BRAIN UPDATE

The coming of age of liquid biopsy in neuro-oncology

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The clinical role of liquid biopsy in oncology is growing significantly. In gliomas and other brain tumours, targeted sequencing of cell-free DNA (cfDNA) from CSF may help differential diagnosis when surgery is not recommended and be more representative of tumour heterogeneity than surgical specimens, unveiling targetable genetic alterations. Given the invasive nature of lumbar puncture to obtain CSF, the quantitative analysis of cfDNA in plasma is a lively option for patient follow-up. Confounding factors may be represented by cfDNA variations due to concomitant pathologies (inflammatory diseases, seizures) or clonal haematopoiesis.

Pilot studies suggest that methylome analysis of cfDNA from plasma and temporary opening of the blood–brain barrier by ultrasound have the potential to overcome some of these limitations. Together with this, an increased understanding of mechanisms modulating the shedding of cfDNA by the tumour may help to decrypt the meaning of cfDNA kinetics in blood or CSF.

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The challenges of liquid biopsy in neuro-oncology

In the late 1970s, evidence was found that cancer patients have higher levels of circulating cell-free DNA (cfDNA) in plasma than healthy subjects.[1] It was later demonstrated that part of this cfDNA is tumour-derived[2] and could be used for the non-invasive detection of cancer mutations,[3,4] resulting in the term 'liquid biopsy' to refer to the non-invasive assessment of tumour genetic profiles using tumour-derived nucleic acids.

In the past decade, liquid biopsies have been increasingly used for response assessment, early detection of treatment resistance, prognostic prediction and treatment decisions, complementing or even replacing tissue biopsies in precision oncology.[5] This is confirmed by the validation of commercial assays of liquid biopsy for clinical use.[6,7] In colon cancer, tumour cfDNA-guided approaches

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reduced adjuvant chemotherapy use without compromising recurrence-free survival.[8] In a prospective study of 1127 patients with non-small cell lung cancer, the detection of tumour cfDNA predicted shorter survival, therapeutic targets identified by sequencing of the cfDNA provided survival advantage, and mutations specific to cfDNA were discovered in a quarter of cases, representing subclonal resistance drivers.[9]

As a result of anatomical barriers (e.g. the blood–brain barrier) that are only partially disrupted by tumours, plasma-based liquid biopsy poses a greater challenge in neuro-oncology[10] (Fig. 1). Among patients with gliomas, <10% had plasma-derived tumour-derived cfDNA, whereas 74–100% had CSF-derived tumour-derived cfDNA.[11] The shedding of cfDNA into the CSF was especially informative in patients with high tumour burden, progressive tumours, or tumours adjacent to ventricles.[12,13] CSF-cfDNA was more informative than plasma-cfDNA for targeted sequencing and mutation detection, identifying at least one tumour-derived genetic alteration in the majority of patients with gliomas or brain metastases.[12–19] The lumbar puncture, however, is an invasive procedure and is not performed in the presence of intracranial hypertension. External ventricular drainage can be a source of CSF when ventriculostomy is required in brain tumour patients. Its use as a source of CSF, however, is hampered by the possibility of obstructions and infections that may increase the amount of cfDNA simulating tumour progression.[20,21]

Thus, in the longitudinal sampling of patients in neuro-oncology, plasma is a preferable source of cfDNA (Fig. 2), as exemplified by patient follow-up with highly sensitive PCR techniques like digital PCR to detect *TERT* mutations, present in >60% of gliomas, with 90% specificity and 62.5% sensitivity.[22] Plasma was also the source to set-up a sensitive, immunoprecipitation-based protocol to analyse the methylome of small amounts of circulating cfDNA, enabling detection of large-scale DNA methylation changes enriched for tumour-specific patterns.[23] This approach was successfully expanded to tumours in the brain, as we will discuss later.[24]

Detecting tumour cell-free DNA

Observations of cfDNA fragment size distributions identified peaks corresponding to DNA associated with nucleosomes (~147 base pairs), suggesting that cfDNA is protected from nuclease digestion through associations with nucleosome core particles. As these associations are tissue-specific, comprehensive analysis of plasma-derived cfDNA allowed predicting nucleosome spacing and transcription factor footprints useful to track their tissue of origin, which in healthy individuals was lymphoid and myeloid cells.[25]

