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REVIEW

Cerebrospinal fluid cell-free tumour DNA as a liquid biopsy for primary brain tumours and central nervous system metastases

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Challenges in obtaining tissue specimens from patients with brain tumours limit the diagnosis and molecular characterisation and impair the development of better therapeutic approaches. The analysis of cell-free tumour DNA in plasma (considered a liquid biopsy) has facilitated the characterisation of extra-cranial tumours. However, cell-free tumour DNA in plasma is limited in quantity and may not reliably capture the landscape of genomic alterations of brain tumours. Here, we review recent work assessing the relevance of cell-free tumour DNA from cerebrospinal fluid in the characterisation of brain cancer. We focus on the advances in the use of the cerebrospinal fluid as a source of cell-free tumour DNA to facilitate diagnosis, reveal actionable genomic alterations, monitor responses to therapy, and capture tumour heterogeneity in patients with primary brain tumours and brain and leptomeningeal metastases. Profiling cerebrospinal fluid cell-free tumour DNA provides the opportunity to precisely acquire and monitor genomic information in real time and guide precision therapies.

Key words: cerebrospinal fluid, circulating cell-free tumour DNA, glioblastoma, brain metastasis, liquid biopsy, brain cancer

Introduction

Genomic characterisation of tumour tissue has been established as crucial for state-of-the-art diagnostic and therapeutic approaches to cancer. However, characterisation of cancer is challenged by constitutive and evolving intra-tumour and inter-lesion heterogeneity, which requires thorough and continuous analysis of genomic complexity over time. This is particularly relevant in brain malignancies where the genomic landscape changes in response to treatment or during relapse and can differ from the primary extra-cranial lesion in the case of brain metastases. Yet, obtaining samples for characterisation and correct diagnosis can be difficult in brain cancer patients. The anatomical location of the tumour limits access due to the risk and complexity of intracranial surgical procedures.

Invasive surgical procedures have been the cornerstone treatment and a diagnostic tool in patients with primary brain tumours and in selected patients with brain metastasis. However, collecting tumour tissue from central nervous system (CNS) malignancies is complex, can be risky, and sometimes unfeasible, at least with purely diagnostic intent. Surgery has a role in improving disease control in patients with primary tumours or with a single, resectable brain metastasis, whereas patients with disseminated systemic disease are frequently not candidates for routine neurosurgical procedures [1, 2]. Moreover, specimens may be small and not representative hampering correct diagnosis or even necessitating multiple surgical samplings to clarify final pathological diagnosis. In addition, the surgical intervention strategy and assessment of the surgical risk–benefit balance depend on the tumour prognosis.

This implies that an intraoperative histological diagnosis may be required possibly delaying the surgical procedure. Repeat surgical interventions may be needed to differentiate tumour pseudoprogression induced by treatment from true relapse. The challenges in obtaining tumour tissue have led physicians to rely on primary archival tumour specimens. Thus, in some cases, therapies for brain cancer are selected based on the molecular characteristics of the primary tumour that can differ from the current tumour manifestation [3, 4].

Plasma cell-free circulating tumour DNA (ctDNA) has been used as a 'liquid biopsy' in the context of tumour genomic characterisation [5–12]. ctDNA is the fraction of the total cell-free DNA that is derived from tumour cells and can be defined by the presence of genomic alterations. ctDNA detected in plasma has shown promise in characterising tumours and allowing patients and their cancers to be monitored over time. Analyses of mutations in plasma ctDNA have demonstrated high concordance with genomic alterations in the tumour [10].

However, in the context of primary brain tumours and brain metastasis, plasma ctDNA has been shown to be in low abundance and present in a limited number of patients [8, 13–16]. Importantly, the cerebrospinal fluid (CSF) is in intimate contact with brain malignancies and has been recently proved to contain ctDNA. The CSF space involves the intracerebral ventricles, subarachnoid spaces of the spine and brain (cisterns and sulci), and the central spinal cord canal. The CSF is renewed three to five times daily and is produced by the choroid plexus. The CSF circulates in a craniocaudal direction from ventricles to spinal subarachnoid space from where it is removed via craniocaudal lymphatic routes and the venous system [17]. The CSF space is separated from the vascular system by the blood–CSF barrier, while the blood–brain barrier is located between the brain parenchyma and the vascular system [18].

