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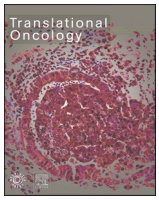

Clinical applications of cerebrospinal fluid liquid biopsies in central nervous system tumors

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ABSTRACT

For patients with central nervous system (CNS) malignancies, liquid biopsies of the cerebrospinal fluid (CSF) may offer an unparalleled source of information about the tumor, with much less risk than traditional biopsies. Two techniques have been adapted to CSF in clinical settings: circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA). CTCs have been employed mostly as a diagnostic tool for leptomeningeal metastases in epithelial tumors, although they may also have value in the prognostication and monitoring of this disease. The ctDNA technology has been studied in a variety of primary and metastatic brain and spinal cord tumors, where it can be used for diagnosis and molecular classification, with some work suggesting that it may also be useful for longitudinal tracking of tumor evolution or as a marker of residual disease. This review summarizes recent publications on the use of these two tests in CSF, focusing on their established and potential clinical applications.

Introduction

With the recent expansion of therapeutic options in oncology, there has been parallel growth in biomarker development, in an attempt to fine-tune the identification of those patients most likely to benefit from specific interventions and deliver precision medicine. The term "liquid biopsies" refers to the detection in bodily fluids, most commonly blood, of biomarkers that provide surrogate information about the state and characteristics of a patient's tumor, potentially substituting the need for histological sampling in the form of a traditional biopsy.

In patients with tumors of the central nervous system (CNS), this ability to bypass surgical access can be particularly interesting, as direct sampling of the tumor may carry a significant risk of morbidity, especially in tumors in deep or eloquent locations and in the setting of multiple lesions. Although blood has been the most extensively studied source of liquid biopsies, cerebrospinal fluid (CSF) offers a very attractive alternative in patients with CNS tumors. While, compared to blood, CSF has the disadvantage of requiring a more invasive procedure for its obtention, the advantages that CSF provides over blood are multiple. First, CSF is in close proximity to or even in direct contact with the tumor (or, in the particular case of leptomeningeal metastases, CSF can directly contain tumor cells), and it appears that this direct contact may be associated with a higher likelihood of detection of tumor-derived material [1]. In addition, in the case of metastatic tumors, this physical proximity may be the key to obtaining information specific to the CNS compartment: CNS metastases may have a different genomic evolution than the primary tumors they derive from [2], which—due to the blood-brain barrier—may not be appropriately captured by liquid biopsies in plasma, but can be detected in CSF. Lastly, CSF has a low baseline cellularity that notably decreases the "noise" that needs to be filtered to detect true tumor material, and numerous studies directly comparing CSF and plasma samples have demonstrated a higher yield for the detection of tumor genomic material in CSF [3–6].

The technologies to obtain tumor data that can be applied to CSF are extensive, including circulating tumor cells, circulating tumor DNA and RNA, circulating metabolites, extracellular vesicles, and detection of tumor-specific proteins through mass spectroscopy or ELISA. The current clinical applications for the majority of these methods are limited; this review will center in the two most commonly employed ones in a clinical setting—circulating tumor cells and circulating tumor DNA—focusing on their existing and potential clinical applications.

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Circulating tumor cells

Introduction

Circulating tumor cells (CTCs) are a subset of quantifiable malignant cells that can be detected in blood and other fluids of patients with solid tumors. In several types of metastatic carcinoma, detection of CTCs in blood has been shown to correlate with prognosis, response to treatment, and tumor burden [7–11], although their use in clinical practice remains controversial [12].

