Blood and cerebrospinal fluid biomarkers in neuro-oncology

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Purpose of review
The purpose of this review is to discuss the value of blood and CSF biomarkers in primary CNS tumors.

Recent findings
Several analytes can be assessed with liquid biopsy techniques, including circulating tumor cells, circulating cell-free tumor DNA, circulating cell-free RNA, circulating proteins and metabolites, extracellular vesicles and tumor-educated platelets. Among diffuse gliomas of the adult, ctDNA in blood or CSF has represented the most used analyte, with the detection of molecular alterations such as MGMT promoter, PTEN, EGFRVIII, TERT promoter mutation and IDH R132H mutation. In general, CSF is enriched for ctDNA as compared with plasma. The use of MRI-guided focused ultrasounds to disrupt the blood–brain barrier could enhance the level of biomarkers in both blood and CSF. The detection of MYD88 L265P mutation with digital droplet PCR and the detection of ctDNA with next generation sequencing represent the best tools to diagnose and monitoring CNS lymphomas under treatment. In meningiomas, the low concentration of ctDNA is a limiting factor for the detection of driver mutations, such as NF2, AKTs, SMO, KLF4, TRAF7, SMARCB1, SMARCE1, PTEN, and TERT; an alternative approach could be the isolation of ctDNA through circulating extracellular vesicles. Liquid biopsies are being used extensively for diagnosis and surveillance of diffuse midline gliomas, in particular with the detection of the driver mutation H3K27M. Last, specific methylome patterns in CSF may allow the distinction of glioblastomas from CNS lymphomas or meningiomas.

Summary
This review summarizes the current knowledge and future perspectives of liquid biopsy of blood and CSF for diagnosis and monitoring of primary CNS tumors.

Keywords
biomarkers, blood, central nervous system lymphomas, cerebrospinal fluid, diffuse gliomas, liquid biopsy, meningiomas, pediatric tumors

INTRODUCTION

Liquid biopsy in cancer patients aims at the detection, analysis and monitoring of tumor-derived analytes that circulate in biofluids, including blood and cerebrospinal fluid (CSF) [1]. Tumor-derived materials in these biofluids consist of circulating tumor cells (CTCs), cell-free DNA (cfDNA), cell-free RNA (cfRNA), tumor-specific proteins and metabolites, extracellular vesicles and tumor-educated platelets (TEPs).

In recent years, there has been an increasing interest for the application of liquid biopsies in both primary and secondary brain tumors with a huge number of studies. In this regard, the RANO (Response Assessment in Neuro-Oncology) Group has performed critical reviews on liquid biopsy in central nervous system (CNS) metastases [2], gliomas [3▪] and CNS lymphomas [4▪▪]. The usefulness of liquid biopsy has been reported in the setting of diagnosis, especially in case of difficult to assess locations for surgical biopsy and/or elderly patients with comorbidities, detection of minimal residual disease after surgery, early response or progression after radiotherapy, chemotherapy or targeted agents, identification of mechanisms of resistance and outcome prediction. Moreover, liquid biopsies can better recapitulate tumor heterogeneity as compared with small surgical specimens and, because of

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KEY POINTS

- Liquid biopsy of blood and CSF in primary CNS tumors may be useful in the setting of diagnosis, detection of minimal residual disease after surgery, early response or progression after radiotherapy, chemotherapy or targeted agents, and outcome prediction.
- Several analytes can be assessed with liquid biopsy techniques, including circulating tumor cells, circulating cell-free tumor DNA, circulating cell-free RNA, circulating proteins and metabolites, extracellular vesicles and tumor-educated platelets.
- The detection of driver molecular alterations in ctDNA by droplet digital PCR or NGS, such as MGMT promoter, PTEN, EGFRVIII, and IDH R132H mutations in diffuse gliomas, MYD88 L265P mutation in primary CNS lymphomas or H3K27M mutation in diffuse midline gliomas, now represents the major advantage of liquid biopsy.
- Global methylation profiling of ctDNA, either in blood of CSF, is an additional tool to differentiate primary CNS tumors such as glioblastomas, primary CNS lymphomas, meningiomas.

the less invasive nature, allow monitoring over time of the molecular changes in tumor cells, that occur spontaneously or induced by a selective pressure of specific targeted agents.