CSF has been explored as a source of ctDNA for precisely characterising brain cancers. Studies reported before the era of high-throughput sequencing showed that some molecular alterations or gene mutations can be detected in the DNA present in the CSF of patients with brain tumours [19–24]. Importantly, massively parallel sequencing methods have recently been used to analyse cell-free tumour DNA from CSF to comprehensively characterise somatic alterations including gene mutations and copy number alterations [15, 25–29] (Table 1).

DNA was isolated from CSF (ranging from 0.75 to 10 ml) usually obtained from a lumbar puncture and DNA sequencing (i.e. droplet digital PCR, targeted sequencing, whole-exome sequencing, or shallow whole-genome sequencing) allowed the identification of ctDNA. Notably, CSF ctDNA enabled the identification of genomic alterations in patients with systemic metastatic burden including brain metastasis, or disease restricted to the brain (primary tumours and brain metastasis) [15, 25–27]. Higher grade brain tumours were more likely to exhibit detectable CSF ctDNA than lower grade ones [26] and, in some cases, the distance of the tumour to CSF spaces could determine the amount of CSF ctDNA [27].

Here, we focus on the studies related to ctDNA obtained from CSF. Nowadays, the CSF liquid biopsy is increasingly allowing molecular diagnoses, providing information on prognosis, facilitating the identification of new actionable genomic alterations,

aiding in monitoring response to therapy, and allowing deconvolution of tumour heterogeneity in patients with CNS cancer (Figure 1).

Primary brain tumours

Diagnostic considerations

Primary brain tumours encompass a large variety of lesions with diverse natural course, response to treatment, and prognosis. The histological grade and molecular genetic make-up determine prognosis, with median overall survivals ranging from <1 year (e.g. in glioblastoma of the elderly) to long-term survival including cures (e.g. pilocytic astrocytoma and other rare circumscribed lesions). The clinical hallmark of glioblastoma is aggressive growth, local invasiveness, and inexorable recurrence [30–32]. In recent years, the development of novel sequencing technologies and DNA methylation profiling coupled to bioinformatics tools has yielded an unparalleled, comprehensive view of the genome and epigenome of brain tumours [33–36].

The 2016 update of the WHO classification incorporated well established molecular parameters into the classification of brain tumours, specifically gliomas. The analysis of the CSF ctDNA of a cohort of diffuse gliomas indicated that they could be subtyped by analysing the IDH1 and IDH2, ATRX, TP53, TERT, H3F3A and HIST1H3B mutational status, facilitating the classification of diffuse gliomas and providing prognostic information [28]. Moreover, the presence of mutations in the TERT promoter found in CSF ctDNA correlated with outcome [37]. In the case of diffuse midline gliomas, the detection of H3F3A and HIST1H3B mutations in the CSF could confirm diagnosis [28]. This is of major relevance since the anatomical location of this type of tumours increases the risk of obtaining surgical specimens.

CSF ctDNA was detected in a large proportion of patients with brain primary tumours (Table 1). However, CSF ctDNA is not found in all brain tumours. For example, in some low grade gliomas, CSF ctDNA was not detected or was not informative [28]. Technological advances may improve sequencing sensitivity in the future, thus reducing the number of non-informative cases.

Therapeutic considerations

ctDNA diagnostic applications with potential therapeutic implications remain limited for adult patients with primary brain tumours. The most relevant biomarker for glioblastoma in terms of choice of therapy remains promoter methylation of the MGMT gene [38]. Efforts at the detection of MGMT promoter methylation in CSF of glioma patients showed higher sensitivity than in plasma [39]. Future applications with therapeutic impact are likely to include the monitoring of EGFRvIII and amplified epidermal growth factor receptor (EGFR) in patients undergoing EGFR-targeted therapy [40]. The evaluation of ctDNA during the follow-up of patients and especially at recurrence can confirm the molecular status and may help to deliver precision therapies.

