidates, of which 3—leucine rich alpha-2-glycoprotein 1 (LRG1), C-reactive protein (CRP), and C9—directly correlated with tumor size and 1, gelsolin, was associated with poorer outcome.

The lack of tumor-specific proteins and the high concentrations of background noise in the serum limit the ability to identify reproducible protein biomarkers. CSF has been viewed as an alternative to serum due to the reduction in background proteins; however, a recent systematic review found the same challenges of inconsistent methodology and results seen with serum analysis.[85] Additionally, both the CSF and systemic proteome are influenced by other pathological and physiological states; therefore, defining the abnormal threshold of individual proteins is not clear, which impacts the validation of a circulating biomarker.

## CLINICAL APPLICATIONS

The ease of integrating a liquid biopsy test into the clinic (Figure 1) and potential clinical benefits has resulted in significant investment into discovering liquid biopsy candidates across all cancers, with promise shown in tumor genotyping at time of diagnosis, detection of minimal residual disease following surgery, predicting response to targeted therapies, and the detection of tumor recurrence.[37,86,87] Despite the potential for circulating biomarkers to be applied at all stages of glioma management (Figure 2), there are no studies to date that demonstrate an impact on clinical decision making.

### Screening

The low incidence of glioma and the lack of evidence for effectiveness of early treatment limit the potential benefits of a screening biomarker.[88] No general population screening tool for brain tumors has been reported. A study investigating a specific screening biomarker in brain tumors reported the use of attenuated total reflection-Fourier transform infrared spectroscopy as a method of triaging patients who present to their primary practitioners with nonspecific symptoms into early or delayed imaging.[89] The test had a sensitivity and specificity of 83.8% and 87%, respectively, in a heterogeneous cohort of 104 patients, 12 of whom had intracranial tumors. However, as a result of

**TABLE 3. Studies Reporting Circulating MicroRNA in Glioma**

| Study | Cases | Controls | Fluid | Study type | miR | Sensitivity | Specificity | Method |
|---|---|---|---|---|---|---|---|---|
| Roth[70] 2011 | 20 | 20 | Blood | Diagnostic | miR-128, miR-125 (down) | 83% | 79% | Taqman low density array (Taqman LDA) |