In this review, we discuss the different types of analytes in both blood and CSF, that can be assessed with liquid biopsy techniques, and may serve as biomarkers in primary CNS tumors.

BLOOD AND CEREBROSPINAL FLUID BIOMARKERS IN BRAIN TUMORS: GENERAL CHARACTERISTICS

Circulating tumor cells

CTCs represent subsets of the tumor cell population, released by primary or metastatic lesions into biofluids, and may retain specific molecular signatures of the original tumor. CTCs are retrieved from body fluids either as single cells or cell clusters, that can contain white blood cells, and be associated with poor prognosis [5]. In general, the abundance of CTCs in peripheral blood is low in comparison to normal cells. The identification and isolation of CTCs are commonly based on the presence of specific cell-surface epithelial markers or biophysical properties. The Food and Drug Administration (FDA)-approved cell-search system uses an antibody against epithelial cell adhesion molecule (EpCAM) and cytokines. This approach has been largely used in leptomeningeal metastases from solid tumors; conversely, it is not effective in gliomas as malignant cells adopt a mesenchymal phenotype instead of an epithelial one. Thus, new techniques are being used; however, their heterogeneity is a limiting factor in order to compare the results of the different studies.

Circulating cell-free tumor DNA

Cell-free tumor DNA (cfDNA) consists of DNA fragments that are released from cells via apoptosis, necrosis or budding DNA. Under physiological conditions, most of these DNA pieces are removed by phagocytosis, while in malignancies, due to the high cell turnover, elevated levels of ctDNA persist in biofluids. ctDNA carries tumor-specific alterations, such as copy number variations, chromosomal rearrangements, point mutations, and so forth. Importantly, less volume of biofluid is needed in comparison to CTCs. Moreover, due to the short-half-life (16 min to 2.5 h), ctDNA could be a real-time marker of changes of tumor burden following the different treatments.

Several studies have found that in primary CNS cancers, the level of ctDNA in blood is generally lower than in non-CNS tumors [6], and moreover, CSF is enriched for ctDNA as compared with plasma because of the limitations posed by the blood–brain barrier (BBB) [7–9]. One approach that is being investigated to enhance the level of biomarkers in blood and CSF is to disrupt the BBB by MRI-guided focused ultrasounds [10]. Different ctDNA detection techniques have been employed, in particular methylation-based polymerase change reaction (PCR), droplet digital PCR (ddPCR) and next-generation sequencing (NGS). In particular, ddPCR is a sensitive and accurate method that can detect single and rare somatic mutations but can detect known targets only through addition of targeted primers and probes. Conversely, NGS may detect unknown genetic alterations, and several platforms may be used for whole-genome, whole-exome or targeted generation sequencing. Recent technological advantages have allowed to perform a global methylation profiling of ctDNA in several brain tumor types [11,12]. Moreover, another epigenetic modification, that can be analyzed in liquid biopsy, is DNA hydroxymethylation [13].

Circulating cell-free RNA

Unlike cfDNA, which is released in the blood by necrotic cells, circulating cell-free RNA (cfRNA) can also be released by living cells through exosome-mediated signaling. Thus, cfDNA and cfRNA in the blood refer to two distinct populations whose

assessment could be combined [14]. Previous studies have been mainly focused on circulating micro-RNAs (miRNAs), that are highly stable and abundant in the blood, whereas few studies have investigated messenger RNA (mRNA) [14]. miRNAs are noncoding RNAs involved in posttranscriptional gene expression regulation through translational expression or mRNA destabilization. Thus far, the usefulness of miRNAs as liquid biopsy biomarkers is limited because of the lack of sensitive and reproducible methods of detection, and the utility of cfRNA methylation has not been fully explored.

