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# Tapping into the genome: the role of CSF ctDNA liquid biopsy in glioma

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**Abstract**

Liquid biopsy has emerged as a novel noninvasive tool in cancer diagnostics. While significant strides have been made in other malignancies using liquid biopsy for diagnosis, disease monitoring, and treatment selection, development of these assays has been more challenging for brain tumors. Recently, research in primary and metastatic brain tumors has begun to harness the potential utility of liquid biopsy—particularly using circulating tumor DNA (ctDNA). Initial studies to identify ctDNA in plasma of brain tumor patients have shown feasibility, but the yield of ctDNA is far below that for other malignancies. Attention has therefore turned to the cerebrospinal fluid (CSF) as a more robust source of ctDNA. This review discusses the unique considerations in liquid biopsy for glioma and places them in the context of the work to date. We address the utility of CSF liquid biopsy for diagnosis, longitudinal monitoring, tracking tumor evolution, clinical trial eligibility, and prognostication. We discuss the differences in assay requirements for each clinical application to best optimize factors such as efficacy, cost, and speed. Ultimately, CSF liquid biopsy has the potential to transform how we manage primary brain tumor patients.

**Keywords**

circulating tumor DNA (ctDNA) | cerebrospinal fluid (CSF) | glioma | liquid biopsy | primary brain tumors

The use of liquid biopsy for noninvasive testing has revolutionized oncology with applications for diagnostics, disease monitoring, and treatment selection. However, liquid biopsy assays for brain tumors have been more challenging to develop than with other solid tumor malignancies. Many factors contribute to this, including low levels of tumor-derived circulating tumor DNA (ctDNA) in plasma, potentially blocked from exiting the central nervous system (CNS) by the blood-brain barrier. In brain tumor patients, the use of cerebrospinal fluid (CSF) for liquid biopsy is a potential alternative to plasma and multiple studies have shown not only increased concentrations of ctDNA in the CSF but also improved rates of detection.[1–6] Hence, CSF ctDNA has shown significant promise for use in clinical diagnostics, longitudinal monitoring, tracking genomic evolution, and prognostication in brain tumor management.

## Cell-Free DNA Background

Cell-free DNAs (cfDNAs) are fragments of DNA that are shed primarily by apoptotic and necrotic cells into biofluids such as blood, CSF, urine, and saliva. ctDNA is the fraction of the cfDNA that originates from cancer cells. ctDNA exists as shorter fragments than nontumor DNA and is amenable to PCR and next-generation sequencing (NGS)-based analyses, with NGS offering greater multiplexing capabilities for mutation profiling.[7,8]

## Plasma Circulating Tumor DNA in Other Cancers

Liquid biopsy has proven to be a reliable tool for the diagnosis, treatment, and prognostication of various types

of cancers including lung, breast, bladder, and colorectal.[9–14] In particular, longitudinal liquid biopsy has made the greatest progress in lung cancer, being used to guide therapy decisions, monitor disease, and evaluate treatment response.[11,12,15–17] Research in liquid biopsy led to FDA approval of multiple liquid biopsy NGS platform tests including FoundationOne® Liquid CDx and Guardant360® CDx. When used to probe plasma for ctDNA, these diagnostic tools accurately confirm targetable genetic alterations in solid tumor malignancies such as nonsmall cell lung cancer (NSCLC), breast cancer, colorectal cancer, ovarian cancer, and prostate cancer.

