ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

Liquid Biopsy for Glioblastoma: Emerging Biomarkers, Clinical Utility, Current Challenges and Future Perspectives - A Narrative Review

Usha Topalkatti[1]; Jhansi Mani Mahadeva[2]

[1]Spartan Health Science University, St. Lucia
[2]Malla Reddy Hospital and Medical College, Hyderabad

Publication Date: 2026/08/19

Abstract:

- ***Background***

Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults and is characterized by extensive molecular and spatial heterogeneity, diffuse infiltration, and frequent recurrence. Current diagnosis and disease monitoring rely primarily on tissue pathology and magnetic resonance imaging (MRI). However, tissue sampling is invasive and may not fully capture the heterogeneous and evolving molecular landscape of the tumor, while MRI may have difficulty distinguishing recurrent disease from treatment-related changes. Liquid biopsy has emerged as a minimally invasive approach for detecting tumor-derived material in biological fluids and may provide opportunities for repeated molecular assessment throughout the disease course.

- ***Objective***

This narrative review summarizes the current evidence regarding liquid biopsy in glioblastoma, with emphasis on circulating tumor DNA, cell-free DNA, circulating tumor cells, microRNAs, extracellular vesicles, and other circulating biomarkers. It further examines their potential applications in molecular characterization, diagnosis, prognostic assessment, treatment-response monitoring, and detection of recurrence.

- ***Methods***

A narrative literature review was conducted using PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. Literature addressing glioblastoma, liquid biopsy, circulating tumor DNA, cell-free DNA, circulating tumor cells, microRNAs, extracellular vesicles, exosomes, cerebrospinal fluid biomarkers, treatment monitoring, and recurrence was identified and critically synthesized. Original research studies, systematic reviews, meta-analyses, and relevant contemporary reviews were considered. Particular attention was given to evidence regarding blood- and cerebrospinal-fluid-derived biomarkers and their potential clinical applications.

- ***Results***

Current evidence indicates that liquid biopsy may provide minimally invasive molecular and longitudinal information in patients with glioblastoma. Circulating tumor DNA and cell-free DNA have been investigated for detection of tumor-associated genetic and epigenetic alterations, while circulating tumor cells, microRNAs, and extracellular vesicles represent additional potential sources of diagnostic and prognostic information. Cerebrospinal fluid may provide greater detectability of tumor-derived material than peripheral blood in selected patients, whereas blood-based approaches offer advantages for repeated and less invasive sampling. Emerging studies also suggest potential applications in monitoring treatment response, tumor evolution, and recurrence. Nevertheless, clinical implementation remains constrained by low circulating tumor fractions, the blood–brain barrier, tumor heterogeneity, methodological variability, and limited prospective validation.

- ***Conclusion***

Liquid biopsy represents a promising complementary approach to conventional tissue pathology and neuroimaging in glioblastoma. Its greatest future value may lie in longitudinal molecular monitoring and integration with MRI, radiomics, artificial intelligence, and other molecular profiling approaches. Standardization of analytical methods and prospective multicenter validation will be necessary before liquid biopsy can be routinely incorporated into clinical practice.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

Keywords: *Glioblastoma; Liquid Biopsy; Circulating Tumor DNA; Cell-Free DNA; Circulating Tumor Cells; MicroRNA; Extracellular Vesicles; Cerebrospinal Fluid; Biomarkers; Precision Medicine*.

How to Cite: Usha Topalkatti; Jhansi Mani Mahadeva (2026) Liquid Biopsy for Glioblastoma: Emerging Biomarkers, Clinical Utility, Current Challenges and Future Perspectives - A Narrative Review. *International Journal of Innovative Science and Research Technology*, 11(8), 1064-1071. https://doi.org/10.38124/ijisrt/26aug582

I. INTRODUCTION

Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults and remains associated with substantial morbidity and mortality despite advances in neurosurgical techniques, radiotherapy, chemotherapy, molecular classification, and supportive care. The biological behavior of GBM is characterized by marked intratumoral heterogeneity, diffuse infiltration into surrounding brain tissue, treatment resistance, and dynamic molecular evolution. These characteristics create major challenges for accurate tumor characterization, prognostic assessment, and longitudinal monitoring.