Circulating proteins and metabolites

Increased secretion of proteins in cancer patients may yield to an elevated level of circulating proteins (membrane receptors and receptors ligands, growth factors, cytokines) in biofluids. Proteomic characterization of tumors via liquid biopsy is feasible by chromatography mass spectrometry.

Similar to proteomic studies, liquid biopsy-based metabolomics may be used to identify and quantify circulating metabolites, such as amino acids, carbohydrates, nucleosides, nucleotides, vitamins, fatty acids and lipids. Because of the high amount of lipids in the brain and their involvement in critical biological functions, lipidomic is an emerging approach in liquid biopsy of brain tumors, even if studies are still very few [15▪,16▪▪].

Extracellular vesicles

Extracellular vesicles are lipid-layer-bound vesicles released into the extracellular space by both normal and tumor cells and are found in biofluids. Extracellular vesicles secreted by tumor cells may be captured by neighboring and distant cells in the microenvironment and facilitate intercellular communications. Machine-learning classification of plasma-derived extracellular vesicles cargo showed 95% sensitivity and 90% specificity in detecting different cancers [17]. Extracellular vesicle content is heterogeneous, as they carry a large repertoire of cargos, including DNA, micro-RNA, mRNA, proteins, metabolites and lipids, together with some surface markers specific to the parental cell of origin. As a biomarker, extracellular vesicles offer a number of advantages: the double-layer lipid membrane allows to cross blood–brain barrier and protects from degradation by nucleases; the biological stability at different temperatures allows a storage without risk of degradation; last, the frequency of cancer mutations, that can be detected in the DNA inside extracellular vesicles, is higher than in ctDNA. Based on size, morphology and method of generation, extracellular vesicles are classified into exosomes, micro-vesicles and apoptotic bodies. Thus far, no standard protocols to specifically isolate and separate exosomes from micro-vesicles are available.

Tumor-educated platelets

Recently, TEPs have been added as another blood-based diagnostic and monitoring opportunity in cancer patients [18]. Platelets may have direct interactions with cancer cells, and also uptake RNA and proteins released by tumor cells [19].

BLOOD AND CEREBROSPINAL FLUID BIOMARKERS IN PRIMARY CENTRAL NERVOUS SYSTEM TUMORS OF THE ADULT

Diffuse gliomas

Research on CTCs in gliomas have been almost confined to glioblastoma with relatively few studies, small sample size and variable sensitivities. Several attempts have been made to characterize and detect CTCs in the blood with the use of antibodies to glial fibrillary acidic protein, amplification of EGFR and activity of telomerase enzymes or cocktails of antibodies, such as STEAM (SOX2, Tubulin beta-3, EGFR, A2B5 and c-MET) [20]. Recently, an interesting approach has been reported using rVAR2 (recombinant malaria VAR2CSA proteins), a ligand for the proteoglycan chondroitin in order to bind to tumor-specific oncofetal chondroitin-sulfate [21]. Overall, by using different technologies, the sensitivities to detect CTCs in the blood of high-grade gliomas and glioblastomas have ranged from 20.6 to 77% [3▪] with a specificity of 91% [22▪▪]. A potential usefulness of liquid biopsy with CTCs has been suggested in the differential diagnosis of pseudoprogression vs. progression or detection of relapse.

ctDNA in blood or CSF has represented thus far the most used analyte in gliomas both in retrospective and prospective studies. Several molecular markers have been detected, such as MGMT promoter, PTEN, EGFRVIII, EGFR, TERT promoter mutation, TP53, ERBB2, MET and IDH R132H mutation [3▪]. In these studies, sensitivity values ranged from 7.4 to 70% with median values around 60% for blood, and from 39 to 95% for CSF. In particular, the detection of the IDH1 R132H mutation in ctDNA of CSF could be of clinical importance in the management of IDH mutant lower grade gliomas, especially in the monitoring of treatment with IDH inhibitors, such as vorasidenib: however, this approach may not be always informative and needs to be improved [23,24▪], In this regard, CSF 2-hydroxyglutarate, the

product of IDH mutation, seems a more valuable biomarker [25▪].