CTCs can be captured through a variety of methods taking advantage of surface markers specific to cells of a certain lineage; for carcinomas, epithelial cell adhesion molecule (EpCAM) is the most widely used target, as it can detect the largest range of tumor types [13]. The most frequently used CTC test is the CellSearch® assay (Menarini Silicon Biosystems), which is FDA approved for use in peripheral blood in patients with metastatic breast, prostate, and colorectal cancer. This method utilizes EpCAM-directed antibodies conjugated with ferrofluid nanoparticles, which allows the magnetic capture of CTCs using specialized equipment. Other immunofluorescent substances are then added to further characterize the cells, including a nuclear marker (4′6-diamidino-2-phenylindole [DAPI] dihydrochloride), confirmatory markers of epithelial cell origin (anti-cytokeratin-phycoerythrin [CK-PE] 8/18 and 9) and a leukocyte marker (anti-CD45 allophycocyanin); the automated system will identify captured cells that are positive for DAPI and CK-PE but negative for CD45 as CTCs, and present them to an operator for final review [14]. Although not as widely used, CTCs can also be detected through flow cytometry techniques utilizing immunofluorescent antibodies against surface cellular markers, such as EpCAM. More recently, a platform based on streptavidin capture of cells marked with a biotin-tagged antibody cocktail against a variety of epithelial and mesenchymal antigens (CNSide™, Biotech) has also been successfully applied to CSF [15]. In addition to the use of surface markers for cell capture, several methods of CTC enrichment have been developed in blood in an attempt to increase the yield of CTC detection against the background of hematopoietic cells; these use biophysical cell properties that differ between tumoral and normal blood cells, such as density, size, deformability and electric charge, to select CTCs [16]. In CSF, reliable CTC capture can be obtained without this kind of enrichment, due to its markedly lower cellularity compared to blood.

Use as a diagnostic tool in leptomeningeal metastases

The clinical applications of CTCs in CSF have focused on their use as a diagnostic tool in patients with leptomeningeal metastases (LM) from solid, extra-CNS tumors. LM, defined by presence of malignant cells in the CSF, are challenging to diagnose using standard methods, which include CSF cytology, magnetic resonance imaging (MRI) of the brain and/or spine, and clinical symptoms. Cytopathologic analysis of the CSF is the gold standard of LM diagnosis, but its sensitivity is around 50 %, requiring multiple samples—usually implying several lumbar punctures—to achieve a sensitivity closer to 90 % [17]. While MRIs have the advantage of being non-invasive, they are subject to significant inter-rater variability [18] that makes them suboptimal for a standalone diagnostic test. In this context, enumeration of CTCs in CSF has emerged as a more sensitive and reliable test to diagnose LM.

Over the last decade, multiple studies have explored the use of CTCs in CSF for LM diagnosis, most of them using the CellSearch® system [19–26], or—in a smaller number of studies—flow cytometry [27–29] or CNSide™ [15] (Table 1). These have demonstrated a sensitivity for the diagnosis of LM ranging from 75 to 100 %, surpassing in all cases the sensitivity of the gold standard of CSF cytology, while maintaining specificity around or over 80 %. These studies employed a variety of cutoffs to consider a sample "positive" for LM diagnostic purposes, in most cases selected arbitrarily; however, two of them employed statistical methods to determine the optimal cutoff suggestive of LM diagnosis and arrived at a similar threshold of 1 CTC/ml [23,25]. Although the current diagnostic criteria for LM do not include the use CSF-CTCs [30], they are becoming more widely used in centers where the technology is available, both in clinical practice and in clinical trials [17].

In addition to the greater sensitivity, another advantage of using CSF CTCs over cytology for LM diagnosis is the longer sample stability after collection with the CellSearch system (samples are stable at room temperature for 96 hours [14]). Besides, it is possible to perform molecular and genomic analyses on the captured cells [15,26,31,32], which has yet to be clinically validated but could potentially be useful in LM cases where the origin of the primary malignancy is unknown and not readily identified by cytology.

Table 1
Summary of published studies investigating CSF CTCs as a diagnostic tool for LM.