The diagnosis and molecular classification of GBM traditionally depend on surgical tissue acquisition followed by histopathological and molecular evaluation. Tissue remains essential for establishing the diagnosis and identifying clinically relevant molecular alterations. However, a biopsy samples only a limited region of a heterogeneous tumor and may therefore fail to represent the complete spatial diversity of tumor populations. Repeated surgical sampling is also invasive and generally impractical during longitudinal disease monitoring. These limitations have stimulated interest in minimally invasive methods capable of providing molecular information throughout the disease course.

MRI remains the principal imaging modality for diagnosis, surgical planning, treatment assessment, and surveillance. Conventional MRI provides important anatomical information regarding tumor location, enhancement, edema, mass effect, and treatment-related changes. Nevertheless, imaging alone may not adequately reflect the molecular state of the tumor. In particular, distinguishing recurrent or progressive GBM from treatment-related changes such as pseudoprogression or radiation-related injury can be challenging. Liquid biopsy has therefore emerged as a potential complementary source of biological information that may supplement radiological and pathological assessment.

Liquid biopsy refers to the detection and analysis of tumor-derived material present in biological fluids. In GBM, investigated analytes include circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), circulating tumor cells (CTCs), microRNAs and other circulating RNAs, extracellular vesicles (EVs), exosomes, proteins, and methylation-associated biomarkers. These analytes can potentially provide information regarding tumor genetics, epigenetics, biological activity, treatment response, and disease evolution.

The concept is particularly attractive in GBM because molecular assessment could theoretically be repeated at multiple time points without requiring repeated craniotomy or stereotactic biopsy. Serial analysis may provide a dynamic representation of tumor evolution and potentially identify molecular changes before they become evident on conventional imaging. CSF may be particularly informative because of its anatomical proximity to intracranial tumors, whereas peripheral blood offers important practical advantages because it can be collected repeatedly with minimal invasiveness.

However, liquid biopsy in GBM presents unique biological and technical challenges. The blood–brain barrier can limit the release of tumor-derived material into systemic circulation, resulting in low concentrations of ctDNA in plasma. Tumor heterogeneity may also produce variable biomarker expression, while differences in sample collection, processing, nucleic-acid extraction, sequencing platforms, and analytical thresholds may affect reproducibility. Consequently, although liquid biopsy has demonstrated considerable promise, its clinical role remains investigational in many applications.

This narrative review summarizes the current evidence regarding liquid biopsy in GBM, focusing on major biomarker classes, biological fluid sources, clinical applications, limitations, and future directions. Particular emphasis is placed on molecular characterization, prognostic assessment, treatment monitoring, recurrence detection, and the potential integration of liquid biopsy with emerging technologies such as radiomics and artificial intelligence.

II. METHODOLOGY

A narrative literature review was conducted to synthesize current evidence regarding liquid biopsy in glioblastoma. Relevant literature was identified through searches of PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar using combinations of the following terms: “glioblastoma,” “glioblastoma multiforme,” “liquid biopsy,” “circulating tumor DNA,” “cell-free DNA,” “circulating tumor cells,” “microRNA,” “circulating RNA,” “extracellular vesicles,” “exosomes,” “cerebrospinal fluid,” “molecular biomarkers,” “treatment response,” “recurrence,” and “prognosis.”

Priority was given to peer-reviewed original research studies, systematic reviews, meta-analyses, and contemporary review articles addressing the biological basis and clinical applications of liquid biopsy in GBM. Literature concerning ctDNA, cfDNA, CTCs, microRNAs, extracellular vesicles, exosomes, circulating proteins, and CSF-derived biomarkers was considered. Studies involving

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

broader glioma populations were included when their findings provided clinically relevant information applicable to GBM.