Few studies have reported a correlation between ctDNA level in blood and tumor volume or contrast enhancement [26], or the usefulness in the monitoring after surgery and adjuvant therapies with radiation and alkylating agents [27]. Other studies described an association between CSF ctDNA and tumor location close to CSF reservoir or cortical surface [28], or imaging findings, such as tumor size or enhancement, tumor progression and tumor spread towards the ventricular or subarachnoid space [9].

The usefulness of a global methylation profiling of cfDNA, either in blood or CSF, has emerged as an additional tool to discriminate gliomas from other primary intracranial or extracranial tumors [29,30▪▪]. Moreover, a serum-based DNA methylation assay may provide an accurate longitudinal monitoring of gliomas under treatment, being able to differentiate pseudoprogression from tumor progression [31]. Last, lower grade gliomas release a smaller amount of ctDNA into CSF in comparison to glioblastoma [3▪].

There are few data on circulating cfRNA in gliomas of adult, and in particular on micro-RNA [32▪▪] and IncRNA [33▪]. Overall, miR-21 seems the most significant and reproducible biomarker.

Several circulating proteins in blood, such as angiogenesis-related proteins (vascular endothelial growth factor – VEGF, primary fibroblastic growth factor – FGF-2, etc.) or extracellular matrix proteins (matrix metalloproteinases – MMPs) or YKL-40 have been evaluated in gliomas with different grade of malignancy and correlated with relapse to therapy or outcome [34▪]. Assessing metabolic markers in glioblastoma is a recent avenue of research, also using artificial intelligence tools [35,36▪].

There is evidence that supports a role for extracellular vesicles in the diagnosis and monitoring of gliomas [37▪,38,39▪]. Extracellular vesicles from glioblastoma may be of help in early detection, prediction of prognosis, monitoring of changes of tumor volume after surgery and influence the microenvironment [40,41,42▪]. Several studies have highlighted the clinical value of extracellular vesicle quantification in glioblastoma that could be achieved also by imaging flow cytometry of fluorescent-labeled extracellular vesicles [43]. Other clinical applications of extracellular vesicles include detection of specific molecular alterations, such as EGFR VIII, IDH1 mutation proteins or miRNAs (e.g. miR-21).

Methylation profiling of glioblastoma-derived extracellular vesicles has been reported to identify the methylation classes of the original tumors, in particular, MGMT promoter methylation status [44].

Few data are available on the value of TEPs. A correlation between changes in platelet concentrations during therapy and outcome in GBM has been observed [45]. Also, TEP-specific RNA signatures of GBM patients were reported to correlate with tumor volume and recurrence vs. pseudoprogression [46].

Central nervous system lymphomas

Standard CSF studies include cytology, flow cytometry, immunoglobulin H gene rearrangement and protein characterization. The sensitivity of CSF cytology to identify lymphoma cells is low (2–32%), whereas flow cytometry increases sensitivity by two to three times. PCR for clonally rearranged immunoglobulin genes may be useful to detect a clonal B-cell population in the setting of negative cytology and flow cytometry. Several proteins are detectable in CSF [4▪▪], and CSF total protein level is associated with prognosis. Of particular importance are cytokine interleukin-10 (IL-10) and the chemokine CXCL13 that are overexpressed in CNS lymphomas in comparison to other brain tumors or inflammatory and demyelinating lesions [47]. Their levels may drop with response to treatment while increasing at relapse, and may be associated with PFS. Also, levels of interleukin-6 (IL-6) are generally higher than those seen in other brain tumors or inflammatory diseases [48]. The detection of IL-10 in the vitreous or aqueous humor is now of increasing importance for the diagnosis of primary central nervous system lymphoma (PCNSL) with ocular involvement [49]. The level of serum lactate dehydrogenase (LDH) is considered a factor associated with tumor burden in systemic high-malignancy lymphomas, but the value as a biomarker in PCNSL is limited.