Study	N	Patient population	Technique	CTC cutoff (cells/mL)	CTC sensitivity	CTC specificity	Cytology sensitivity	Cytology specificity
Subirá (2012)	78	Clinical suspicion of LM from EST	FC	NR	75.5 %	96.1 %	65.3 %	100 %
Nayak (2013)	51	Clinical suspicion of LM from EST	C	>0	100 %	97.2 %	66.7 %	100 % (GS)
Lee (2015)	38	Confirmed or clinically suspected LM from BC	C	1.9	80.95 %	84.62 %	66.67 %	100 % (GS)
Subirá (2015)	144	Clinical suspicion of LM from EST	FC	NR	79.8 %	84 %	50 %	100 %
Tu (2015)	18	Confirmed LM (in MRI) from LC	C	0.2	77.8 %	100 %	44.4 %	NR
Milojkovic Kerklaan (2016)	29	Clinical suspicion of LM from EST with normal/inconclusive MRI	FC	0.25	100 %	100 %	61.5 %	100 %
Jiang (2017)	21	Clinical suspicion of LM from NSCLC	C	>0	95.2 %	100 %	61.5 %	100 %
Lin (2018)	95	Clinical suspicion of LM from EST	C	1	93 %	95 %	29 %	NR
Torre (2020)	20	Clinical suspicion of LM from solid tumors (18 patients with epithelial tumors, 2 patients with melanoma)	C	1	88.9 %	100 %	77.8 %	100 %
Van Bussel (2020)	81	Clinical suspicion of LM from EST with normal/inconclusive MRI	C	0.86	94 %	100 %	76 %	100 % (GS)
Darlix (2022)	40	Clinical and/or radiographic suspicion for LM from BC	C	1	100 %	77.3 %	88.9 %	100 % (GS)
Wooster (2022)	10	Clinical and/or radiographic suspicion for LM from BC	CNS	1	100 %	83 %	100 %	100 %

BC: breast cancer, C: CellSearch® assay, CNS: CNSide™, CTC: circulating tumor cell(s), EST: epithelial solid tumors, FC: flow cytometry, GS: gold standard, LC: lung cancer, LM: leptomeningeal metastases, MRI: magnetic resonance imaging, NR: not reported, NSCLC: non-small cell lung cancer.

Use as a marker of disease burden

Another major difference between CSF cytology and CTCs is the quantitative nature of the latter, which allows for its potential use as a monitoring and prognostic tool. After early CSF CTC studies described dynamic changes in CTC count during chemotherapy [31,33], some prospective LM clinical trials started incorporating this test as an exploratory endpoint. A phase I/II trial of intrathecal trastuzumab for breast cancer LM reported a decrease in CTCs in response to treatment, and a subsequent increase before clear radiographic progression in several patients [32]. In a phase I trial of proton craniospinal irradiation for LM from solid tumors, patients with a higher CSF CTC count prior to treatment, as well as those with smaller decreases in the counts with therapy, had poorer progression-free survival in the CNS [34]. Similarly, two retrospective studies have demonstrated decreased survival in patients with higher CSF-CTC counts at the time of LM diagnosis [35,36]. Although further clinical validation is needed, these results suggest that CSF CTCs could be used as a surrogate marker of disease burden and could provide a more nuanced assessment of disease response than the current methods, restricted by their limitations in consistency (MRI) and their binary nature (CSF cytology).

Limitations

As the most frequently used method for CTC detection—the CellSearch platform—relies on the presence of EpCAM, cancers of non-epithelial origin cannot be captured with this technique, including hematologic malignancies (which can however be detected by standard flow cytometry), melanoma and primary brain tumors. In melanoma, a common source of LM, the CellSearch system has successfully been adapted using melanoma markers in CSF samples [37], but this assay has yet to be clinically validated. In gliomas, a number of CTC detection methods have been explored [38], but none of these have been applied to CSF. Even for epithelial cancers, the variability of markers over time is a potential limitation; for example, it has been shown that CTCs in the blood of patients with metastatic breast cancer can lose EpCAM as the cell transitions to a mesenchymal-like phenotype in advanced disease [39]. Besides, an additional disadvantage of the CellSearch platform is that it requires specialized equipment and operator training [14], although this may mean the results are more reproducible between institutions.

Table 2
Selection of published studies investigating clinical applications of CSF ctDNA.