Cancer patients have higher levels of cfDNA in plasma compared to healthy subjects because of the high turnover of tumour cells.[26] Necrotic and apoptotic cells shed DNA fragments in extracellular fluids including plasma and CSF, again reflecting the genetic and epigenetic features of the cell of origin.[27] Increasing sequencing depth and using corrective algorithms allow to partially compensate for the presence of limited amounts of tumour cfDNA.[28]

Paediatric brain cancers

Liquid biopsy has made considerable progress in the follow-up of paediatric patients. In medulloblastomas, low-coverage whole-genome sequencing (WGS) of CSF-derived cfDNA used to detect minimal residual disease (MRD), unveiled tumour mutations at baseline in 83% of cases with metastatic spreading of the disease.[29] During therapy, persistence of MRD in cfDNA was associated with increased risk of progression. A similar fraction of altered cfDNA in the CSF was found by targeted sequencing (MSK-IMPACT) in a cohort of paediatric patients with medulloblastoma and other brain tumours.[30] However, in another cohort including 258 patients

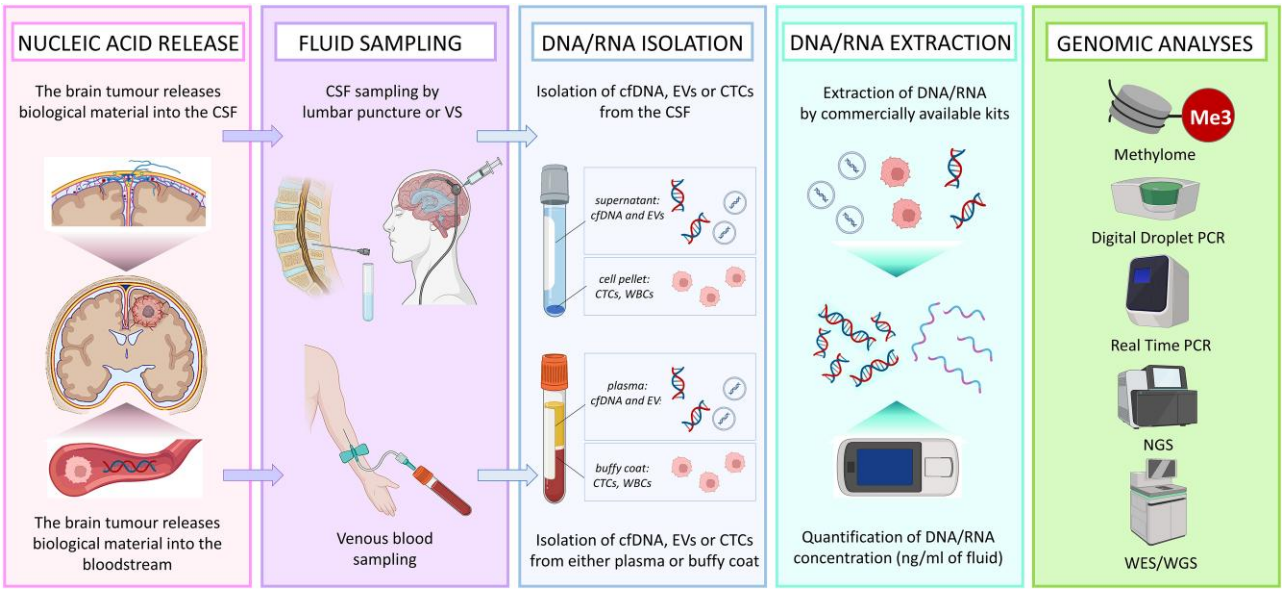

Figure 1 The workflow of plasma-based and CSF-based liquid biopsy in neuro-oncology. Tumour-derived material (cfDNA, EVs, CTCs) is released in both CSF and plasma. Samples of both fluids can be obtained in the clinic by lumbar puncture or ventricular shunts (CSF) or venous blood sampling (plasma). Fresh biological fluids should be centrifuged at different speeds according to different protocols, depending on the material, in order to separate the supernatant from the pellet and isolate the material of interest. Nucleic acids can be extracted from either EVs or CTCs and then quantified by fluorometric assay or qPCR. Once good-quality material has been obtained, a plethora of genetic analyses can be performed, from whole-genome sequencing to methylomics. cfDNA = cell-free DNA; CTCs = circulating tumour cells; EVs = extracellular vesicles; NGS = next-generation sequencing; VS = ventricular shunt; WBCs = white blood cells; WES = whole exome sequencing; WGS = whole genome sequencing. Created with Biorender.com.