## Plasma Circulating Tumor DNA in Glioma

The initial work on ctDNA from brain tumors began in plasma. In a study using digital droplet PCR (ddPCR) to search for plasma ctDNA in patients across various tumor types, Bettegowda et al. found that for glioma, compared with other malignancies, there was significantly lower ctDNA and postulated that the blood-brain barrier may be the primary obstacle to ctDNA detection in plasma.[18] Despite technical challenges, both NGS and ddPCR have recently emerged as feasible methods for assessing plasma ctDNA in glioma (Table 1). By using the Guardant360 NGS targeted assay on 419 primary brain tumor (PBT) patients, Piccioni et al. detected at least one sample with positive plasma ctDNA for a given patient in approximately 50% of primary brain tumors (PBT) and 55% of glioblastomas (GBM) when accounting for serial testing.[19] Also utilizing a NGS platform, Bagley et al. found elevated levels of plasma cfDNA in patients with glioma and demonstrated the potential for correlating this quantitative elevation of cfDNA to tumor burden and progression-free survival.[20,21] However, if plasma cfDNA assays are not run against a matched normal, high levels of plasma cfDNA positivity must be interpreted with caution; known rates of clonal hematopoiesis can lead to accumulation of nontumor-derived somatic mutations in hematopoietic cells resulting in significant background noise in plasma, especially after radiation therapy or chemotherapy.[22] Alternatively, ddPCR targeting specific mutations, such as H3K27M and TERT promoter mutations, has also been successful in diagnosing glioma.[1,23] Recently, Muralidharan et al. utilized a novel ddPCR assay focused on the two most common TERT promoter mutations and demonstrated improved sensitivity and specificity for plasma ctDNA detection and longitudinal monitoring in glioma.[24] While there has been progress in detecting plasma ctDNA in glioma with both NGS and ddPCR approaches, the relatively low quantity of glioma ctDNA in plasma amid a significantly greater amount of nontumor-derived cfDNA poses a significant obstacle. Due to the challenges in detecting ctDNA in plasma, to increase the likelihood of success for the assay, often targeted approaches are needed, limiting its utility for diagnosis and monitoring tumor evolution.

## CSF Circulating Tumor DNA in Glioma

### Overview

Recent work has brought increased attention to the use of CSF as an alternative to plasma for cfDNA in brain tumor patients. In contrast to plasma-based tests where the search for ctDNA is hindered by high levels of background nontumor-derived cfDNA necessitating deep sequencing, CSF approaches benefit from a more acellular, sterile environment where ctDNA is enriched and depth of sequencing is less of a technical limitation.[2,25] For both PBTs and CNS metastatic disease, several groups have now confirmed that CSF is more optimal for ctDNA sequencing with CSF harboring higher ctDNA levels than plasma while also maintaining high concordance of mutational profile to the CNS specific tissue (Table 1).[2,4,5] Studies have also demonstrated that the likelihood of CSF ctDNA detection increases in patients with active disease, high tumor burden, lesions proximal to CSF spaces, and leptomeningeal disease.[2,5,26] In one of the first studies assessing the utility of ctDNA as a liquid biopsy in the CSF, Wang et al. detected ctDNA in the CSF of 26/35 (74%) primary brain tumor patients by utilizing an assay on CSF from the surgical cavity at time of resection.[25] With this new untapped source of genomic data, we must now determine how CSF liquid biopsy can be best utilized to help address major challenges in the field of neuro-oncology including diagnosis, longitudinal monitoring, tumor evolution, clinical trial eligibility, and prognostication. Each of these clinical objectives requires different approaches to sequencing ctDNA to optimize efficacy, cost effectiveness, and turnaround time.

### Diagnosis

While tumor tissue is ideal for diagnosis in glioma patients, there are several clinical scenarios where tissue sampling is high risk or clinically unjustified due to factors such as anatomical location (e.g. brainstem gliomas) or multifocal recurrent disease. There are also instances where tissue biopsy is nondiagnostic and NGS fails due to low tumor content. In these cases, CSF ctDNA via lumbar puncture offers an alternative mechanism for diagnosis or investigation of a molecular target. Here, the goal would be to utilize a highly sensitive genomic panel containing the most common disease-defining alterations in glioma to make a molecular diagnosis; the detection of any disease-defining mutation is monumental for these patients in whom a tissue diagnosis is not possible. In the most recent WHO classification update, molecular markers are defining features of some pathologies including the IDH mutation for glioma subtypes and the H3K27M alteration in diffuse midline gliomas.[27] Existing targeted agents such as BRAF/MEK inhibitors as well as new therapeutic developments such as IDH inhibitors and ONC201 are often studied and exclusively utilized in patients with tumors containing these disease-defining mutations, necessitating accurate molecular diagnosis to get patients on novel treatments.[28–30] For molecular diagnosis, ddPCR emerges as a relatively inexpensive yet highly sensitive test and has already shown to