The literature was critically synthesized according to biomarker category and potential clinical application. Particular attention was given to diagnostic and molecular characterization, prognostic assessment, treatment-response monitoring, recurrence detection, and longitudinal assessment. Current methodological limitations and barriers to clinical translation were also evaluated. Because this was a narrative review, no formal meta-analysis or quantitative pooling of study outcomes was performed.

Narrative reviews benefit from clearly stating their importance, objectives, literature-search approach, referencing strategy, and level of supporting evidence; these principles are reflected in the SANRA framework for improving the quality of narrative review articles.

III. BIOLOGICAL BASIS OF LIQUID BIOPSY IN GLIOBLASTOMA

Tumors continuously release biological material into surrounding tissues and biological fluids through processes including apoptosis, necrosis, active secretion, and vesicle-mediated communication. In GBM, this material may include fragmented DNA, RNA, intact tumor cells, extracellular vesicles, proteins, and other molecules that reflect aspects of tumor biology. Liquid biopsy seeks to isolate and characterize these components to obtain information about the tumor without direct tissue sampling.

The biological utility of liquid biopsy depends strongly on the anatomical relationship between the tumor and the sampled fluid. Peripheral blood is readily accessible and can be repeatedly collected, but tumor-derived material may be present at very low concentrations because of the blood–brain barrier and dilution within a large pool of normal circulating nucleic acids. Plasma-based cfDNA sequencing in glioma has therefore historically demonstrated lower tumor fractions than those observed in many extracranial malignancies.

CSF represents a potentially more informative compartment because it is anatomically closer to the central nervous system. Studies have demonstrated that CSF can contain detectable tumor-derived DNA and other molecular biomarkers, supporting its use for molecular characterization and longitudinal monitoring. A systematic review and meta-analysis of ctDNA in adult glioma found higher apparent diagnostic performance in CSF than plasma, although the difference did not reach statistical significance in the pooled analysis.

The liquid-biopsy concept therefore extends beyond a single biomarker. Different analytes may provide complementary information: ctDNA may reflect tumor-specific genetic alterations, cfDNA may provide broader genomic or epigenomic information, CTCs may provide cellular information, and extracellular vesicles may carry DNA, RNA, and proteins protected within lipid membranes.

This multimodal nature makes liquid biopsy particularly attractive for a biologically heterogeneous tumor such as GBM. Rather than replacing tissue pathology, liquid biopsy may ultimately serve as a complementary longitudinal tool that captures molecular information between major clinical events.

IV. MAJOR LIQUID BIOPSY BIOMARKERS IN GLIOBLASTOMA

- *Circulating Tumor DNA and Cell-Free DNA*

Circulating cell-free DNA consists of extracellular DNA fragments released into biological fluids, whereas circulating tumor DNA represents the fraction originating directly from tumor cells. In GBM, ctDNA may contain tumor-specific mutations, copy-number alterations, methylation patterns, and other molecular features that can potentially be used for tumor characterization and monitoring.

Early studies demonstrated that detecting plasma ctDNA in brain tumors can be challenging because of the relatively low concentration of tumor-derived DNA. Nevertheless, large clinical datasets have shown that plasma ctDNA alterations can be detected in a subset of patients with GBM. In an analysis of 419 patients with primary brain tumors undergoing clinical plasma ctDNA testing, somatic alterations were identified in approximately half of patients overall, with detection in approximately 55% of glioblastoma cases.

The sensitivity of plasma-based approaches may depend strongly on tumor burden, molecular characteristics, assay design, and the quantity of plasma analyzed. A recent study investigating larger plasma volumes in IDH-wild-type GBM demonstrated that increasing cfDNA yield substantially improved ctDNA detection, illustrating how preanalytical factors can influence liquid-biopsy performance.

