Review



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# The Use of Circulating Tumor DNA Assays and Liquid Biopsies in Pediatric Brain Tumors: A Review

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**Abstract:** Pediatric brain tumors are the leading cause of cancer-related death in children. Diagnosis currently relies on surgical biopsy to obtain tumor tissue for histological analysis. There is a great need for less invasive approaches to molecularly profile tumors and monitor therapeutic response. Liquid biopsy analysis of circulating tumor biomarkers in biofluids like cerebrospinal fluid (CSF) and blood offers a promising minimally invasive strategy. This review discusses recent advances in developing liquid biopsy assays using next-generation sequencing, digital PCR, and emerging techniques to detect circulating tumor DNA (ctDNA), cells (CTCs), and exosomes in pediatric brain tumors. The ability to use these circulating biomarkers for non-invasive diagnosis, therapeutic targeting, tracking response to therapy, and understanding resistance mechanisms is examined. Technical and clinical validation challenges to translate liquid biopsies into routine clinical practice are also discussed.

**Keywords:** medulloblastoma; circulating tumor DNA; exosomes; DIPG

## 1. Introduction

Pediatric brain tumors encompass a heterogeneous group of over 120 distinct histological subtypes, accounting for approximately 25% of childhood cancers [1]. The most common malignant pediatric brain tumors include medulloblastoma, high-grade glioma, ependymoma, and atypical teratoid/rhabdoid tumors (AT/RT) [2]. Despite advances in multimodal therapy over past decades, pediatric brain tumors remain the leading cause of cancer-related death in children, with 5-year overall survival around 70% compared to 83% for other pediatric cancers [1,3].

The current standard-of-care for diagnosis and monitoring of pediatric brain tumors centers on neuroimaging and histopathological analysis of tumor tissue obtained via surgical biopsy or resection [4]. However, these approaches have significant limitations. Neuroimaging lacks accuracy in distinguishing post-treatment effects like radiation necrosis from residual/recurrent tumor [5]. Repeated surgical sampling is often not feasible or safe, especially for brainstem or deep seated tumors. Molecular characterization of the entire tumor landscape from a single biopsy specimen is also challenged by intra- and inter-tumor heterogeneity [6,7]. There is a critical need for less invasive diagnostic and monitoring approaches that enable genomic profiling of pediatric brain tumors to guide personalized therapy.

Liquid biopsy analysis of circulating tumor biomarkers in biofluids like cerebrospinal fluid (CSF) and blood has emerged as a promising minimally invasive strategy to address limitations of current management approaches for pediatric brain tumors [8–10]. Liquid biopsies analyze circulating tumor materials like cell-free DNA (cfDNA), circulating tumor cells (CTCs), exosomes, proteins, and microRNAs [11]. As surrogates for the tumor's molecular profile, circulating

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biomarkers allow for non-invasive diagnosis, therapeutic targeting, tracking response to therapy, and studying resistance mechanisms without repeated invasive biopsies. This review discusses recent advances in developing liquid biopsy assays to detect circulating biomarkers in pediatric brain tumors, their current and potential clinical utility, and the challenges that must be overcome to translate these technologies into routine clinical practice.

## Circulating Tumor DNA

Cell-free circulating tumor DNA (ctDNA) are short nucleic acid fragments released into circulation by apoptotic and necrotic tumor cells [12]. CtDNA carries the same genomic alterations as the parent tumor, enabling non-invasive detection of clinically relevant mutations without repeated biopsies [13,14]. Another major advantage of ctDNA analysis is that it captures DNA from multiple tumor sites, overcoming sampling bias from single site tumor biopsies of heterogeneous cancers [15].

Diffuse midline gliomas like diffuse intrinsic pontine glioma (DIPG) have been a focus for ctDNA analysis as their location often precludes surgery for obtaining tumor tissue. Several studies in DIPG patients showed droplet digital PCR (ddPCR) could reliably detect H3K27M mutations in cfDNA from CSF and plasma, facilitating non-invasive diagnosis and therapeutic monitoring [16–18].