The *MYD88* (myeloid differentiation primary response gene 88) L265P mutation is the most frequent and specific mutation (up to 88% of cases) in biopsies of PCNSL, being less frequent (30%) in systematic diffuse large B-cell lymphomas and absent in primary brain tumors and brain metastases [50].

The sensitivity for the detection of MYD88 mutation with PCR is 4–57% in the blood, and 63–92% in the CSF. MYD88 mutation is also detectable in 69% of vitreous aspirates of vitroretinal lymphomas, and may be considered as an additional biomarker in the diagnosis of PCNSL with ocular environment [51]. Administration of steroids prior to liquid biopsy may reduce the detection rate of MYD88 in the CSF [52].

The detection of ctDNA with NGS at diagnosis of PCNSL has a sensitivity of 24–78% in plasma, and 63.5–100% in the CSF with a specificity of 96–100% [4▪▪,53]. Importantly, the positivity in plasma before and after treatment correlates with relapse and a

shorter PFS and OS [54▪▪,55▪▪] and has been shown useful for monitoring the targeted therapy with ibrutinib [56,57].

A number of micro-RNAs could be potential biomarkers for diagnosis and prognosis [58▪], but the value needs to be confirmed in prospective studies.

DNA methylation profiling has not shown major differences between PCNSL and systemic diffuse large B-cell lymphoma (DLBCL), whereas eight methylation markers that could distinguish PCNSL from other primary malignant brain tumors (mainly GBM), have been identified [59].

Meningiomas

In contrast to other brain tumors, in meningiomas, the lack of a blood–brain barrier makes possible the passage of ctDNA in the blood; however, the low concentration has limited in most cases the detection of driver gene mutations, such as *NF2, AKT1, SMO, KLF4, TRAF7, PIK3CA, SMARCB1, SMARCE1, CDK2A/B, PTEN* and *TERT*. In a recent study [60], none of these mutations were detected in 11 grade 1 or 2 meningiomas, while in 2 out of 6 recurrent high-grade tumors, a NF2 mutation was detected in the blood. In another series [61▪], only 1 out of 28 grade 1 or 2 meningiomas had detectable mutations in the cfDNA of blood. Detectable levels of miR21 were found in the blood of meningioma patients [61▪], and moreover, miR497 has been suggested as a potential biomarker for high-grade meningiomas [62].

A DNA methylation signature unique to meningiomas, as compared with other brain tumors, has been discovered in the blood of 155 meningioma patients [63▪], thus allowing the creation by means AI of models for identifying and predicting tumor recurrence.

The isolation of ctDNA through circulating extracellular vesicles could be an alternative approach for liquid biopsy. Ricklefs *et al.* (2022) [64] showed that plasma extracellular vesicles were significantly higher in meningioma patients with grade 1–3 tumors (n.46) as compared with matched healthy controls (n.18); moreover, these levels dropped significantly after complete resection.

BLOOD AND CEREBROSPINAL FLUID BIOMARKERS IN PRIMARY CENTRAL NERVOUS SYSTEM TUMORS OF CHILDREN

Pediatric-type diffuse low-grade and high-grade gliomas

Liquid biopsies have been used quite extensively for diagnosis and surveillance of pediatric low-grade and high-grade diffuse gliomas [65–67,68▪]. In a series of 258 pediatrics patients, mostly high-grade gliomas, molecular alterations were detected in 9/46 CSF samples, and 3/230 plasma samples [69]. The presence of BRAF V600E mutation in blood and CSF of 29 pediatric low-grade gliomas has been reported [70]. Interestingly, in a small study in pediatric patients with Langerhans cell histiocytosis, the level of BRAF V600E ctDNA correlated with disease stage and treatment relapse. Thus, this molecular alteration holds promise to become a prognostic and predictive biomarker in liquid biopsy for monitoring BRAF-mutated entities [71]. In pediatric pilocytic astrocytomas, serum-specific miRNAs levels have been positively correlated with tumor size and response to therapy [72]. Recently miRNA-containing exosomes have been detected in pediatric gliomas [73].