Specific molecular alterations investigated in plasma include IDH1, TERT promoter alterations, EGFR alterations, and EGFRvIII. A recent ddPCR study evaluating plasma samples from glioma patients demonstrated that selected alterations could be detected in plasma and that total cfDNA levels were associated with tumor grade and survival-related variables, although longitudinal changes did not consistently track postoperative progression.

CSF-based ctDNA may offer greater sensitivity in selected patients. Reviews of CSF liquid biopsy have emphasized its potential for molecular diagnosis, treatment selection, response assessment, tracking tumor evolution, and prognostication.

Thus, ctDNA represents one of the most extensively studied liquid-biopsy analytes in GBM, but its clinical value

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

depends on the biofluid sampled, tumor characteristics, assay sensitivity, and timing of collection.

➢ *Circulating Tumor Cells*

Circulating tumor cells are intact tumor cells that can enter the circulation and potentially provide information that cannot be obtained from cell-free nucleic acids alone. CTC analysis may theoretically permit evaluation of cellular phenotype, tumor-associated markers, molecular alterations, and treatment resistance.

However, CTC detection in GBM is technically challenging. The number of tumor cells entering the peripheral circulation can be extremely low, and the blood–brain barrier may restrict their release. Furthermore, conventional CTC enrichment strategies developed for systemic cancers may not be directly applicable to brain tumors because GBM cells may lack universally expressed epithelial markers.

Despite these limitations, CTCs remain an area of active investigation as a potential component of liquid biopsy. Their potential value may increase when combined with molecular assays capable of identifying tumor-specific genetic or phenotypic characteristics.

The broader liquid-biopsy literature supports the investigation of CTCs alongside circulating nucleic acids and extracellular vesicles rather than viewing any single analyte as sufficient for comprehensive tumor characterization.

➢ *MicroRNAs and Circulating RNA*

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and are involved in tumor proliferation, invasion, angiogenesis, metabolism, and treatment resistance. Their stability in biological fluids makes them attractive candidates for liquid-biopsy applications.

In GBM, altered miRNA expression patterns have been investigated in serum, plasma, CSF, and extracellular vesicles. Certain miRNAs have been associated with tumor presence, biological aggressiveness, prognosis, and therapeutic response. Because individual miRNAs may have limited specificity, current research increasingly considers panels or composite signatures rather than single-marker approaches.

A recent systematic review and meta-analysis specifically evaluating the diagnostic accuracy of miRNA-based liquid biopsies in GBM highlights the increasing maturity of this field while also reflecting the need to evaluate heterogeneous assay methodologies and biomarker signatures collectively.

Future applications may involve combining circulating miRNA signatures with ctDNA, EV cargo, imaging biomarkers, and clinical variables to improve diagnostic and prognostic performance.

➢ *Extracellular Vesicles and Exosomes*

Extracellular vesicles are membrane-enclosed particles released by cells into biological fluids and include several subtypes, with exosomes representing a well-studied population. EVs contain molecular cargo including DNA, mRNA, miRNA, circular RNA, proteins, and other molecules. Because their lipid membranes can protect molecular cargo from degradation, EVs have attracted substantial interest as liquid-biopsy biomarkers in GBM.

EVs are particularly interesting because they may reflect both tumor-cell biology and interactions between the tumor and its microenvironment. Studies have identified GBM-associated EV populations in plasma and have explored their potential for diagnosis, prognosis, and treatment monitoring.

Clinical studies have reported increased circulating EV concentrations in patients with GBM compared with controls. In one study, plasma EV concentrations were higher in GBM patients and decreased following surgery; higher EV levels were also associated with shorter overall and progression-free survival.

EV-associated molecular cargo may also provide tumor-specific information. For example, EGFRvIII RNA has been detected in circulating extracellular vesicles, and longitudinal measurements in a small number of patients demonstrated changes associated with disease progression and treatment response.