Liu et al. evaluated the clinical utility of cerebrospinal fluid (CSF)-derived cell-free DNA (cfDNA) analysis by low-coverage whole-genome sequencing (lcWGS) to detect measurable residual disease (MRD) in 123 children with medulloblastoma enrolled on a prospective clinical trial (SJMB03) [19]. The authors collected 476 serial CSF samples at diagnosis, during therapy, and after treatment completion. Tumor-associated copy number variations (CNVs) in cfDNA were measured as a surrogate for MRD.

The study found MRD was detectable in 85% of metastatic and 54% of localized medulloblastoma cases at diagnosis. The number of MRD-positive patients declined with therapy, yet those with persistent MRD had significantly higher risk of progression [19]. Importantly, MRD detection preceded radiographic progression in 50% of relapsed cases by a median of 8 months. Serial cfDNA analysis revealed divergent CNVs at relapse versus diagnosis in 80% of evaluable cases, suggesting clonal evolution [19]. Two cases highlighted the utility of cfDNA profiling to retrospectively detect dual pathologies (medulloblastoma with high-grade glioma) and identify the relapsed tumor clone at diagnosis.

The authors conclude CSF-derived cfDNA analysis has clinical utility for MRD detection and predicting treatment response in pediatric medulloblastoma. Findings support incorporating cfDNA evaluation into future clinical trials to guide personalized therapy based on molecular response [19]. This study provides a foundation for CSF liquid biopsy analysis in CNS cancers. Ongoing research must address assay sensitivity, standardization, and prospective validation.

For other pediatric brain tumors, personalized ctDNA assays using patient-specific mutations identified via tumor sequencing have shown the ability to track ctDNA dynamics in medulloblastoma, ependymoma, CNS neuroblastoma and embryonal tumors [20]. Besides primary tumors, whole exome sequencing of CSF ctDNA was able to detect clinically relevant alterations in 76% of CNS metastasis cases from extracranial solid tumors [21]. Methylation profiling of ctDNA has also revealed epigenetic signatures that improve diagnosis and classification of pediatric CNS tumors [22].

Overall, ctDNA assays using advanced next-generation sequencing, digital PCR, and epigenetic techniques have demonstrated potential for providing molecular stratification of pediatric brain tumors in a non-invasive manner. This enables more personalized therapeutic decisions tailored to each child's tumor genomic and epigenetic profile. However, ctDNA levels are often low, particularly for small or well-encapsulated tumors, posing challenges for detection and accurate quantification [23]. Even highly sensitive ddPCR assays can have false negatives. Larger clinical validation studies are needed to establish criteria and thresholds for clinical interpretation of ctDNA results across diverse pediatric brain tumor types. Standardization of sample collection, processing protocols, and analytical validation of assays at each center is also critical prior to widespread clinical implementation.

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## Circulating Tumor Cells

Intact circulating tumor cells (CTCs) offer a complementary source of tumor material to ctDNA for liquid biopsy analysis, providing a more complete picture of the cancer genome and transcriptome [24]. CTCs allow assessment of cellular phenotypes and drug sensitivity for guiding targeted therapy [25]. However, isolation and detection of rare CTCs presents technical hurdles, particularly for non-epithelial cancers like brain tumors where well-defined surface markers are lacking [26,27]. Recent progress has been made in developing microfluidic platforms to capture viable CTCs from peripheral blood of glioblastoma patients, though further technology development is needed to improve CTC recovery for use in pediatric cases [28,29]. Analysis of CTC clusters rather than single CTCs has also shown promise for improved yield and ability to culture cells ex vivo for functional testing [30].

## Circulating Tumor Exosomes & MicroRNAs

In addition to ctDNA and CTCs, exosomal vesicles and microRNAs (miRNAs) released by tumor cells are being explored as circulating biomarkers for pediatric brain tumors [31,32]. Exosomes contain DNA, RNA, and proteins that reflect molecular signatures of the tumor [33]. miRNA transcripts regulate gene expression and can be detected in biofluids as potential biomarkers of diagnosis, subtype, and prognosis [34–36]. However, standardized methods for isolation and analysis have not yet been established. Future studies must determine the clinical value and validity of these circulating biomarkers relative to ctDNA.