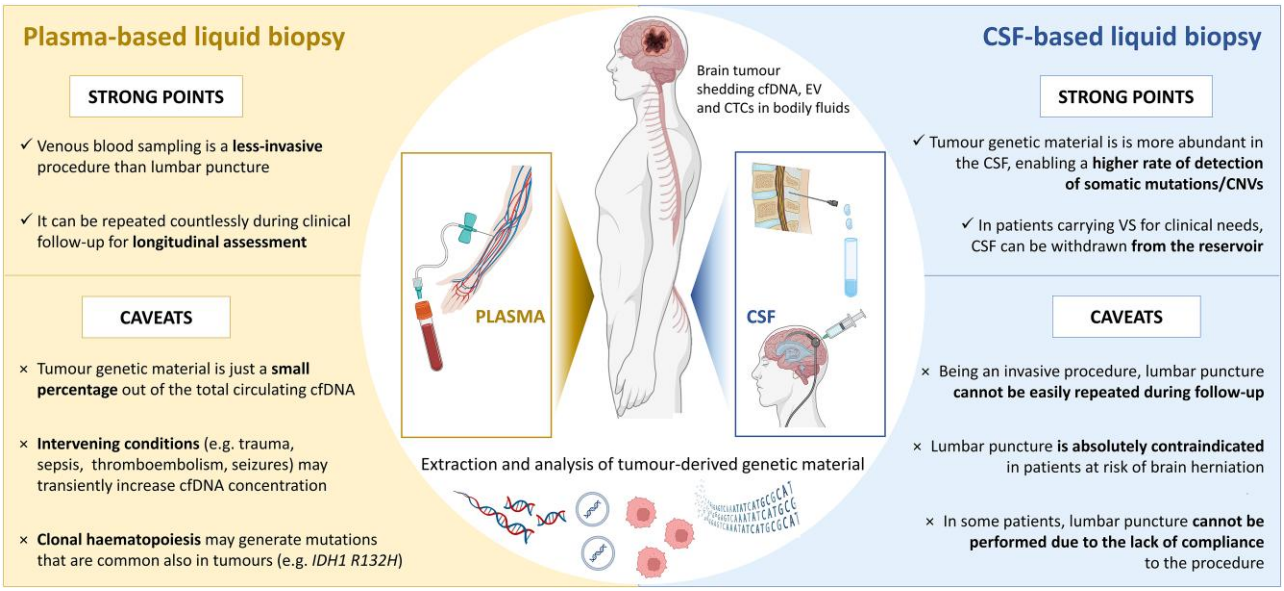

Figure 2 Strong points and caveats of plasma-based (*left*) and CSF-based (*right*) liquid biopsy of cfDNA. Both plasma and CSF can be precious sources of genetic material shed by brain tumours in bodily fluids. On one hand, venous blood sampling is less invasive than CSF withdrawal, and can be easily repeated during clinical follow-up, and namely during routine outpatient visits. On the other hand, CSF generally contains larger amounts of tumour-derived genetic material and lesser amounts of genetic material released from normal cells, resulting in higher rates of mutation detection. Moreover, the total amount of cfDNA in plasma may also transiently increase due to intervening conditions (e.g. venous thromboembolism,[90] inflammation,[91] sepsis,[91,92] focal epilepsy[94]), which should be accounted for during result interpretation. cfDNA = cell-free DNA; CNVs = copy number variations; CTCs = circulating tumour cells; EVs = extracellular vesicles; VS = ventricular shunt. Created with Biorender.com.

with different diagnosis (mostly high-grade gliomas) using ultra-low pass WGS copy number enabled the detection of molecular alterations in 9/46 CSF, 3/230 plasma and 0/153 urine samples.[31]