| Wang[100] 2012 | 10 | 10 | Plasma | Diagnostic<br>Prognostic | miR-21 (up)<br>miR-128 (down) | 90% | 100% | Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) |
| Yang[68] 2013 | 177 | 137 | Serum | Diagnostic | miR-15b, miR-23a, miR-133a, miR-150, miR-197, miR-548b-5b (down) | 88% | 97% | Taqman LDA |
| Manterola[101] 2014 | 75 | 55 | Serum | Diagnostic | miR-320, miR-574-3p, RNU6-1 (up) | 87% | 86% | Taqman LDA |
| Wu[102] 2015 | 83 | 69 | Serum | Diagnostic | miR-29 (down) | 68% | 77% | qRT-PCR |
| Sun[103] 2015 | 151 | 53 | Serum | Diagnostic | miR-128 (down) | 86% | 88% | qRT-PCR |
| D'Urso[104] 2015 | 30 | 30 | Plasma | Diagnostic | miR-15b (up) | 98% | 98% | qRT-PCR |
| Zhi[69] 2015 | 90 | 110 | Serum | Diagnostic<br>Prognostic | miR-15b-5p, miR-16-5p, miR-19b-3p, miR-20a-5b, miR-106a-5p, miR-130-3p, miR-181b-5p, miR-208a-3p (up) | 93% | 92% | Taqman LDA |
| Lai[71] 2015 | 136 | 50 | Serum | Diagnostic<br>Prognostic | miR-210 (up) | 91% | 72.5% | qRT-PCR |
| Zhang[105] 2015 | 50 | 51 | Plasma | Diagnostic<br>Prognostic | miR-221, miR-222 (up) | 73%<br>85% | 80%<br>87% | qRT-PCR |
| Shao[106] 2015 | 70 | 70 | Plasma | Prognostic | miR-454 (up) | 99% | 82% | qRT-PCR |
| Wei[107] 2016 | 33 | 33 | Serum | Diagnostic | miR-125 (down) | 78% | 75% | qRT-PCR |
| Regazzo[108] 2016 | 15 | 10 | Serum | Diagnostic | miR-497, miR-125b (down) | 89% | 67% | qRT-PCR |
| Xiao[109] 2016 | 112 | 54 | Plasma | Diagnostic<br>Prognostic | miR-182 (up) | 58% | 85% | qRT-PCR |
| Yue[64] 2016 | 64 | 45 | Serum | Diagnostic<br>Prognostic | miR-205 (down) | 86% | 92% | qRT-PCR |
| Siegal[66] 2016 | 28 | | Serum | Monitoring | miR-10b, miR-21 | ns | ns | qRT-PCR |
| Huang[110] 2017 | 100 | 50 | Serum | Diagnostic<br>Prognostic | miR-376a<br>miR-376b<br>miR-376c (down) | 81%<br>82%<br>90% | 82%<br>78%<br>50% | qRT-PCR |
| Zhao[111] 2017 | 106 | | Serum | Prognostic | miR-106a-5p, miR-182, miR-145-5p (overall survival) miR-222-3p, miR-20a-5p, miR106a-5p, miR-145-5p, miR-182 (progression-free survival) | ns | ns | NGS |
| Ebrahimkhani[47] 2018 | 12 | 10 | Serum exosomes | Diagnostic | miR-182-5p, miR-486-5p (up) miR-328-3p, miR-339-5p, miR-340-5p, miR-485-3p, miR-543 (down) | AUC 91.3% | ns | Illumina RNA NGS |
| Swellam[65] 2019 | 20 | 20 | Serum | Diagnostic<br>Prognostic | miR-221<br>miR-222 | 90%<br>90% | 90%<br>85% | qRT-PCR |
| Morokoff[67] 2020 | 91 | 17 | Serum | Diagnostic<br>monitoring | miR-320e, miR-223,<br>miR-21 | 99.8% | 97.8% | Nanostring, ddPCR |