Disease	Study	Patient population	Method	Key findings
BM (and others)	De Mattos-Arruda (2015)	8 BM, 4 GBM	ddPCR, NGS (Sn=100 %)	• Higher VAF in CSF ctDNA than plasma ctDNA • CSF ctDNA detects CNS private mutations not captured by plasma ctDNA (1 pt) • VAF increases with progression and decreases with treatment response (5 pts)
	Momtaz (2016)	8 melanoma BM, 3 ECD (BRAF-mutant)	dPCR (Sn=55 %)	• CSF ctDNA concentration increases with progression and decreases with treatment response (2 pts)
	Pentsova (2016)	32 BM, 11 gliomas, 1 ependymoma	NGS (Sn=63 % for BM, 50 % for PBT)	• CSF ctDNA detected in 20/32 (63 %) BM and 6/12 (50 %) PBT • Detection of emergent drug-resistance mutations in 4/12 BM patients with CNS progression on TKI
LM	Nevel (2020)	21 NSCLC LM	NGS	• Continuous relationship between CSF ctDNA concentration and risk of death
	Zhao (2019)	35 LM	NGS (Sn=100 %)	• CSF ctDNA detected in 35/35 (100 %) pts (vs 25/35 [71 %] with positive CSF cytology)
	White (2021)	48 LM/BM samples (30 pts: 22 LM, 8 BM)	NGS (Sn=93 %, Sp=100 %)	• CSF ctDNA accurate in LM diagnosis in 45/48 (94 %) samples (vs 36/48 [75 %] in CSF cytology) • CSF ctDNA detected in 3/3 pts with CSF-abutting BM but no LM
	Fitzpatrick (2022)	24 BCE LM	ulpWGS (Sn=100 %)	• CSF ctDNA detectable in 24/24 (100 %) pts (vs 14/24 [58 %] with positive CSF cytology) • CSF ctDNA suppression after IT therapy predictive of survival (12 pts) • CSF ctDNA concentration and VAF increase with progression and decrease with treatment response (12 pts)
Glioma	Martinez-Ricarte (2018)	20 diffuse gliomas	ddPCR, NGS (Sn=85 %)	• CSF ctDNA allowed for glioma subtyping in 17/20 (85 %) pts
	Pan (2019)	57 brainstem gliomas	NGS (Sn=83 %)	• CSF ctDNA allowed for glioma subtyping in 47/57 (83 %) pts
	Fujioka (2021)	34 diffuse gliomas	ddPCR (Sn=59 %)	• CSF ctDNA allowed for glioma subtyping in 20/34 (59 %) pts
	Miller (2019)	85 diffuse gliomas	NGS (Sn=50 %)	• CSF ctDNA detection associated with tumor burden, radiographic progression, and survival
	Cantor (2022)	24 DMG (prospective CT)	ddPCR (Sn=97 %)	• Decrease in CSF ctDNA associated with longer PFS (13 pts with nonrecurrent tumor)
Other primary brain tumors	Bobillo (2021)	7 CNS lymphoma (1 primary, 6 secondary)	NGS, WES (Sn=86 %, Sp=100 %)	• VAF increases with progression and decreases with treatment response (4 pts) • CSF ctDNA detected in one pt with systemic lymphoma but negative CSF flow cytometry, who later developed CNS lymphoma involvement
	Liu (2021)	123 medulloblastomas (prospective CT)	sWGS (Sn=64 %, Sp=100 %)	• Detectable CSF ctDNA (MDR) during/after treatment associated with subsequent progression

BC: breast cancer, BM: Brain metastases, CNS: central nervous system, CSF: cerebrospinal fluid, CT: clinical trial, ctDNA: circulating tumor DNA, ddPCR: digital droplet polymerase chain reaction, dPCR: digital polymerase chain reaction, DMG: diffuse midline gliomas, ECD: Erdheim-Chester disease, GBM: glioblastoma, IT: intrathecal, LM: leptomeningeal metastases, MRD: measurable residual disease, NSCLC: non-small cell lung cancer, PBT: primary brain tumors, PFS: progression-free survival, pt: patient, sWGS: shallow whole genome sequencing, Sn: sensitivity, Sp: specificity, TKI: tyrosine kinase inhibitor, ulpWGS: ultra-low-pass whole genome sequencing, VAF: variant allelic frequency, WES: whole exome sequencing. The percentage of cases with detectable CSF-ctDNA among patients included in each study is reported as sensitivity, when reported; as criteria for patient selection varied among studies, these numbers should be interpreted with caution. Specificity is reported in those studies that analyzed CSF-ctDNA in control patients (without CNS malignancies).