Diffuse midline gliomas, more frequent in childhood, are characterized by the H3K27M mutation of the H3 histone gene that has been analysed by digital PCR in cfDNA from CSF and plasma of patients enrolled in the NCT03416530 trial.[32] Decrease of the mutant allelic frequencies (MAF) in the CSF was associated with prolonged progression-free survival (PFS) ($P < 0.004$), while 25% increase or more of MAF was predictive of progression in half of the patients, both in plasma and CSF.[32]

Extracellular vesicles

Machine-learning classification of plasma-derived extracellular vesicles (EVs) cargo, including immunoglobulins, revealed 95% sensitivity and 90% specificity in detecting different cancers not originating in the brain.[33] Initial evidence supports a role for extracellular vesicles in the diagnosis and clinical follow-up of gliomas.[34,35] Meningiomas release sizable amounts of extracellular vesicles and their amount decreases after surgery.[36] *In vitro* extracellular vesicle DNA from meningioma cultures reflect the genetic, epigenetic and mutational landscape of the tumour of origin.[36]

Extracellular vesicles from glioblastoma (GBM) patients can help predict prognosis,[37–42] mirror modifications of tumour volume before and after surgery,[43] and express specific GBM markers.[44] They contained EGFRvIII[45,46] and, intriguingly, amounts of the von Willebrand factor significantly higher than in healthy controls.[47]

Extracellular vesicles may play a role in conditioning the GBM immune milieu. Exosomes, one type of extracellular vesicles, can transport TGF-β, one major immune suppressive cytokine produced by GBM.[48] In a cohort of patients receiving a dendritic cell vaccination, IL-8 (but also TGF-β) mRNA in plasma exosomes, positively correlated with immunological response to glioma antigens.[49] On the other hand, exosomes in the CSF of GBM patients contain the LGALS9 ligand that inhibits antigen processing and presentation by dendritic cells by binding their TIM3 receptor.[50] Recent data suggest extracellular vesicle-mediated interactions of GBM and natural killer cells showing that circulating immune vesicles 'carry unique tumour-specific signals'.[51]

Clinical studies reporting on extracellular vesicles in liquid biopsy are summarized in Table 1. Overall evidence for a prominent role of EVs for diagnosis or clinical follow-up of brain tumours in the peripheral blood or the CSF requires more data. For instance, qalectin-3 binding protein in blood plasma and plasma-derived extracellular vesicles was at significantly higher levels in GBM patients than in healthy controls, but detection accuracy in predicting patient mortality was considerably higher in plasma than plasma extracellular vesicles (75% versus 45%, respectively).[52]

Circulating tumour cells

The presence of circulating tumour cells (CTCs) in peripheral blood originating in primary brain tumours has been described years ago but their scarcity has limited their clinical use.[53–56] On the contrary, selection of CTCs in CSF can play a relevant role in disease staging and follow-up in patients affected by medulloblastoma[57] and leptomeningeal metastases (LM).[58] In LM, CSF-CTCs were detected in 43/95 samples and one CSF-CTC/ml provided a diagnostic threshold with high sensitivity and specificity.[58] A CTCs assay based on epithelial cell adhesion molecule (EpCAM) immunoflow cytometry in CSF of patients with suspected LM showed higher sensitivity than cytology (94% versus 76%).[59] In LM patients treated by proton cranio-spinal irradiation, the presence of <53 CTCs in 3 ml of CSF was associated with longer PFS (12 versus 6 months; $P < 0.01$).[60]

The cfDNA, however, showed higher mutation rates (43.6% versus 19.8%) and MAF (41.1% versus 13.0%; $P = 0.0001$) than CTCs.[61]

Tracking glioma spatial heterogeneity and branched evolution

Gliomas and GBM are heterogeneous tumours composed of several clonal cell populations with different genetic and transcriptional profiles.[62] Because of this, biopsy samples obtained from a single area may not represent the entire tumour while liquid biopsies might provide a more comprehensive representation of tumour molecular profiles. In 1 of 13 gliomas studied by shallow WGS, molecular alterations were detected in CSF cfDNA and not in one corresponding surgical specimen.[63] In 17 gliomas, 34% of the mutations found by targeted sequencing were detected in the CSF only.[64]