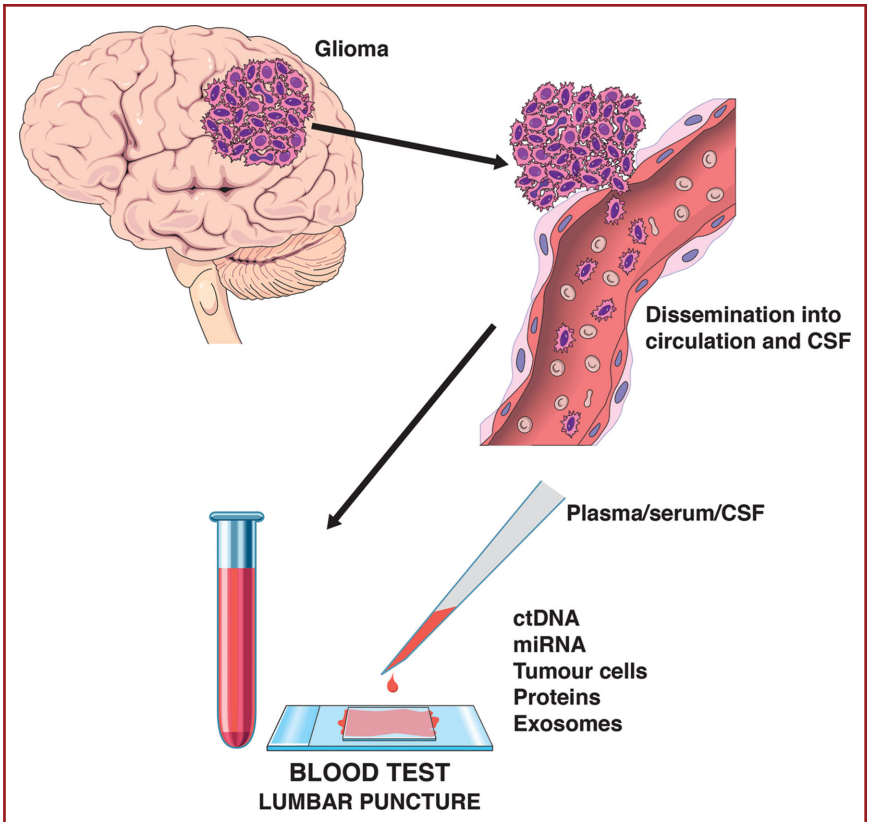


**FIGURE 1.** *Circulating factors are disseminated into the systemic circulation through several potential mechanisms including endothelial disruption, as whole tumor cells, exosomes, or via carrier molecules. Factors are also secreted directly into the CSF. Subsequently, serum, plasma, or CSF can be sampled and analyzed for one or more biomarkers.*

this screening method, 16% of symptomatic patients would be falsely reassured by a negative result. Since this test is designed for symptomatic patients after presentation to a clinic, it is unlikely to significantly change the time to initial diagnosis.

## Tissue Diagnosis and Tumor Subclassification

Liquid biopsy has been considered as an alternative to direct surgical tissue sampling if it is not possible due to tumor location or patient comorbidities with high risk. Currently, it is unlikely that liquid biopsy will replace histopathology for initial tumor diagnosis in the routine setting. Surgery is usually required to reduce mass effect and tumor burden. Two potential roles of liquid biopsy may be to assist in diagnosis of tumors located in eloquent or nonoperable locations such as the brainstem, or as a companion in diagnostics by identifying high-risk groups within LGGs that may benefit from early adjuvant treatment.

## Molecular Characterization

Accurate molecular profiling is essential for diagnosis, prognostication, and potential therapeutics in all cancer types, including glioma. A circulating biomarker has the advantage of being able to be repeated at multiple time points, providing "real-time" genomic information that can overcome temporal and spatial heterogeneity of malignancies.[37,90] Additionally, as cancer therapeutics pivot towards targeted treatments, the blood-based stratification of patients into clinical trials is becoming an increasing important application of liquid biopsies. Currently, 2 ongoing trials, the Tumour Characterisation to Guide Experimental Targeted Therapy and Guardant Originates in ZIpangu Liquid Biopsy Arrival trials, have matched patients with advanced solid organ tumors to clinical trials based on ctDNA analysis using multigene cancer panel sequencing.[36,91] Early reports from both trials have found good concordance between tumor tissue and ctDNA for identification of actionable mutations; however, patients with glioma were not included in either trial. Miller et al[28] reported the ability to monitor genomic evolution via CSF in 5 glioma patients following surgery. However, the lack of effective targeted treatments, difficulties of routine CSF sampling, and low concentrations of glioma ctDNA within the plasma are current challenges in applying this clinically.