Circulating tumor DNA (ctDNA)

Introduction

Cell-free DNA refers to the DNA fragments released into bodily fluids after cell death, most commonly derived from leukocytes. In patients with cancer, a proportion of cell-free DNA stems from the malignant cells, and can be distinguished from non-tumor DNA by its shorter fragmentation patterns (tumor DNA is composed of 145-base-pair fragments, compared to 167 base pairs in non-tumor DNA) [40,41]. This section of genomic material is known as circulating tumor DNA (ctDNA), and it has extensive applications when detected in blood and other fluids for many non-CNS malignancies [42]. In patients with primary and metastatic brain tumors, ctDNA can be detected in the CSF, potentially providing information specific to the CNS compartment. Table 2 summarizes the key findings of those studies that have demonstrated applications of CSF ctDNA that are clinically relevant.

Clinical applications

a) *Brain metastases*

CSF ctDNA may be a particularly useful tool for patients with brain metastases (BM), given the potential for targeted therapies specifically directed to their CNS lesions. For example, patients with non-small cell lung cancer (NSCLC) with driver alterations such as *epidermal growth factor receptor* (*EGFR*) mutations or *anaplastic lymphoma kinase* (*ALK*) rearrangements can receive brain-penetrant tyrosine kinase inhibitors (TKI) as a first-line therapy for their brain metastases; and patients with BM from human epidermal growth factor receptor 2 (HER2)-positive breast cancer may benefit from the addition of the HER2 inhibitor tucatinib to a combination regimen after failing previous lines of therapy [43]. However, the genomic evolution of brain metastases may diverge from that of the primary tumor they derive from; one study comparing genomic data from BM tissue and matched primary tumors found over 50 % of brain metastases samples had actionable mutations that were not present in the primary tumor [2]. Having updated information on the mutational status of the brain metastases can be paramount to devising an improved treatment plan; however, many BM never undergo resection, particularly for patients with multiple, small lesions that are suitable for radiation or systemic therapies.

In these cases, CSF ctDNA could be a useful tool to guide treatment decisions without the risks of surgery, with rates of detection similar or even superior to plasma ctDNA in small studies [3–5], and the added advantage over plasma that CSF may more accurately reflect intracranial as opposed to extracranial disease. Case reports and other small studies have described the effective tailoring of BM treatment based on mutational targets detected in CSF ctDNA [44–46], including one case where concurrent plasma ctDNA analysis failed to show the relevant mutation [45]. Another potential use of CSF ctDNA could be as a dynamic, quantitative measurement of CNS tumor burden, similar to CTCs: separate studies have found changes in the variant allelic frequency (VAF) of relevant genomic alterations in CSF tracking with the status of CNS disease at the time (increasing in cases of CNS tumor progression, and decreasing in cases of CNS treatment response), again in a very small number of patients (6 total) [3,47,48].

Despite how promising the use of CSF ctDNA could be for brain metastases, further research with larger numbers of patient samples is needed, and several issues need to be addressed prior to its implementation in clinical practice. The critical question of whether CSF ctDNA adequately represents parenchymal brain metastases remains to be fully answered, although several small studies have shown at least partial concordance between mutations detected in CSF and matched surgical BM samples [5,49]. In addition, the reported rate of CSF ctDNA positivity for BM patients is around 60 % [50,51], suggesting that not every BM patient may benefit from this test. A recent study suggests that selecting patients with larger BM and lesions closer to the ventricles may increase the yield [51], but more research is needed to better optimize the use of CSF ctDNA in patients with BM.

b) *Leptomeningeal metastases*

Patients with LM could reap similar benefits from CSF ctDNA to their parenchymal counterparts in terms of molecularly tailored treatments, with the significant advantage that in LM the disease is by definition in the CSF, and therefore CSF ctDNA likely reflects the entirety of the tumor and follows the characteristics of the disease more accurately. As a result, there have been more studies investigating CSF ctDNA in LM than in BM, although patient numbers remain overall small, in part due to the rarity of this disease. Similarly to BM, some case reports detail successful treatment changes in response to mutations detected in CSF ctDNA [44,52], and a few studies report a similar correlation between CSF ctDNA levels and disease status, with increases at the time of progression and decreases in response to treatment [53–55]. Moreover, CSF ctDNA has been explored as a prognostic tool in LM, with a few studies in NSCLC showing a correlation between CSF ctDNA levels and presence of specific genomic alterations and survival [35,56,57].