Under the selective pressure of alkylating chemotherapy, GBM cells are subject to hypermutation and branched clonal evolution.[65] Since only a minor proportion of GBM patients undergo second surgery at recurrence,[66] liquid biopsies offer the chance to reassess tumour molecular profile and to monitor tumour evolution during treatment with MAF in plasma and CSF, as suggested by preliminary evidence in brainstem paediatric gliomas[67–69] (Table 2). Along the same line, longitudinal liquid biopsies of plasma might support neuroimaging in response assessment (e.g. pseudoprogression versus true progression), a clinically relevant point raised by the liquid biopsy task-force of the RANO (Response Assessment in Neuro-Oncology) consortium.[70]

Recently, evidence has emerged that liquid biopsies might also have a prognostic role, given that tumour cfDNA detection in the CSF is a strong independent predictor of reduced overall survival.[15] In plasma, a first study in 42 GBM patients showed correlations between shorter PFS and cfDNA higher than 13.4 ng/ml.[71] In a larger subsequent study on 62 GBM using a higher threshold (25. 2 ng/ml), higher values of cfDNA were also associated with shorter overall survival.[72] This observation was challenged by data on another cohort of GBM patients, showing correlations between cfDNA levels and disease progression but not between cfDNA levels before surgery and survival[73] (Table 2).

Identifying therapeutic targets

Although in glioma patients the number of actionable molecular alterations for targeted therapies is limited,[74] the potential for their non-invasive identification is appealing. Studies of patient cohorts of primary brain tumours showed that actionable genetic alterations—including *IDH1*, *BRAF*, *EGFR* and *NRAS* mutations—could be identified from plasma cfDNA in 18–24% of patients.[75,76]

Liquid biopsies can also be considered for targeted therapy of brain metastases, which show divergence in terms of genetic profile[77,78] and immune microenvironment[79] with respect to the primary tumour. As an example, by identifying *EGFR* mutations as an intervening mechanism of resistance to EGFR inhibitors,[80,81] liquid biopsies might provide a chance for a prompt switch in therapy.

The observation that MAF in the CSF decreases with response to treatment and increases at recurrence[82,83] confirms further the potential of CSF-based liquid biopsies.

The 'background noise' in plasma-based liquid biopsy

The levels of cfDNA can change during the day and in the presence of stress.[84,85]

A confounding factor for interpreting the results of plasma liquid biopsies is represented by clonal haematopoiesis (CH), which may lead to the accumulation of non-tumoural somatic mutations in haematopoietic stem cells.[86] If interpreted as tumour-associated genetic alterations in cfDNA analyses, they could steer toward inappropriate therapeutic decisions.[86,87] CH mutations are quite common in the general population, associated with ageing (9.5–18.4% beyond 70 years old), prior radiotherapy or chemotherapy, and increased risk of developing haematological malignancies and cardiovascular diseases.[87,88] They mainly include mutations in epigenetic modulators (*DNMT3A*, *TET2* and *ASXL*), and in genes often altered in solid (*KRAS*, *GNAS*, *NRAS* and *PIK3CA*) and haematological tumours (*JAK2*, *PPM1D*, *TP53*, *SF3B1*, *SRSF2*, *IDH1* and *IDH2*). Because of CH, the finding of *IDH1* and *IDH2* mutations in plasma-derived cfDNA of patients with gliomas may require investigation of their origin by sequencing the DNA from white blood cells (and tumour tissue, when available).[86] The relevance of CH was demonstrated by the finding of mutations in peripheral blood but not in tumour tissue in 17 of 18 patients tested.[89] Of note, CH is more frequent after chemoradiotherapy and is associated with shorter overall survival.