## Tracking Response to Therapy & Resistance Mechanisms

A major potential clinical utility of liquid biopsies is tracking response to therapy and unraveling resistance mechanisms through longitudinal genomic profiling of circulating biomarkers [8,37]. Serial analysis of ctDNA/CTC dynamics can detect emergence of resistance mutations or shifts in clonal populations during therapy that may evade detection on imaging [38,39]. Several studies in DIPG and medulloblastoma patients have shown changes in ctDNA levels correlate with disease status on imaging and outcomes [16,18,40]. Whisker plot digital PCR was able to differentiate pseudoprogression from true progression in DIPG based on distinct ctDNA patterns [41].

For relapsed medulloblastoma, whole exome sequencing of serial plasma samples revealed convergent evolutionary shifts leading to SKMYC/RUNX2 translocations as a resistance mechanism [42]. Integrated genomic and transcriptomic profiling of sequentially collected ctDNA could provide crucial insights into clonal evolution underlying therapeutic resistance and disease progression in pediatric brain tumors to guide next line targeted therapies [43]. Liquid biopsies thus have potential to provide molecular guidance for adapting therapy in real-time to attack the cancer's evolving weaknesses.

## Current Challenges and Future Outlook

Despite tremendous promise, several challenges remain to be addressed before liquid biopsies can be widely integrated into routine clinical care for pediatric neuro-oncology patients. Detection sensitivity and specificity of circulating biomarkers, particularly low abundance ctDNA, remains variable. Sample size requirements, specialized assays, and dedicated bioinformatics expertise may restrict feasibility for routine use at many centers. Standardization of protocols for specimen collection, processing, and analytical validation of assays is essential to ensure consistent, high-quality results [44].

Most published studies have used small retrospective cohorts, limiting assessment of clinical utility and validity. Large prospective multicenter trials are critically needed to determine how best to integrate liquid biopsy platforms into clinical decision algorithms based on correlations with imaging, therapeutic response, and patient outcomes [45,46]. Cost-effectiveness and clinical utility of liquid biopsies compared to standard-of-care tissue biopsies must be established as well. Regulatory hurdles for approval of liquid biopsy assays will need to be overcome too.

Despite these challenges, liquid biopsies are poised to transform the diagnostic and therapeutic landscape for pediatric brain tumors. Rapid technological progress in ctDNA and CTC analysis, combined with disciplined clinical validation studies, can pave the way for regulatory approval,

clinical adoption, and insurance coverage of liquid biopsies. Non-invasive blood tests could soon provide a faster, safer and more comprehensive snapshot of a child's brain tumor compared to surgical biopsies. Liquid biopsy-directed therapeutic selection and monitoring to counter evolving resistance may one day be a reality. This could herald a new era of precision medicine guided by liquid biopsies to improve clinical outcomes for children with brain cancer.

## 5. Conclusions

Liquid biopsy through analysis of ctDNA has shown considerable promise as a minimally invasive approach to facilitate genomic characterization, therapeutic targeting, and monitoring of pediatric brain tumors. Assays using NGS, ddPCR and emerging platforms have been able to detect tumor-specific alterations and quantify tumor burden in biofluids of patients with various primary and metastatic pediatric brain malignancies. While early results are encouraging, larger prospective clinical studies are needed to validate sensitivity, clinical utility, and cost-effectiveness prior to routine integration of ctDNA testing into the standard-of-care management for pediatric CNS cancers. Standardization of methodologies and analytical validations will be important to achieve consistent and reliable results. As the technology matures, ctDNA-guided approaches have immense potential to enable more precise diagnosis, improve risk-stratification, and allow early detection of residual disease and relapse in an effort to deliver personalized therapies and improve outcomes for children with brain cancer.

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