**Table 1.** Selected Studies Using Blood and CSF Cell-Free DNA for Liquid Biopsy in Brain Cancer

| | Reference | Disease Subtype | Number of Patients | Sequencing Methods | Sequencing Target | Pertinent Results | Clinical Applicability |
|---|---|---|---|---|---|---|---|
| Blood | Bettegowda et al. 2014 | Solid Tumors | 640 | ddPCR | Tumor Guided | Detection of ctDNA in < 10% gliomas | Diagnosis |
| | Piccioni et al. 2019 | PBT | 419 | NGS | Guardant360 (54,68, 73 gene panels) | Detection of ≥ 1 somatic mutation in plasma ctDNA in 211/419 (50%) patients | Diagnosis |
| | Bagley et al. 2020 | Newly diagnosed GBM | 42 | NGS | Guardant360 (74 gene panel) | Detection of ≥ 1 somatic mutation in plasma ctDNA in 11/20 (55%) patients | Prognosis<br>Longitudinal Monitoring |
| | Bagley et al. 2021 | Newly diagnosed IDH WT GBM | 62 | N/A | N/A | Elevated levels of cfDNA associated with inferior PFS and OS | Prognosis |
| | Muralidharan et al. 2021 | Glioma | 157 | ddPCR | TERT promoter mutation | Test Sensitivity of 62.5% and Specificity of 90% | Diagnosis<br>Longitudinal Monitoring |
| CSF | Pan et al. 2015 | PBT & CNS Metastases | 7 | ddPCR Targeted Amplicon | Tumor Guided | Detection of diagnostic mutations in CSF in 6/7 (86%) patients | Diagnosis |
| | De Mattos-Arruda et al. 2015 | PBT & CNS Metastases | 12 | ddPCR & NGS | Tumor Guided<br>MSK IMPACT (341 gene panel) | Detection of CSF ctDNA in 12/12 (100%) cases<br>MAF in CSF tracked with treatments and disease burden | Diagnosis<br>Longitudinal Monitoring |
| | Wang et al. 2015 | PBT | 35 | ddPCR & WES | Tumor Guided | Detection of CSF ctDNA in 26/35 (74%) patients | Diagnosis |
| | Pentsova et al. 2016 | PBT & CNS Metastases | 53 | NGS | MSK IMPACT (341 gene panel) | Detection of CSF ctDNA in 20/32 (63%) CNS metastases and 6/12 (50%) PBTs | Diagnosis<br>Evolution |
| | Huang et al. 2017 | Pediatric brain tumors | 11 | ddPCR | Histone H3 mutations | Test Sensitivity of 87.5% and Specificity of 100%<br>Detection of H3K27M in CSF of 4/6 (66.7%) DMGs | Diagnosis |
| | Martinez-Ricarte et al. 2018 | Glioma | 20 | ddPCR & NGS | Targeted glioma panel | Detection of CSF ctDNA in 17/20 (85%) | Diagnosis |
| | Panditharatna et al. 2018 | Pediatric HGG/DMG | 48 | ddPCR | H3K27M mutation | Detection of H3K27M in CSF and plasma in 88% of DMGs<br>Detection of H3K27M ctDNA in CSF of 20/23 (87%) H3K27M mut. tumors | Diagnosis<br>Longitudinal Monitoring |
| | Mouliere et al. 2018 | Glioma | 13 | sWGS | N/A | Detection of CSF somatic copy number alterations in 5/13 (38.4%) patients | Diagnosis |
| | Pan et al. 2019 | Primary Brainstem Tumor | 57 | NGS | Panel of 68 glioma-associated genes | Detection of ≥ 1 somatic tumor specific mutation in CSF in 47/57 (82.5%) patients | Diagnosis |
| | Miller et al. 2019 | Glioma | 85 | NGS | MSK IMPACT (341, 410, 468 gene panels) | Detection of CSF ctDNA in 42/85 (49.4%) patients and associated with disease burden and adverse outcome | Diagnosis<br>Prognosis<br>Evolution |
| | Escuerdo et al. 2020 | Medulloblastoma | 13 | ddPCR | Tumor Guided | Detection of CSF ctDNA in 10/13 (76.9%) patients | Diagnosis<br>Longitudinal Monitoring |
| | Izquierdo et al. 2021 | Pediatric HGG/DMG | 32 | ddPCR | Tumor Guided | Detection of ≥ 1 somatic mutation in CSF ctDNA in 6/9 (67%) samples | Diagnosis |