Other factors creating background noise in neuro-oncology include venous thromboembolism,[90] inflammation,[91] sepsis[92] and trauma.[92] In the plasma of septic patients, a positive correlation was present between the levels of myeloperoxidase, an enzyme released by activated neutrophils, and the amounts of cfDNA. NETosis, a specific form of cell death in which activated neutrophils release neutrophil extracellular traps (NETs) in response to inflammation, infection or hypoxia. Myeloperoxidase, released by activated neutrophils, was found significantly correlated to cfDNA levels in septic patients.[92] NETosis can also be the underlying cause of the increased release of cfDNA in autoimmune diseases like lupus erythematosus and in rheumatoid arthritis.[93]

Of relevance in neuro-oncology is also the observation of increased cfDNA in the blood of patients with epileptic seizures. This was signalled in 2013: the difference in cfDNA concentrations between patients and controls was limited but reached statistical significance.[94] The values, however, were considerably higher than expected (μg/ml rather than ng/ml), suggesting the presence of contaminating nuclear DNA. Confirmation in other patient cohorts would help to validate these observations.

Increasing the clinical translation of liquid biopsy in neuro-oncology

The large majority of tumour cfDNA does not contain tumour-defining mutations. Epigenetic profiles, on the other hand, are widely distributed in the genome[95] and their evolution is modulated by the number of cell divisions as in ageing and cancer, where DNA methylation loss becomes more frequent.[96,97] The potential of methylation analysis in brain tumour diagnostics is well illustrated by the development of a DNA methylation-based classification of CNS tumours founded on a machine-learning approach.[98] The use of advanced methylation-based sequencing allowed the identification of specific methylation signatures and accurate classification of brain tumours from circulating cfDNA.[24] A similar result

Table 1 A selection of studies on liquid biopsy, listed based on order of publication, suggesting the potential use of extracellular vesicles for prognostic stratification and tumour monitoring in patients with primary and secondary CNS tumours

Reference	*n*	Population	Tumour type	Biological fluid	Biomarker	Methods	Clinical application	Main findings of the study
Koch *et al.*[37]	11	Adults	GBM	Plasma	MV count	Flow cytometry, Electron microscopy	Response assessment	MV count helped to distinguish between tumour progression and pseudoprogression
Shi *et al.*[38]	70	n.r.	Grade 2 astrocytoma, Grade 2 ependymoma, GBM	Plasma CSF	miR-21	TEM, WB, RT-PCR	Prognosis	miR-21 levels can predict PFS and OS
Evans *et al.*[39]	16	Adults	GBM	Plasma	MV count	Flow cytometry, Cryoelectron microscopy	Prognosis	MV count can predict PFS and OS
Manda *et al.*[46]	96	Adults	HGG	Serum	EGFRvIII	BCA protein assay, TEM, flow cytometry, RT-PCR	Prognosis	EGFRvIII expression in EVs correlates with OS
Indira Chandran *et al.*[44]	82	Adults	HGG and LGG	Plasma	SDC1	NTA, TEM, ProSeek multiplex proximity extension assay, ELISA	Prognosis	SDC1 expression in EVs correlates with OS
Osti *et al.*[43]	68	Adults	GBM and other CNS tumours	Plasma	EVs count and size, proteomic profile	NTA, TEM, mass spectrometry	Response assessment	EVs can assist in GBM diagnosis and monitoring
Zhong *et al.*[40]	147	Adults	HGG	Serum	miR-29b	RT-PCR	Prognosis	miR-29b levels correlate with OS
Batool *et al.*[35]	40	Adults	HGG	Plasma	EGFRvIII	ddPCR	Response assessment	EGFRvIII mutant copies assisted in response assessment
Khristov *et al.*[41]	82	Adults	GBM	Plasma	IL13Ra2	ELISA	Prognosis	IL13Ra2 levels in plasma correlate with OS
Dobra *et al.*[42]	222	Adults	GBM, meningiomas, brain metastases	Serum	MMP-9	NTA, TEM, WB, LC-MS, ELISA	Prognosis	MMP-9 levels correlate with OS

ddPCR = digital droplet PCR; ELISA = enzyme-linked immunosorbent assay; EVs = extracellular vesicles; GBM = glioblastoma; HGG = high-grade gliomas; LC-MS = liquid chromatography–mass spectrometry; LGG = low grade gliomas; MV = microvesicles; *n* = number of patients included; n.r. = information not reported; NTA = nanoparticle tracking analysis; OS = overall survival; PFS = progression-free survival; RT-PCR = reverse transcription PCR; TEM = transmission electron microscopy; WB = western blot.