Table 1. Studies detecting ctDNA in CSF

Patient population	Sequencing technologies	Method for CSF collection/ amount collected	Tumour-derived DNA found in CSF (% of cases)	Main findings	Comparison with plasma ctDNA	Extra-cranial disease analysed	Refs
Four glioblastoma, 2 medulloblastoma, and 17 brain metastases (from breast and NSCLC)	Targeted capture massively parallel sequencing, digital droplet PCR	Lumbar puncture, cerebral shunts, autopsy/	58–60	CSF ctDNA is enriched in brain tumours and produces better results than plasma ctDNA.	✓	✓	[15]
Thirty-five primary brain and spinal cord tumours	Targeted capture massively parallel sequencing, whole-exome sequencing in four cases	Cerebral shunts (during surgical procedure)/average of 4.8 ml (range=0.75–10 ml)	57–88	All adjacent tumours to the CSF reservoir had CSF ctDNA detectable	–	–	[26]
Twelve primary brain tumours and 41 brain metastases	Targeted capture massively parallel sequencing	Lumbar puncture and one sample Ommaya reservoir/5 ml of CSF	50–63	Drug-resistance mutations in patients whose CNS disease progresses during kinase inhibitor therapy is identified in CSF ctDNA.	–	✓	[27]
A vestibular schwannoma, a meningioma, five brain metastases and three leptomeningeal metastases	Targeted amplicon sequencing and digital PCR	Lumbar puncture, cerebral shunts (during surgical procedure)/1–10 ml of CSF	85	Tumour mutations were detectable in the CSF ctDNA of patients with different types of brain tumours	✓	–	[25]
Twenty primary diffuse glioma tumours	Targeted amplicon sequencing and digital PCR	Lumbar puncture before surgery, two samples cisterna magna, one sample cerebral shunt/2 ml of CSF	85	A sequencing platform to simultaneously test seven genes <i>IDH1</i> , <i>IDH2</i> , <i>TP53</i> , <i>ATRX</i> , <i>TERT</i> , <i>H3F3A</i> , <i>HIST1H3B</i> in the CSF ctDNA allowing the subclassification of diffuse glioma	–	–	[28]
Thirty-eight TERT mutant glioblastoma (34 primary and 4 recurrent glioblastoma)	Sequenced uni-directionally on an Ion Torrent PGM NGS system, digital droplet PCR	Directly after opening the dura (durotomy), through dissection of the convexity subarachnoid space/2–4 ml of CSF	78–98	CSF ctDNA identifies <i>TERT</i> promoter mutations	✓	–	[37]
Thirteen glioma tumours	Untargeted, low-coverage WGS (<0.4x) to detect SCNAs	Lumbar puncture/10 ml of CSF	39	Combining analyses of SCNAs with DNA fragmentation allows detection of ctDNA in CSF using sWGS data at low cost	–	–	[29]

CNS, central nervous system; GBM, glioblastoma; LM, leptomeningeal metastasis; NSCLC, non-small cell lung cancer; SCNAs, somatic copy number alterations; s-WGS, shallow whole-genome sequencing.

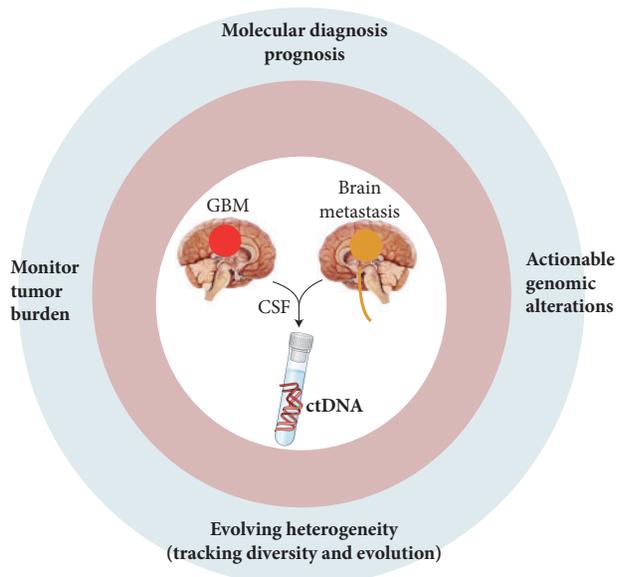


Figure 1. Potential use of CSF ctDNA as a liquid biopsy for primary brain tumours and brain metastasis.

Brain metastases

Diagnostic considerations

Brain metastases from solid tumours are more frequent than primary brain tumours. They may occur in 20%–40% of advanced stage cancers, particularly in lung cancer, breast cancer and melanoma [41–43]. Recent reports on the branched evolution of cancer at different sites including metastasis to the brain have reinforced the need of sequential molecular profiling across the disease trajectory [4, 44]. Brain metastases exhibit different genomic alterations than the primary extra-cranial tumours [4] indicating that the brain lesion-specific genomic alterations should be identified to select the optimal therapeutic approach [4, 45]. CSF ctDNA and not plasma ctDNA can be a good surrogate marker in such situations since ctDNA from brain lesions is enriched in the CSF.