**Table 1.** Continued

| Reference | Disease Subtype | Number of Patients | Sequencing Methods | Sequencing Target | Pertinent Results | Clinical Applicability |
|---|---|---|---|---|---|---|
| Fujioka et al. 2021 | Diffuse Glioma | 34 | ddPCR | IDH1, TERT promoter, H3K27M mutations | Detection of diagnostic mutations in intracranial CSF in 20/34 (71%) compared to detection in 28/34 (82%) in tumor samples | Diagnosis |
| Liu et al. 2021 | Medulloblastoma | 123 | sWGS | N/A | Tumor Associated CNV in CSF as viable surrogate for minimum residual disease | Longitudinal Monitoring |

ddPCR, digital droplet PCR; PBT, primary brain tumor; NGS, next-generation sequencing; MAF, mutant allele fraction; GBM, glioblastoma; WT, wild type; PFS, progression-free survival; OS, overall survival; WES, whole-exome sequencing; DMG, diffuse midline glioma; HGG, high-grade glioma; sWGS, shallow whole-genome sequencing; CNV, copy number variation.

be effective for identifying specific mutations in the CSF of pediatric patients with midline gliomas.[1,31,32] An approach with a ddPCR assay targeting a few common glioma mutations such as TERT or H3K27M would be reasonable since the diagnostic priority is finding the disease-defining mutation, not necessarily capturing the full genomic profile. On the other hand, a targeted NGS glioma-specific panel would provide more information by casting a wider net for possible mutation capture to detect this "rule in" mutation, most likely at the cost of lower sensitivity. Studies utilizing NGS panels with cancer hotspot mutations or glioma-associated genes in CSF from glioma patients have also shown reliability in identifying tumor-specific mutations that would allow diagnostic capabilities.[3,5,6] Martinez-Ricarte et al. used a glioma-specific panel on CSF and found ctDNA in 17/20 (85%) patients.[6]

Perhaps the ideal sequencing method for initial diagnosis would be combining NGS and ddPCR in a tiered approach. NGS has the potential advantage of not just identifying the disease-defining alterations but also uncovering the genomic landscape of the tumor which may be useful for treatment decisions and in some situations, prognosis (e.g. CDKN2A/B deletion in IDH mutant tumors). If a sample is negative for CSF ctDNA via an NGS panel, then a higher sensitivity ddPCR assay could be attempted to "rescue" the negative result. A challenge with this tiered approach however is that there must be residual material to test (CSF, extracted cfDNA, or residual library). For patients with recurrent disease, where a targetable mutation is to be confirmed or identified, an NGS approach would be preferable to ddPCR due to the known genomic heterogeneity seen in primary brain tumors.

## Longitudinal Monitoring

Another area where CSF liquid biopsy has the potential to be practice-changing is for longitudinal monitoring of disease response. Biomarkers in neuro-oncology are very limited and there are few established biomarkers for tracking disease burden over time. Having a reliable biomarker to gauge tumor response could contribute toward time-sensitive treatment decisions. This is particularly important in high-grade gliomas where monitoring disease burden using traditional imaging-based methods is challenging, especially trying to differentiate progression of disease from pseudoprogression or pseudo-response in patients who have received concurrent chemo-radiation or those on VEGF inhibitors such as bevacizumab.[33–35] While advanced imaging techniques using a variety of modalities have been developed to help distinguish these imaging changes, to date no validated imaging modality exists. Hence, to help guide therapeutic decision-making, an assay for longitudinal monitoring designed for disease-defining mutations (e.g. TERT promoter alterations in GBM) needs to be developed that is both relatively inexpensive and rapid, allowing for modification of treatments in a short time frame. Both goals can be achieved with ddPCR. In contrast, standard NGS panels are more costly with longer turnaround times, rendering them less effective for longitudinal monitoring. Several groups have shown the potential of probing CSF for

ctDNA with ddPCR in CNS tumors to track the mutant allele fraction and correlate to disease treatment status.[4,36]

Alternatively, shallow whole-genome sequencing (sWGS) has emerged as a possible sequencing solution. Brain tumors such as GBM have been shown to be copy number-driven diseases and can be assessed through sWGS where cfDNA is able to be sequenced at low coverage (1-2x) identifying arm level amplifications and deletions as well as disease-defining focal events.[37] Recent studies of sWGS in other CNS malignancies have shown promise in accurately correlating liquid biopsy to primary tissue copy number variation (CNV) profiles.[38,39] Mouliere et al. used low-coverage sWGS in a small cohort of gliomas to detect somatic copy number alterations in 5/13 CSF samples and further correlated the levels of CSF cfDNA with the presence of somatic copy number alterations.[40] Most recently, Liu et al. utilized sWGS to show CNV as a surrogate for minimum residual disease in medulloblastoma patients and demonstrated how this novel marker decreased with treatment and carried higher risk of disease progression with persistent levels of ctDNA.[41] By tracking CSF ctDNA dynamics and correlating to not only disease burden but also therapeutic assessments, longitudinal monitoring may have profound applications in determining treatment efficacies and possibly revolutionize how we evaluate response to glioma therapies. Overall, the future of longitudinal monitoring of glioma via liquid biopsy most likely resides in methods such as ddPCR and sWGS, which provide cheaper and faster results compared with more complex and costly NGS targeted panels.