Table 2 A selection of relevant studies, listed in order of publication, suggesting a potential application of cfDNA-based liquid biopsies for purposes of response assessment and prognostic stratification in patients with primary and secondary CNS tumours

Reference	*n*	Population	Tumour type	Biological fluid	Biomarker	Sequencing method	Clinical application	Main findings of the study
De Mattos-Arruda *et al.*[14]	12	Adults	Primary and secondary CNS tumours	CSF	Mutations and CNVs	NGS ddPCR	Response assessment	MAF of CSF tcfDNA decreased after surgery and increased at progression
Panditharatna *et al.*[12]	48	Children AYA	Diffuse midline gliomas	Plasma	H3K27M mutations	ddPCR	Response assessment	MAF tracked tumour evolution and predicted relapse
Juratli *et al.*[18]	38	Adults	Newly-diagnosed and recurrent GBM	CSF Plasma	TERTp mutations	ddPCR	Prognosis	MAF are predictive of OS
Miller *et al.*[15]	85	Adults	LGG and GBM	CSF	tcfDNA detection	NGS	Prognosis	The presence of tcfDNA was associated with shorter OS
Escudero *et al.*[57]	13	Children Adolescents	Medulloblastoma	CSF Plasma	tcfDNA	WES ddPCR	Response assessment	CSF tcfDNA allows the study of minimal residual disease
Muralidharan *et al.*[22]	157	Adults	Lower grade gliomas and GBM	Plasma	TERTp mutations	ddPCR	Response assessment	MAF paralleled clinical and radiological evolution
Fontanilles *et al.*[73]	52	Adults	Newly diagnosed GBM or gliosarcoma	Plasma	cfDNA concentration	–	Response assessment	Median cfDNA concentrations tracked tumour evolution
Bagley *et al.*[72]	62	Adults	Newly diagnosed GBM	Plasma	cfDNA concentration	–	Prognosis	Preoperative cfDNA concentration correlated with PFS and OS
Liu *et al.*[29]	134	Children	Newly diagnosed medulloblastoma	CSF	tcfDNA detection	NGS	Response assessment Prognosis	Patients with detectable tcfDNA during treatment had worse PFS
Li *et al.*[78]	92	Adults	Newly diagnosed NSCLC with brain metastases	CSF Plasma	tcfDNA detection	NGS	Response assessment Prognosis	Patients with CSF tcfDNA at baseline had shorter OS
Cantor *et al.*[32]	28	Children	H3K27M-mutant gliomas	CSF Plasma	H3K27M mutations	ddPCR	Response assessment Prognosis	MAF variations correlate with PFS and predict targeted therapy response
Kojic *et al.*[19]	12	Children Preadolecents	Malignant primary brain tumours	CSF	tcfDNA	WES ddPCR	Response assessment	ctDNA correlated with disease course and clinical outcomes

AYA = adolescents and young adults; cfDNA = cell-free DNA; CNVs = copy number variations; ddPCR = digital droplet PCR; GBM = glioblastoma; LGG = lower grade gliomas; MAF = mutant allelic frequencies; *n* = number of patients included; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; OS = overall survival; PFS = progression-free survival; tcfDNA = tumour-derived cell-free DNA; TERTp = TERT promoter; WES = whole exome sequencing.

was replicated by another group, who developed a machine learning algorithm to distinguish patients with or without glioma based on a cfDNA-derived methylation signature.[99]

In vitro, epigenetic profiling was also used to characterize the DNA cargo of glioma extracellular vesicles.[100] *In vivo*, extracellular vesicles may be released by neuronal-like subpopulation of glioma interconnecting with the surrounding normal astrocytes and neurons through microtubes, a key strategy favouring invasion.[101] Extracellular vesicles are more abundant in GBM patients than in controls and their protein cargo may allude to GBM biology.[43]

Of note, blood platelets have the ability to take up secreted RNA-containing membrane vesicles derived from glioma cells.[102] Recent data showed that tumour-educated platelet (TEP) RNA-based blood tests enable the detection of 18 cancer types. Specifically, the detection rate in GBM was 51%.[103]

As mentioned earlier, the blood–brain barrier limits the shedding of biomarkers, such as cfDNA, from brain tumours into the bloodstream, hampering their detection by conventional assays. Transcranial magnetic resonance-guided focused ultrasound (MRgFUS) can transiently open the blood–brain barrier, providing an opportunity for less invasive access to brain pathology. Meng *et al.*[104] showed first-in-human proof-of-concept in nine GBM patients by finding that MRgFUS acutely enhances plasma cfDNA (2.6 ± 1.2-fold, $P < 0.01$, Wilcoxon signed-rank test).