Trunk mutations, present in all cancer cells, as well as private genomic alterations, present in just a subpopulation of cells or in specific metastatic lesions, can be identified in the CSF [15, 27]. This allows opportunities for deconvolving tumour heterogeneity. CSF and plasma ctDNA were compared in a series of samples that included multiregional metastatic sites from postmortem specimens of patients with disseminated breast cancers including brain metastases [15]. For example, mutations found in the CSF ctDNA allowed to discern the origin of leptomeningeal and brain metastasis implants separately in a patient with Li Fraumeni syndrome and two concurrent tumours, a metastatic breast cancer and esthesioneuroblastoma [15]. CSF ctDNA analysis captured trunk mutations and, importantly, private mutations to the brain and to the meningeal deposits. These observations highlight the potential applications of CSF ctDNA to complement diagnosis of brain metastasis.

Therapeutic considerations

Several targeted therapeutic agents have demonstrated clinical activity against established brain metastases [46–55] and

monitoring actionable mutations and therapy resistance using CSF ctDNA appears to be an application of CSF-based liquid biopsies that could be close to clinical practice.

First- (erlotinib and gefitinib) and second-generation (afatinib) EGFR tyrosine kinase inhibitors (TKI) have shown activity against brain metastasis from non-small-cell lung cancers (NSCLC) that harbour *EGFR* mutations [56–58]. A number of third-generation EGFR-TKI that also target mutant EGFR T790M, which confers therapeutic resistance, are in various phases of clinical investigation to target brain metastases (osimertinib, rociletinib, ASP-8273, HM-61713). In anaplastic lymphoma kinase (ALK) gene-rearranged (ALK)-NSCLC, second-generation ALK inhibitors with increased potency such as alectinib and ceritinib have apparently superior CNS penetration compared with crizotinib and share significant therapeutic potential [53–55]. Breast cancer studies have focussed primarily on targeted therapies [e.g. lapatinib, pertuzumab, ado-trastuzumab emtansine (T-DM1)] used for HER2-positive cancers [51, 52, 59, 60]. In patients with melanoma and brain metastases, substantial clinical activity has been observed with BRAF and MEK inhibitors, e.g. dabrafenib plus trametinib [49, 61], resulting in an intracranial response rate of nearly 60% [61]. Ongoing clinical trials exploit the cytotoxic T-lymphocyte-associated antigen 4 and programmed death 1 pathways as target for immune checkpoint inhibitor therapy [50, 62]. Actionable genomic alterations with potential therapeutic implications have been identified in the CSF ctDNA [15, 25–27], including EGFR, ALK, HER2, BRAF-targetable kinases, and others associated with DNA integrety such as *BRCA1* and *BRCA2* [63].

Analysis of CSF ctDNA has also shown gene mutations associated with therapy resistance [15, 27, 64]. Drug-resistance mutations in patients whose CNS disease progressed during TKI therapy (EGFR, ALK, HER2, or BRAF) were identified in CSF ctDNA in one-third of cases [27]. This included a *NRAS* G12R mutation in the CSF of a BRAF V600E-mutant (and *NRAS*-negative) melanoma; a *PIK3CA* H1047R mutation in the CSF of a HER2-amplified breast cancer patient, potentially associated with trastuzumab resistance; and an *EGFR* T790M mutation in the CSF of a patient with *EGFR*-mutant NSCLC who did not respond to a second-generation EGFR-TKI [27]. *ESR1* mutations can confer resistance to aromatase inhibitor therapy in advanced estrogen receptor-positive breast cancers, but not to fulvestrant [65, 66]. A clinical trial is evaluating *ESR1* mutations in plasma ctDNA to predict the efficacy of a change of the hormone therapy (aromatase inhibitor changed to fulvestrant) (ClinicalTrials.gov Identifier: NCT03079011). Translation of this type of clinical trial design to the setting of patients with brain metastasis is envisioned using liquid biopsies. In the context of multiple metastases and discordant clinico-radiological findings, analysing a single-lesion biopsy is inadequate in guiding the selection of targeted therapy [67]. Parallel analyses of serial CSF and plasma ctDNA samples may be warranted.

Although the most common initial clinical presentation of metastatic HER2-positive breast cancer is with extra-cranial metastases, CNS progression occurs in a substantial proportion of patients during the course of the disease [68]. It has also been shown that extensive extra-CNS disease control, with HER2 targeting, might drive high incidence of CNS progression [69, 70]. This situation remains a major challenge where genomic analysis

of ctDNA in the CSF might allow interrogation of the molecular status of progressive CNS metastasis. For example, in a case vignette, CSF ctDNA analysis captured CNS genomic alterations in patients with absent or minimal extracranial tumour disease burden, where plasma ctDNA profiling did not play a diagnostic role [15]. A HER2-positive metastatic breast cancer patient with divergent responses of brain metastases underwent autopsy. Copy number alteration testing of three spatially separated brain metastases, in addition to CSF ctDNA and plasma ctDNA sampling, showed *ERBB2* amplification, a hallmark of HER2-positive breast cancer in CSF ctDNA and not in the plasma analysis [15].