Recent work has focused on enriching tumor-derived EV subpopulations to improve specificity. Plasma EV research has identified multiple potential cargos, including miRNAs, mRNAs, circular RNAs, DNA, and proteins, while newer approaches use immunocapture, microfluidics, and other enrichment techniques to isolate tumor-associated vesicles.

Despite this promise, EV analysis faces substantial challenges, including vesicle heterogeneity, contamination by non-tumor-derived vesicles, inconsistent isolation procedures, and variability in biomarker expression. Standardization will therefore be essential for clinical translation.

V. BLOOD VERSUS CEREBROSPINAL FLUID AS LIQUID-BIOPSY SOURCES

The choice of biological fluid is a major determinant of liquid-biopsy performance in GBM.

Peripheral blood is the most convenient source because venipuncture is minimally invasive, inexpensive, widely available, and suitable for repeated sampling. These characteristics make blood particularly attractive for longitudinal monitoring. However, the concentration of tumor-derived material in plasma can be low because of the blood–brain barrier and dilution by abundant DNA and RNA released from non-tumor tissues.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

CSF may provide a richer source of tumor-derived material because of its anatomical proximity to intracranial tumors. CSF liquid biopsy has been investigated using ctDNA, EVs, miRNAs, proteins, and other biomarkers and may provide information regarding molecular alterations and tumor evolution.

The comparative evidence for ctDNA suggests that CSF can offer higher analytical performance than plasma in selected glioma populations. However, CSF collection generally requires lumbar puncture or another invasive procedure and may not be appropriate for every patient. Consequently, the optimal clinical strategy may ultimately involve selecting the biological fluid according to the clinical question, disease location, patient condition, and required monitoring frequency.

Rather than considering blood and CSF as competing approaches, future research should evaluate them as complementary sources of biological information.

VI. CLINICAL APPLICATIONS OF LIQUID BIOPSY IN GLIOBLASTOMA

- *Molecular Characterization*

One of the most important potential applications of liquid biopsy is non-invasive molecular characterization. Genetic alterations detected in ctDNA or other tumor-derived material may provide information about tumor biology when tissue is unavailable, insufficient, or difficult to obtain.

Potentially informative alterations include IDH, TERT promoter, EGFR, EGFRvIII, and other genomic changes. Plasma ctDNA studies have demonstrated that molecular alterations can be detected in a subset of patients with GBM, while CSF ctDNA may provide greater sensitivity for intracranial disease.

However, liquid biopsy should currently be regarded as complementary to tissue-based diagnosis rather than a replacement for histopathology. Its future role may be particularly valuable when tissue is limited or when molecular reassessment is required during disease progression.

- *Prognostic Assessment*

Liquid-biopsy biomarkers may also provide prognostic information. Total cfDNA levels, ctDNA detection, EV concentrations, and specific molecular signatures have been associated with clinical outcomes in different studies.

For example, studies of plasma EVs have reported associations between elevated EV concentrations and shorter overall and progression-free survival.

Similarly, plasma cfDNA levels have been investigated as potential indicators of tumor burden and prognosis. However, associations vary among studies, and biomarkers should not yet be interpreted as universally validated prognostic tools.

The greatest potential may lie in multimarker models that integrate liquid-biopsy measurements with established clinical variables, molecular classification, and imaging findings.

- *Treatment-Response Monitoring*

Longitudinal monitoring is one of the most attractive applications of liquid biopsy because blood or CSF can potentially be collected repeatedly during treatment.

Changes in ctDNA or other circulating biomarkers may reflect alterations in tumor burden or molecular activity. EV-associated biomarkers have similarly been investigated for changes during treatment. In one study of EGFRvIII-containing circulating EV RNA, longitudinal measurements increased with progression and decreased following treatment in a small number of patients, providing proof-of-concept for dynamic monitoring.

However, the relationship between circulating biomarker levels and radiological response is not always straightforward. Postoperative changes, treatment-related cell death, biomarker clearance, and temporal differences between molecular and radiological responses may influence interpretation.