Diffuse midline gliomas

In recent years, the techniques to detect driver mutations in CSF and blood of patients with diffuse midline gliomas have been standardized [74,75]. These patients are the best candidates for liquid biopsies because of the high risk of morbidity following surgical biopsies of brain stem.

Primary driver mutations have been detected in CSF ctDNA in a series of brainstem gliomas, including DIPG [76]. Of particular interest is the possibility to detect in ctDNA of CSF and blood the driver mutation H3K27M [77▪▪], whose levels have been correlated either with contrast-enhancing areas [78] or, more importantly, in NCT03416530 trial with PFS, and the possibility to distinguish pseudoprogression from true progression [79▪▪].

Medulloblastomas

A recent study has reported that ctDNA is more abundant in CSF than plasma, reflects the tumor genomic alterations, facilitates medulloblastoma-subgroup and risk stratification, and the detection of minimal residual disease (MRD) [80,81]. The evolution of CSF ctDNA over time may allow to predict the disease dissemination vs. a localized relapse [82–84,85▪]. Somatic mutations have been detected by ddPCR in eight medulloblastomas and three ependymomas [86▪]. CTCs could play a relevant role in disease staging and follow-up [87]. miRNA21 changes have been reported to regulate pathways of tumorigenesis, such as SHH and WHT [88]. Moreover, extracellular vesicle isolation from the cell media supernatant of medulloblastoma cell lines allowed the identification of specific extracellular vesicle-derived miRNA [89].

Table 1. Clinical trials on liquid biopsy in gliomas[a]

Adult gliomas				
Trial ID	**Type of study**	**Aim of the study**	***N***	**Endpoints**
NCT04931732 (circTeloDIAG)	Observational	To evaluate the performances of the diagnostic test 'circTeloDIAG' at time of initial surgery for gliomas	150	Primary: - sensitivity and specificity of the circTeloDIAG assay in finding IDH mutation, TERT mutation and a marker correlated with ATRX loss
NCT05133154 (GLIOLIPSY)	Observational Three cohorts: - Lower grade gliomas - High-grade gliomas - Nontumor disease	To evaluate the presence of CTCs in a preoperative blood sample for the three cohorts	50	Primary: - proportion of patients with CTCs (>0) in a preoperative sample for the three following groups Secondary: - Platelets RNA profile in a preoperative sample for the three groups - Number, characteristics of CTCs and platelets profile in a postoperative sample for the three groups
NCT05383872	Observational	To evaluate the safety and efficacy of targeted blood–brain barrier disruption with Exablate Model 4000 Type 2.0/2.1 for liquid biopsy in subjects with glioblastoma	57	Primary: - Adverse events - correlation between patterns obtained in the panel of biomarkers evaluated in the resected tumor tissue and/or biopsy and blood sample collected post-BBBD Secondary - to demonstrate at least a two-fold increase in circulating free DNA following BBBD
NCT05964153	Observational	Analysis of the ctDNA in blood patients affected by gliomas	10	Primary: - to correlate the results of the tissue samples with those of the blood samples, the concentration of ctDNA found, and the magnetic resonance images prior to the biopsies - to compare time, money, and the impact on the patient (recovery time after biopsies, pain, sequelae) between the liquid biopsy procedure and the conventional tissue biopsy procedure
NCT05536024	Observational (registry)	To combine radiomics and liquid biopsy technology to improve the diagnosis of glioma	500	Primary: - AUC value of prediction Dice coefficient for evaluating segmentation performance
NCT05630664 (SOPRANO)	Observational Two cohorts: - High-grade gliomas - Meningiomas	To evaluate the value of ctDNA in plasma as a marker of tumor evolution	90	Primary: - ctDNA correlation with PFS Secondary: - ctDNA correlation with OS - correlation between change in ctDNA concentration after surgery and PFS and OS correlation between change in ctDNA concentration 1 month after radiotherapy completion and tumor volume changes, as well as clinical status changes - ctDNA concentration changes at progression

Table 1 (*Continued*)