## Tumor Evolution

High-grade gliomas harbor mutations in multiple core growth factor signaling pathways. In many types of cancer, such mutations offer targets for therapy. Unfortunately, attempts to target these signaling pathways in gliomas have been largely unsuccessful to date. It is possible the lack of success in treating high-grade glioma is due to a lack of fundamental understanding of the selective pressures promoting the emergence of dominant lethal clone(s) that likely possess infiltrative properties allowing them to proliferate and dominate end-stage disease. As tumors progress and spread, their clonal composition evolves. Liquid biopsies offer a unique opportunity to tap into the molecular profile of late-stage disease. This may enable us to track the clonal evolution of these tumors and identify the upregulation of specific genetic alterations which may play a critical role causing the widespread infiltrative disease, a hallmark of late-stage disease. By relying exclusively on tissue biopsy, clinicians typically have at most one or two time points to assess the changes in mutational profile for a given glioma. Tracking the emergence (and the presumed disappearance) of genomic alterations would shed tremendous insight on the biology of glioma and help us understand not only how the tumor will behave but how it may respond to specific therapies, especially with the potential of adaptive, acquired resistance to targeted agents such as BRAF inhibitors.[42] ctDNA from CSF would allow access to significantly more time points permitting in-depth phylogenic analyses of tumor evolution.

An ideal way of assessing genomic evolution might include "untargeted" panels where the assay is agnostic to the primary tumor tissue's mutational profile. Targeted sequencing methods such as ddPCR that can track only a few mutations would not provide the necessary comprehensive overview to assess tumor evolution. To monitor tumor evolution, the goal would be to surveil a multitude of genetic hotspots over time to characterize molecular modifications. This comprehensive tracking can be accomplished with cancer NGS panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS). Pentsova et al. was an early study that analyzed CSF from both primary and secondary brain tumors (metastatic disease) using a large untargeted NGS panel. They found a high percentage of positive CSF ctDNA cases (63% metastatic and 50% PBT) and cases indicating glioma evolution or molecular alterations secondary to chemotherapy.[43] Pan et al. examined 57 patients with an untargeted NGS panel of 68 glioma-associated genes in brainstem gliomas and demonstrated not only high rates of positive mutation capture in CSF ctDNA but also mutations present in CSF ctDNA that were not found in the primary tissue.[3] In our Memorial Sloan Kettering Cancer Center (MSK) study, using a comprehensive untargeted NGS panel, we were able to show detection of ctDNA in the CSF of 42/85 (49.4%) patients with gliomas and identify strong evidence of molecular tumor evolution with the emergence of new alterations over time.[5] Inroads have also been made using whole-exome sequencing on the CSF in primary brain tumors.[25,44,45] While more work is needed to establish the feasibility of WES and WGS, in cases where there is a high burden of tumor in the CSF, we believe that these broader, more comprehensive genomic approaches are likely feasible and have the potential to unlock key insights into the clonal dynamics of high-grade glioma.

## Clinical Trial Eligibility

The future use of CSF liquid biopsy in glioma will most likely include a role in determining clinical trial eligibility with the updated 2021 WHO classification of CNS tumors further integrating molecular markers with histology for tumor characterization and grading. For example, if a sample is histologically determined to be low-grade but on molecular studies is identified to be *IDH* wildtype with either a *TERT* promoter mutation, *EGFR* amplification, or 7 gain/10 loss copy number changes, the tumor is classified as a glioblastoma and the patient is potentially eligible for further clinical trials.[27] However surgical tissue is not always available, especially at time of recurrence. Molecular mutations identified in CSF liquid biopsy may provide a novel mechanism to identify clinical trial eligible patients. Here, rapid determination of targetable mutations is essential, and NGS hotspot panels with a slower turnaround time may have more limited utility. Methods such as ddPCR focused on molecular markers including *TERT* promoter mutation and *EGFR* amplification would be effective, fast approaches. Alternatively, sWGS is also a viable sequencing option, for example in assessing for chromosome 7 gain/10 loss. CSF may be particularly useful for confirming the presence of a targetable mutation for trial