Therefore, liquid biopsy is most likely to become clinically useful as a complementary tool alongside MRI rather than as an independent replacement for imaging.

- *Recurrence and Progression Monitoring*

Recurrent GBM remains a major clinical challenge. MRI is essential for surveillance but may provide ambiguous findings after surgery, radiotherapy, and chemotherapy. In particular, pseudoprogression and radiation necrosis can resemble recurrent tumor.

Liquid biopsy may potentially provide molecular evidence of tumor activity during periods when imaging is inconclusive. ctDNA and EV-associated biomarkers are being investigated for their ability to identify molecular changes associated with progression.

The ability to detect recurrence before clear radiological progression would represent a major clinical advance. However, this application requires prospective longitudinal studies demonstrating that molecular detection provides meaningful lead time and improves patient outcomes.

- *Detection of Molecular Evolution and Treatment Resistance*

GBM evolves under therapeutic pressure. Tumor populations that survive initial treatment may acquire or select for molecular characteristics associated with resistance.

Serial liquid biopsy could theoretically provide a dynamic representation of this evolution. Repeated analysis of ctDNA, EV cargo, or circulating RNA may reveal

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

emerging molecular alterations that are not represented in the original surgical specimen.

This could eventually support treatment adaptation and selection of patients for molecularly targeted clinical trials. CSF ctDNA is particularly promising in this context because it may capture tumor-derived genomic information more effectively than plasma in selected patients.

At present, however, most applications remain investigational and require validation before molecular changes detected in liquid biopsy can routinely guide treatment decisions.

VII. INTEGRATION OF LIQUID BIOPSY WITH IMAGING AND ARTIFICIAL INTELLIGENCE

The future of liquid biopsy may not involve using a single biomarker in isolation. Instead, integration with imaging, radiomics, artificial intelligence, and molecular pathology could provide a multidimensional representation of tumor biology.

MRI provides spatial information regarding tumor location, morphology, enhancement, edema, and treatment-related changes. Radiomics can convert imaging information into quantitative features describing tumor heterogeneity and spatial characteristics. Liquid biopsy provides complementary molecular information that may change over time.

Artificial intelligence and machine-learning methods could potentially integrate these heterogeneous data sources into predictive models. For example, an integrated model could combine MRI-derived radiomic features, ctDNA alterations, EV-associated RNA, molecular pathology, and clinical variables to estimate recurrence risk or treatment response.

Such multimodal approaches are conceptually attractive because GBM is simultaneously a spatially heterogeneous and molecularly dynamic disease. However, the development of these models requires large, standardized datasets and rigorous external validation.

VIII. LIMITATIONS AND CHALLENGES

Despite substantial progress, several scientific, technical, and clinical barriers currently limit the routine use of liquid biopsy in GBM.

- *Blood–Brain Barrier and Low Tumor Fraction*

The blood–brain barrier is a major limitation of peripheral liquid biopsy. Tumor-derived DNA may enter the circulation at very low concentrations, making detection difficult with conventional assays. Plasma cfDNA studies have therefore emphasized the importance of highly sensitive analytical methods and optimized sample collection.

Recent work suggests that increasing the amount of plasma analyzed may improve ctDNA detection in GBM, supporting the importance of preanalytical optimization.

- *Tumor Heterogeneity*

GBM contains multiple genetically and phenotypically distinct cellular populations. A circulating biomarker may therefore represent only a subset of the tumor and may not capture the complete molecular landscape.

Although liquid biopsy could theoretically sample tumor-derived material from multiple regions, low biomarker concentrations and variable shedding may limit this advantage.

- *Methodological Variability*

Differences in blood or CSF collection, processing time, storage, nucleic-acid extraction, sequencing platforms, PCR methodology, EV isolation, and analytical thresholds can produce substantial variability between studies.