Adult gliomas				
Trial ID	**Type of study**	**Aim of the study**	***N***	**Endpoints**
NCT05695976 (GRETeL)	Observational	To define longitudinal genomic alterations in patients with glioblastoma To determine if plasma ctDNA or cell free DNA is associated with disease recurrence, survival, tumor characteristics, and/or peripheral immunosuppression	100	Primary: - Median cell free DNA and ctDNA concentration at preradiation and postradiation, as well as median change in cell free DNA/ctDNA concentration - Median levels of cell free DNA collected longitudinally after completion of radiation Secondary: - Median number of ctDNA fragments obtained from tumor tissue, preradiation serum specimen, and postradiation serum specimen, and healthy controls - association between degree and frequency of ctDNA shedding and clinical characteristics, such as molecular findings, histopathological characteristics, corticosteroid use, and bevacizumab use - to quantify the level of immune infiltration in the tumor, and assess its association with PFS, OS, and ctDNA detection - to assess the prognostic value of chromosomal instability/CAN burden, and assess its association with ctDNA from plasma - to determine if peripheral T-cell function and stemness or autoantibodies are association with ctDNA levels, or TME composition
NCT05925218	Observational	Feasibility of measuring both the burden and key molecular features of HGG through profiling of plasma ctDNA	70	Primary: - To detect ctDNA in samples from HGG patients before treatment - To measure changes in levels of specific ctDNA fragments following RT and CT
NCT04274283 (BRAIN MATRIX)	Observational (patient registry)	Evaluation of concordance of matched tumor, CSF, and blood samples	1000	Primary: - Time (date from biopsy) to integrated histological–molecular diagnosis (date of whole genome diagnosis and epigenomic classification)
NCT05281731	Observational	Ability to acquire tumor genetic and molecular signatures after opening the BBB using sonobiopsy device	40	Primary: - Feasibility of sonobiopsy as measured by change in ctDNA level - Number of matched mutations between the postsonobiopsy sample and the tumor tissue sample
Pediatric gliomas				
NCT05934630	Observational All children and AYA patients ≤21 years of age are eligible in the following cohorts: - medulloblastoma - nonmedulloblastoma - embryonal brain tumors - atypical teratoid rhabdoid tumors - ependymoma - CNS germ cell tumors - diffuse midline glioma, H3K27M-altered - Diffuse hemispheric glioma, H3 G34-mutant - Pineoblastoma - diffuse leptomeningeal glioneuronal tumor - diffuse brainstem glioma - pilocytic astrocytoma - choroid plexus carcinoma	To compare the performance of CSF ctDNA liquid biopsy in finding molecular alterations shared with matched blood samples	Not declared	Primary: - Within specific histologies with paired samples, estimate the concordance of mutation detected in the tumor tissue vs. CSF ctDNA across samples collected within 8 weeks of each other Secondary: - To estimate the frequency of detection of CSF ctDNA across different types of pediatric CNS tumors in association with various disease states - To track and estimate the correlation between the levels of CSF ctDNA and disease response as determined by clinical and imaging criteria across different disease types

AUC, area under curve; AYA, adolescent/young adult; BBB, blood–brain barrier; BBBD, blood–brain barrier disruption; CAN, copy number alterations; CT, chemotherapy; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; HGG, high-grade gliomas; OS, overall survival; PFS, progression-free survival; RT, radiotherapy; TME, tumor microenvironment.
ClinicalTrialGov access until 30 June 2024.

CONCLUSION

The different liquid biopsy approaches each have advantages and disadvantages. Thus far, no clinically validated biomarker for managing primary CNS tumors exists, mainly because of the small sample size and heterogeneity of techniques across the studies. There is need, in the future, of a standardization of protocols of biofluid collection and storage, and techniques of molecular analysis [90▪]. In particular, the various assays should be evaluated through well organized centralized testing. Importantly, clinical trials (Table 1) should incorporate molecular liquid biopsy endpoints in order to compare traditional endpoints head-to-head with molecular biomarkers and ultimately identify potential surrogate endpoints.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
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