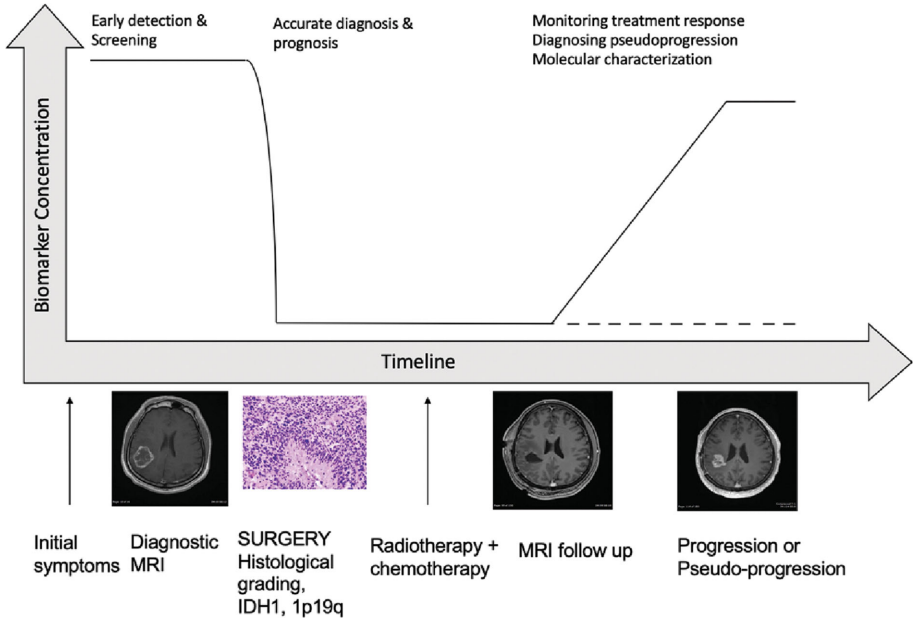


**FIGURE 2.** *Theoretical patient timeline demonstrating the potential clinical applications of a circulating biomarker in glioma. Biomarker levels are elevated at the time of diagnosis followed by a reduction after surgery. Levels remain low during the monitoring period until disease recurrence, which is confirmed by magnetic resonance imaging findings. The dashed line indicates low levels of biomarker concentration in the presence of pseudoprogression, suggesting a role in differentiating between this treatment effect and tumor progression.*

**TABLE 4. Summary Table of Advantages and Disadvantages of Each Circulating Biomarker Type**

| Circulating factor | Advantages | Disadvantages |
|---|---|---|
| CTCs | Highly specific<br>Allows molecular classification | Requires elaborate isolation techniques with no standard method of glioma CTCs classification rare |
| ctDNA | Highly specific<br>Allows molecular classification<br>Known isolation methods<br>Higher circulating levels than CTCs | Low sensitivity of detection<br>May only represent a subpopulation of tumor cells |
| Exosomes | Allows analysis of contents (RNA, DNA, proteins)<br>Able to cross the blood-brain barrier | High background of nontumor exosomes in the circulation<br>Nonstandardized methods of isolation |
| miRNA | Higher sensitivity of detection<br>May aid in prognostication and monitoring of glioma progression | Nonspecific<br>No standard methods of isolation and normalization |
| Protein | Protein expression directly related to cellular functions | Nonspecific and complex background influenced by other pathological, immunological, or physiological states |

## Monitoring

The ability of circulating biomarkers to monitor tumor status during the follow-up phase more reliably than imaging has been well reported in breast, melanoma, and lung cancers[32,37,92]; however, there is scarce literature on this application in glioma. Bernardi et al[80] and Vietheer et al[77] found no relationship between tumor status and the nonspecific protein markers YKL-40 and GFAP, respectively, during the monitoring period. Circulating immunological markers including cytokines, T-cell, and blood-based mutational burden have been used to predict benefit, monitor response, and identify the mechanism of resistance following immunotherapy.[87,93,94] There are only a few reports on

circulating immune markers in patients with brain tumors[95,96]; however, as progress is made in immunotherapies for glioma, these markers may become important tools for monitoring response to treatment.

Our research group has recently reported exploratory studies on the potential utility of a diagnostic serum miRNA signature as a monitoring biomarker for tumor status in the postoperative period that may be useful in distinguishing true progression from pseudoprogression, especially in the setting of bevacizumab therapy.[66,67] However, these biomarkers need to be further validated in carefully planned trials to determine if they can substantially contribute to the clinical management and decision making for glioma patients.

## SUMMARY

Identifying an accurate circulating biomarker for use in glioma patients is a complex but potentially highly rewarding area of interest in neuro-oncology. Many different methods have been suggested, each with their advantages and disadvantages, with no single biomarker test seemingly optimal (Table 4). Although population screening for brain tumors may be appealing, the clinical application of a circulating biomarker in glioma patients at this stage appears to be in tumor subclassification, molecular characterization, and monitoring for tumor progression. Further understanding of tumor biology and advances in detection techniques may lead to identification of an ideal biomarker for all gliomas; however, the heterogeneous nature suggests that a combination of approaches may be required to provide the many clinical benefits. Additionally, the field of radiogenomics and advanced imaging techniques is expanding with potential synergistic benefits of using both improved radiological techniques and circulating biomarkers. Combined research efforts in biobanking tissue, plasma, serum, and other body fluids are essential to increase our understanding in this rapidly expanding field.

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