An additional use of CSF ctDNA in LM is as a diagnostic tool that, like CSF CTCs, could potentially overcome the limitations of CSF cytology and neuroimaging. Several studies have investigated this application of CSF ctDNA, finding that “positive” ctDNA—defined in most studies as detection of any cancer-related genomic alteration in CSF—can diagnose LM with very high sensitivity, between 92 and 100 % [1,55,58,59]. An important problem of using CSF ctDNA for LM diagnosis is that LM and BM frequently coexist, and as explained above ctDNA can be detected in the CSF of BM patients even in the absence of LM; therefore, its diagnostic value may be reduced for patients with BM in whom concurrent LM is suspected. For example, one of the aforementioned studies included 8 patients with parenchymal BM but without clinical or radiographic suspicion of LM, and found presence of ctDNA in all 3 cases where the BM were abutting the ventricles (and in none of the 5 patients whose BM were not) [1]. Whether presence of CSF ctDNA in BM could be indicative of early preclinical LM (and not just BM) remains to be investigated; for now, there is no definitive way to distinguish between LM and BM based exclusively on CSF ctDNA, which may limit its use as a diagnostic instrument.

c) *Gliomas*

Gliomas are the most common malignant primary brain tumors, and the tumor type for which CSF ctDNA has been most widely studied. In gliomas, CSF ctDNA is a feasible and reliable tool for molecular diagnosis. While detecting specific tumor alterations does not carry the same therapeutic implications in gliomas as it does in BM—because, unfortunately, targeted therapies are not considered a first-line treatment for gliomas except in rare circumstances—current guidelines for glioma classification rely heavily on molecular alterations, including a number of gliomas with disease-defining molecular alterations, such as histone 3 (H3) K27-altered diffuse midline gliomas or isocitrate dehydrogenase (IDH)-mutant low grade gliomas [60]. In cases where a biopsy is risky or not feasible, CSF ctDNA can capitalize on this molecular approach and provide a less invasive means of arriving at a specific diagnosis, which will then have therapeutic and prognostic implications. Several studies have preliminarily demonstrated the feasibility of classifying gliomas using CSF ctDNA data, in particular in regard to *IDH* [6,61-64] and *H3* mutations [61-63,65,66]; H3 K27M-mutant diffuse midline gliomas are an especially interesting target for this approach, as their location frequently makes surgical access difficult. It is important to note that many of these studies utilized intracranial CSF (obtained intraoperatively or from a ventricular shunt or reservoir), and the diagnostic sensitivity of a lumbar puncture may be lower [66]. The likelihood of detecting CSF ctDNA in gliomas is higher in cases with active, aggressive

disease [67,68]; hence, to use this tool to longitudinally monitor disease response (when tumor burden may be lower), more sensitive assays may need to be developed. Despite this limitation, several studies have demonstrated that, as was the case with metastatic disease, the yield of CSF ctDNA in gliomas is higher in CSF than in plasma [3,6].

In addition to assisting in diagnosis and molecular classification, CSF ctDNA could have a role in the longitudinal monitoring of gliomas. Several studies have outlined a dynamic correlation between the amount of CSF ctDNA and changes in disease burden [3,69,70], including a recent clinical trial in H3 K27M-mutant tumors where CSF ctDNA was used as an auxiliary tool to confirm treatment response and identify subsequent tumor progression [71]. For high-grade gliomas, an attractive theoretical application of this observation could be the potential to differentiate between pseudoprogression—the relatively common appearance of radiographic changes mimicking tumor progression following treatment with radiation—and true tumor progression: longitudinal CSF ctDNA sampling could add to the existing advanced imaging tools used for this purpose, none of which are able to reliably make the distinction. However, this potential application will need to be specifically investigated, possibly through prospective studies, before it can be implemented in clinical practice.