This is particularly problematic for EV-based assays, where isolation and characterization methods vary considerably. Recent reviews emphasize the need for standardized EV protocols and improved tumor-derived EV enrichment.

- *Small and Retrospective Studies*

Much of the literature consists of relatively small cohorts, single-center studies, and retrospective analyses. These designs provide important proof-of-concept evidence but may not adequately establish clinical utility.

Larger prospective multicenter studies are necessary to determine whether liquid-biopsy biomarkers improve diagnostic accuracy, clinical decision-making, treatment monitoring, or patient outcomes.

- *Lack of Standardized Biomarker Panels*

There is currently no universally accepted liquid-biopsy biomarker panel for GBM. Different studies investigate different genes, miRNAs, proteins, EV markers, and combinations thereof.

Standardization of clinically relevant targets and analytical thresholds will be necessary for reproducible clinical implementation.

- *Clinical Interpretation*

Detecting a molecular alteration does not automatically establish clinical significance. A biomarker must demonstrate reproducibility, appropriate sensitivity and specificity, temporal relevance, and meaningful association with clinical outcomes before it can be incorporated into decision-making.

Therefore, future studies should prioritize clinically meaningful endpoints rather than focusing solely on analytical detection.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

➢ *Cost and Accessibility*

Highly sensitive sequencing, digital PCR, EV isolation, and specialized molecular assays may require considerable infrastructure and technical expertise. Cost and availability may therefore limit adoption, particularly in resource-constrained healthcare systems.

IX. FUTURE PERSPECTIVES

The future development of liquid biopsy in GBM will likely depend on combining biological innovation with analytical standardization and prospective clinical validation.

First, standardized protocols for blood and CSF collection, processing, storage, and biomarker analysis are required. Harmonization would allow results from different institutions to be compared more reliably and facilitate multicenter validation.

Second, greater attention should be directed toward multimodal biomarker strategies. Instead of relying on a single molecule, future assays may integrate ctDNA, methylation patterns, circulating RNA, EV cargo, proteins, and CTC-associated information.

Third, tumor-derived EV enrichment represents an important area of development. Recent studies have explored immunocapture, microfluidic methods, and other approaches to enrich tumor-associated vesicles from plasma, potentially improving the tumor specificity of EV-based assays.

Fourth, CSF liquid biopsy deserves continued investigation, particularly for patients in whom sufficient tumor-derived material cannot be detected in peripheral blood. CSF ctDNA may be especially valuable for molecular characterization and longitudinal monitoring of intracranial disease.

Fifth, artificial intelligence may facilitate integration of liquid-biopsy measurements with MRI, radiomics, clinical variables, and tissue-based molecular data. Machine-learning models could potentially identify complex biomarker combinations associated with recurrence, treatment response, or survival.

Finally, prospective multicenter clinical studies should determine whether liquid biopsy actually changes clinical management and improves patient outcomes. Analytical performance alone is insufficient; clinical utility, cost-effectiveness, reproducibility, and impact on treatment decisions must be demonstrated.

X. CONCLUSION

Liquid biopsy represents a promising and rapidly evolving approach to the molecular assessment of glioblastoma. By analyzing tumor-derived DNA, RNA, circulating tumor cells, extracellular vesicles, and other biomarkers in biological fluids, liquid biopsy may provide information that complements conventional tissue pathology and MRI.

Current evidence supports several potential applications, including molecular characterization, prognostic assessment, treatment-response monitoring, detection of recurrence, and tracking of tumor evolution. CSF may provide greater access to tumor-derived material in selected patients, whereas peripheral blood offers substantial practical advantages for repeated longitudinal sampling. Emerging EV-based technologies and highly sensitive ctDNA assays further expand the potential clinical applications of liquid biopsy.

Nevertheless, important limitations remain, including low circulating tumor fractions, the blood–brain barrier, intratumoral heterogeneity, methodological variability, lack of standardized protocols, and insufficient prospective validation. Consequently, liquid biopsy should currently be considered a complementary investigational tool rather than a replacement for tissue diagnosis or neuroimaging.