Leptomeningeal metastases

Diagnostic considerations

Leptomeningeal metastasis, defined by the multifocal seeding of the leptomeninges by malignant cells, is a rare but often rapidly fatal manifestation of advanced cancer [71–73]. Diagnosing leptomeningeal metastasis relies on clinical symptomatology, MRI, and on detecting malignant cells in the CSF through CSF cytology. Its incidence is increasing and prognosis remains poor despite radiotherapy, systemic and intrathecal chemotherapy, and precision treatments in molecularly selected patients [71, 72].

Two principal diagnostic applications of ctDNA studies in patients with leptomeningeal metastasis emerge: first, detecting CSF ctDNA in patients with leptomeningeal metastasis may complement diagnostic profiling in patients with negative cytology, second, identifying actionable genomic alterations in CSF ctDNA has the potential to define an optimal targeted therapy [15]. CSF ctDNA revealed mutations in 50% of patients with primary brain tumours despite their CSF being negative for malignant cells [27], further, among patients with brain metastases, somatic mutations were found in 100% of patients with positive cytology and in 25% of patients with negative cytology [27].

A pivotal study compared CSF profiling with CSF cytology results in the same CSF extraction [15]. Analysis of three metastatic breast cancer patients with clinical signs and symptoms suggestive of leptomeningeal metastasis showed that CSF ctDNA analysis was more sensitive than cytology in detecting leptomeningeal metastasis, and leptomeningeal infiltration was confirmed during autopsy [15]. A molecular case report compared paired profiling of matched CSF ctDNA and plasma ctDNA from a patient with HER2-positive metastatic breast cancer. The patient developed CNS progression and leptomeningeal metastasis whereas the systemic extracranial metastases showed a clinical and radiological response to treatment with T-DM1 [74]. CSF ctDNA revealed an enrichment of *ERBB2* amplification, *MYC* amplification and *PIK3CA* and *TP53* driver gene mutations, presumably reflecting CNS progression whereas decreasing mutant allelic fractions of selected mutations in plasma ctDNA likely reflected a partial clinical response in the extracranial compartment [74]. In metastatic melanoma spreading to the leptomeninges, CSF examination using PCR-based techniques has been successfully used for diagnosis and monitoring response to therapy based on the detection of driver mutations, e.g. affecting *BRAF* [64, 75].

Further work is warranted to consolidate CSF ctDNA as a complementary tool for the diagnosis and characterization of leptomeningeal metastasis. Accordingly, the EANO ESMO guideline advises caution in over-interpreting ctDNA detected in CSF as a proof of leptomeningeal seeding.

Therapeutic considerations

Recent and ongoing studies address the role of CSF ctDNA in patients with *EGFR*-mutant NSCLC and leptomeningeal metastasis [27, 64]. Forty NSCLC patients with suspected leptomeningeal metastasis were profiled, including 35 patients with a confirmed leptomeningeal metastases [64]. *EGFR* T790M and *MET* amplification were detected in 21% and 39% in CSF ctDNA, respectively, suggesting a resistance profile to EGFR-TKI associated with leptomeningeal disease. The BLOOM study (ClinicalTrials.gov Identifier: NCT02228369) investigates osimertinib, an oral, irreversible third-generation EGFR-TKI selectively active against the EGFR T790M resistance mutation [76]. Encouraging activity has been seen in patients with leptomeningeal metastasis from NSCLC and results of *EGFR*-mutant ctDNA analyses are being awaited (ClinicalTrials.gov Identifier: NCT02228369) [77]. Thus, ctDNA analysis should be considered for the EGFR and T790M status in the CSF at diagnosis and in case of suspicion of progression of NSCLC leptomeningeal metastases to guide the therapeutic decision.