Artificial intelligence

Artificial Intelligence (AI) and, specifically, machine learning shows considerable potential also for the elaboration of data gained by liquid biopsy.[105] For instance, in patients with colorectal cancer ($n = 72$), a multimodal liquid biopsy resulting from a machine learning algorithm combining analysis of CTCs, exosomes and cfDNA outperformed each of the three biomarkers in predicting overall survival.[106] Notably, the machine learning-generated classifier that uses whole genome methylation sequencing showed highest detection sensitivity out of 10 machine-learning classifiers trained on the same samples.[107]

An 'upstream' size selection (an example of cfDNA fragmentomics) can help enrich tumour cfDNA that are typically shorter than normal cfDNA fragments. By selecting *in silico* DNA fragments between 90 and 150 base pairs in length, Mouliere and colleagues[108] achieved a more than 2-fold median enrichment of tumour cfDNA in ~95% of cases, increasing the detection rate of clinically actionable mutations and copy number alterations in plasma cfDNA.

Furthermore, machine learning-guided extraction of MRI features of brain tumours, characterizing their texture,[109] could complement quantitative data obtained by cfDNA improving the clinical follow-up and the rationale for therapeutic decisions.

Outstanding questions

In previous reports a negative association of baseline CSF cfDNA concentration with survival was independent of demographic or clinico-pathological data.[15] A similar trend was reported with plasma cfDNA.[72] This raises the intriguing possibility that cfDNA release is depending on intrinsic biological features of the tumour and not just on its size. Thus, to make progress in the interpretation of liquid biopsy data a deeper insight into mechanisms of cfDNA release into the CSF or the bloodstream is desirable.

The process of nucleosome eviction might be one relevant mechanism of cfDNA increase in the periphery worth further investigation. Different genes are responsible for nucleosome eviction and consequent chromatin reshaping that increases the availability of DNA stretches for interaction with transcription factors in promoter or enhancer regions of the genome. Bromodomain protein 4 (BRD4) is one such factor, facilitating nucleosome eviction by acetylating the critical residue for nucleosome stability H3 K122.[110] Notably, BRD4, also implied in enhancing epistasis by maintaining 3D chromosomal interactions,[111] is overexpressed in GBM and together with other BET bromodomain proteins is required for GBM proliferation.[112]

Another mechanism highly relevant to glioma biology is hypoxia.[113,114] Hypoxic repression of endothelial nitric-oxide synthase transcription is coupled with nucleosome eviction.[115] Nucleosome-free DNA regions (NFRs) can be established by nucleosome eviction in hypoxia-inducible promoters by hypoxia-inducible transcription factors and nucleosome re-assembly can take place hours after reoxygenation.[116] NFRs are typical configurations of chromatin in active gene promoters, and each NFR in a promoter region encompasses 100–500 base pairs, corresponding to 1–2 nucleosomes.

Final remarks

The present status of liquid biopsy in neuro-oncology is summarized in Fig. 2. Analysis of CSF may provide information on differential diagnosis when surgery is not recommended or has not been informative. Plasma is more amenable to clinical follow-up, helping to decipher brain tumour modulation by tissue microenvironment and therapeutic challenges. Technical advances at three levels have the potential to improve the informative potential of plasma cfDNA: methylation profiling, temporary blood–brain barrier disruption, and increased knowledge of the biology of cfDNA shedding by the tumour.

Literature search

Data for this Update were identified by searches of PubMed, and references from relevant articles using the search terms 'liquid biopsy', 'cell free DNA', 'glioma', 'brain metastases', 'liquid biopsy and CSF', 'liquid biopsy and plasma'. Abstracts and reports from meetings were not included. Only articles published in English between 1977 and March 2023 were included.

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