The most promising future direction may involve integrating liquid biopsy with MRI, radiomics, artificial intelligence, molecular pathology, and clinical data to create dynamic, multimodal models of glioblastoma biology. With standardized methodologies and rigorous prospective validation, liquid biopsy could become an important component of personalized and longitudinal neuro-oncology care.

REFERENCES

[1]. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016.
[2]. Baxter PA, et al. Significance of liquid biopsy in glioblastoma—a review. *J Biotechnol.* 2019.
[3]. Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. *Nature.* 2019.
[4]. Mair R, Mouliere F, Smith CG, et al. Measurement of plasma cell-free mitochondrial tumor DNA improves detection of glioblastoma in patient-derived orthotopic xenograft models. *Cancer Res.* 2019.
[5]. Miller AM, et al. Analysis of cell-free circulating tumor DNA in 419 patients with glioblastoma and other primary brain tumors. *Clin Cancer Res.* 2019.
[6]. Sullivan RJ, et al. Liquid biopsy in glioblastoma: opportunities, applications and challenges. 2019.
[7]. Nabors LB, et al. Extracellular vesicles as a platform for liquid biopsy in glioblastoma patients.
[8]. Miller AM, et al. Cerebrospinal fluid circulating tumor DNA as a liquid biopsy for central nervous system malignancies. *Curr Opin Oncol.* 2020.
[9]. Friedman JS, Hertz CA, Karajannis MA, Miller AM. Tapping into the genome: the role of CSF ctDNA liquid biopsy in glioma. *Neurooncol Adv.* 2022.
[10]. Garrido-Castro AC, et al. Liquid biopsy and glioblastoma. 2023.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug582

[11]. Mouliere F, et al. Clinical utility of plasma cell-free DNA in gliomas. *Neuro-Oncology Advances.* 2022.
[12]. Tumor-Derived Biomarkers in Liquid Biopsy of Glioblastoma. 2022.
[13]. The Current Landscape of Glioblastoma Biomarkers in Body Fluids. 2023.
[14]. Highly Sensitive EGFRvIII Detection in Circulating Extracellular Vesicle RNA of Glioma Patients. 2022.
[15]. Circulating extracellular vesicles as biomarker for diagnosis, prognosis, and monitoring in glioblastoma patients. 2024.
[16]. Chandran VI, et al. Extracellular vesicles in glioblastoma: a challenge and an opportunity. *NPJ Precis Oncol.* 2024;8:103.
[17]. Cerebrospinal Fluid Liquid Biopsies in the Evaluation of Adult Gliomas. 2024.
[18]. Circulating Tumor DNA in Adults With Glioma: A Systematic Review and Meta-Analysis of Biomarker Performance. 2022.
[19]. Plasma ctDNA liquid biopsy of IDH1, TERTp, and EGFRvIII mutations in glioma. 2024.
[20]. Impact of Higher Cell-Free DNA Yields on Liquid Biopsy Testing in Glioblastoma Patients. 2025.
[21]. Aibaidula A, et al. Plasma extracellular vesicles as liquid biopsies for glioblastoma: biomarkers, subpopulation enrichment, and clinical translation. *Int J Mol Sci.* 2025;26:11686.
[22]. Niznik T, et al. Exploring the diagnostic test accuracy of microRNAs as potential biomarkers for glioblastoma: systematic review and meta-analysis. *Neurosurgery*. 2025.
[23]. Liquid Biopsy-Derived Tumor Biomarkers for Clinical Applications in Glioblastoma. 2025.
[24]. Extracellular vesicles as liquid biopsy tools for personalized therapeutic goals in glioblastoma. *Brain.* 2026.
[25]. Baethge C, Goldbeck-Wood S, Mertens S. SANRA—a scale for the quality assessment of narrative review articles. *Res Integr Peer Rev.* 2019;4:5.