Road to clinical practice

To integrate the assessment of ctDNA obtained from CSF liquid biopsies into current standards of care, several questions and controversies have to be addressed. For almost all primary brain tumours, extent of resection is an important prognostic factor. Thus, situations where a surgical intervention is not an option, but a diagnosis would still be welcome, are rare. These might include patients with major comorbidities thought to be at high risk of complications, e.g. those with high bleeding risk for various reasons. Furthermore, there are instances where initial stereotactic biopsies of brain lesions are not informative and where non-neoplastic lesions, e.g. neuroinflammatory or neuroinfectious diseases are a differential diagnosis. In these cases, detection of tumour-defining genomic alterations in the CSF ctDNA may greatly aid in further management. Furthermore, DNA methylation profiling may represent a novel approach that will undoubtedly also be explored for confirming tumour diagnoses from small tissue samples, including CSF [36]. Future studies will also need to determine in how far serial assessments of ctDNA load in the CSF may aid in situations where response assessment based on MRI alone remains challenging, including brain tumours treated with immunotherapy, and help clinical decision making.

The situation is different for patients with brain metastases from solid tumours. For brain metastases from unknown primary tumours, either rapid neurosurgical intervention as clinically needed or initial work-up by chest abdomen CT or FDG-PET are standard procedures [78] whereas liquid biopsies have so far not assumed a role. However, patients with new brain lesions detected by neuroimaging who are known to suffer from a malignancy are not routinely sent for neurosurgical resection unless

this is thought to be in the best interest of the patient, e.g. because there are concerns regarding the validity of the radiological diagnosis, or because the patient is neurologically symptomatic. In such circumstances, notably with tumours with targetable lesions or in patients pre-exposed to chemotherapy or targeted therapy, it may be of major interest to ascertain whether the molecular tumour profile has changed, to select the most appropriate treatment. In such scenarios molecular tumour profiling from ctDNA from the CSF could become most valuable.

Similar considerations apply to patients with known leptomeningeal metastases, and targeted treatments are available for the major primary tumours associated with leptomeningeal metastasis: lung cancer, and breast cancer, and melanoma. The role of ctDNA in the CSF remains controversial if neither MRI nor routine CSF studies suggest the presence of leptomeningeal metastasis. In such situations, it can at present not be clarified whether ctDNA detected in the CSF signifies leptomeningeal tumour cell seeding. Thus, further studies are required to show that ctDNA in the CSF alone may justify treatment directed against leptomeningeal metastasis [79]. However, the identification of the EGFR mutation or T790M in the CSF of patients with CNS metastases help guide the therapeutic decision in NSCLC patients.

Regarding the current data, ctDNA should be explored for the diagnosis and in case of suspicion of progression of leptomeningeal metastases. CSF and plasma ctDNA should be evaluated in parallel.

Current limitations

Current data on ctDNA are mainly reported in small cohorts of patients, including sometimes different primary tumours. Analyses of large cohorts of patients should be carried out. Technical issues such as the potential blood contamination in the CSF sample or the minimum time interval between surgery and CSF analysis for ctDNA have to be evaluated. Confirmation studies are needed to validate the role of ctDNA analysis for the diagnosis and follow-up of patients. Importantly, the feasibility of the CSF analysis in patients with brain tumours have to be considered when the lumbar puncture is contra-indicated due to risk of herniation related to the presentation of the space-occupying CNS tumours, or abnormal coagulation.

Discussion

Conclusions and future perspectives

Increasing understanding of the genomic and epigenomic characteristics of primary brain tumours and brain and leptomeningeal metastases has uncovered the extraordinary complexity of these tumours [4, 48, 80, 81]. Nevertheless, identifying biomarkers to assist in the diagnosis, prognosis, prediction of targeted therapy responses, serial monitoring and mechanisms of therapy resistance for patients with CNS malignancies [80] remains challenging, in part because of difficulties in accessing CNS tumour-derived tissue.

CNS malignancies demonstrate considerable spatial and temporal intra-tumour and inter-tumour heterogeneity. For patients with primary brain tumours or with brain metastases, identifying

and monitoring brain-specific characteristics through CSF ctDNA may expedite the design of targeted therapies. Yet, no liquid circulating biomarkers have been validated and integrated into clinical practice for primary brain tumours or brain metastases. CSF ctDNA is a promising instrument to evaluate CNS malignancies in real-time and guide therapeutic management of patients.

The treatment of human cancer has shifted towards a precision medicine paradigm, in which the selection of a targeted therapy will rely upon the genetic anomalies in individual patients. We predict that characterising brain tumours will be feasible using CSF ctDNA in the near future. In addition, combining plasma ctDNA with CSF ctDNA, morphological analyses and imaging methods would ideally be complementary for patients with brain metastases and systemic disseminated disease. Thus, liquid biopsy approaches based on CSF are opening new avenues for the better managing of brain cancer patients.

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Disclosure

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