Besides the "quantitative" aspect of CSF ctDNA monitoring, repeated sampling can also provide an updated molecular profile reflecting tumor evolution, for example at the time of progression [50,67]. This is potentially relevant for clinical trial enrollment, not only in the context of targeted therapies (such as using CSF ctDNA to confirm the existence of an *EGFR* alteration for a trial testing a new EGFR inhibitor), but also to potentially "upgrade" patients' diagnoses and enable trial eligibility: for example, CSF ctDNA could reveal the development of a *CDKN2A/2B* deletion in a patient with an IDH-mutant tumor, or it could demonstrate *EGFR* amplification or a *telomerase reverse transcriptase* (*TERT*) promoter mutation in IDH-wildtype diffuse glioma, both scenarios in which the tumor would automatically be classified as a grade 4 regardless of other histologic characteristics [60]. It is therefore possible that future clinical trials will start incorporating CSF ctDNA results as an accepted avenue in their inclusion criteria, particularly in recurrent disease, when the yield is likely to be higher (given more advanced disease) and the indication for surgical intervention is less straightforward.

d) *Other primary brain tumors*

For primary brain tumors where, unlike gliomas, treatments have the potential to be curative, having a highly sensitive tool to monitor disease burden after initial therapy could help select those patients who may benefit from further treatment. This concept of measurable residual disease (MRD) is well established in many hematologic malignancies, where next-generation sequencing of bone marrow samples can be used to identify patients at high risk of recurrence to implement early additional treatment [72]. CSF ctDNA could be used in a similar fashion in primary brain tumors such as CNS lymphoma, the neurological counterpart of hematologic malignancies. In addition to the potential detection of specific genomic alterations (*MYD88*) as a complementary diagnostic marker for CNS lymphoma [73,74], a few studies (some prospective) have demonstrated decreases or resolution of CSF ctDNA levels after successful treatment of CNS lymphoma, and increases with tumor progression that frequently predate the detection by standard tools (MRI and/or CSF flow cytometry) [75–77].

A similar approach has been used in medulloblastoma, another potentially curable malignant CNS tumor. A large retrospective review of banked CSF samples from a prospective trial showed a tendency towards subsequent progression in patients with post-treatment MRD in the form of positive CSF ctDNA [78]. In addition, MRD-positive patients had significantly worse progression-free survival than patients without MRD at different points in their sequential treatment course, arguing for the integration of CSF ctDNA into future clinical trial design in medulloblastoma [78].

Conclusion

Liquid biopsies can be a remarkably rich source of up-to-date information in CNS malignancies, with very minimal risk compared to the surgical access of brain and spinal cord tumors. Although slightly less accessible than blood, CSF has been shown to be superior to plasma in detecting tumor-derived genomic material in both primary and metastatic brain tumors; for the latter, CSF has the distinct advantage of reflecting the disease inside the CNS compartment as opposed to the status of the systemic malignancy. Both CTCs and ctDNA in CSF have successfully been used in clinical practice for the diagnosis and monitoring of brain tumors; while retrospective research suggest these tests can be used in parallel and likely provide complementary information [79], further studies investigating their concurrent use are needed to determine their relative roles in the management of patients with CNS malignancies. In any case, while more research is needed to validate the clinical applications of these technologies, they have the potential to change the field of neurooncology. It is conceivable that, in the future, serial CSF analyses will become part of the routine diagnosis and monitoring of certain CNS malignancies, potentially facilitated by the implantation of ventricular reservoirs—in an analogous way as how serial bone marrow biopsies are currently performed as part of the routine care of many hematologic tumors. For now, a number of clinical trials are incorporating CSF biomarkers as central parts of their designs [80–83], and it is likely that the uses of these test will continue to expand in the near future.

CRediT authorship contribution statement

Maria Diaz: Conceptualization, Data curation, Writing – original draft. **Sofia Chudsky:** Data curation, Writing – review & editing. **Elena Pentsova:** Writing – review & editing. **Alexandra M. Miller:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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