enrollment at progression of disease when we often don't have tissue to test. For example, there are many experimental receptor tyrosine kinase inhibitors that are tested in patients with recurrent gliomas. However, in GBM there are fluctuations in the level of expression and heterogeneity of receptor tyrosine kinases over the disease course.[5,46,47] Absence of the target can potentially sway clinical trial outcomes and subject patients to unnecessary risk/toxicity from experimental agents they are unlikely to benefit from. By using a ddPCR primer on CSF confirming a specific alteration in the original tumor, one could ensure that the target is still present in the recurrent tumor. Obtaining CSF ctDNA at the time of progression would also offer an opportunity to do NGS to improve understanding of tumor evolution over time (though this would take too long for trial enrollment).

### Prognostication

CSF liquid biopsy may also be a future prognostic biomarker in glioma. In our study of CSF ctDNA in gliomas, there was a strong association between ctDNA detection and both disease burden and adverse outcome.[5] Similarly, Liu et al. found with sWGS that tumor-associated CNVs in the CSF of medulloblastoma patients after radiation therapy was associated with significantly worse progression-free survival.[41] These studies reinforce the importance of a dedicated prospective clinical trial to validate CSF ctDNA as a prognostic biomarker in glioma.

### Technical Considerations of CSF Retrieval and Molecular Testing

Although lumbar puncture is significantly lower risk than surgery with rare complications of infection, site bleeding, and more commonly post procedural headache, there are additional safety considerations for patients with PBTs. In the case of space occupying intracranial lesions such as gliomas, caution must be exercised before the procedure to evaluate for raised intracranial pressure. Particular attention is required when assessing the risk of lumbar puncture if there is mass effect in the posterior fossa due to the potential of cerebellar tonsillar herniation from a pressure gradient. Standard cytology assessment of CSF can be performed with volumes as low as 2 to 3 milliliters, although laboratories routinely request higher volumes to optimize yield. However, cytological testing is often negative in glioma patients, bringing the assay's utility in this disease into question.[43] In contrast, the abovementioned molecular tests including NGS panels, ddPCR, and sWGS can be analyzed at volumes of 3 milliliters.[5,32,41] It is possible that for a subset of patients with limited CSF ctDNA, greater volumes of CSF will lead to higher likelihood of success for the assay, but currently that has not been shown to be the case.[48] Future research will also be important to elucidate the reproducibility of these novel molecular tests. In our Memorial Sloan Kettering study, a small cohort of glioma patients showed high concordance of CSF ctDNA genomic profiles at two time points of lumbar puncture and subsequent ventriculoperitoneal shunt placement.[5] Procedures such as lumbar puncture or surgery can introduce blood into CSF samples. However, this contamination is unlikely to influence molecular studies due to separation of the cell pellet and supernatant by centrifugation.

## Conclusion

The relatively recent discovery of CSF ctDNA in primary brain tumor patients has a myriad of practical clinical applications including diagnostics, longitudinal monitoring, tracking tumor evolution, determination of clinical trial eligibility, and even prognostication. Moreover, each of these objectives requires an individualized sequencing approach ranging from highly sensitive, focused ddPCR to broader relatively inexpensive assessments of copy number variations (sWGS) to comprehensive NGS panels to WGS. While preliminary studies have shown feasibility for most of these CSF sequencing techniques in PBT patients, almost all studies have been small and retrospective, rendering the true clinical utility unknown. In order to further the clinical use of these validated CSF assays, subsequent prospective clinical trials are critical. One such prospective clinical trial is currently being launched at Memorial Sloan Kettering assessing the prognostic value of ctDNA positivity in the CSF of newly diagnosed GBM patients pre- and postsurgery and the utility of CSF ctDNA for differentiating true vs. pseudoprogression after concurrent chemo-radiation. We also have a trial under review at the MSK IRB which is a prospective study implanting Ommaya reservoirs to enable frequent and low-risk CSF collections for longitudinal monitoring of patients with diffuse midline gliomas to determine CSF dynamics in response to therapy.

While prospective data are currently lacking, we are encouraged by the early success of the abovementioned approaches and believe that CSF cfDNA has the potential to markedly advance the field of neuro-oncology by: (1) enabling molecular diagnosis when tumor tissue is not readily accessible or biopsies are nondiagnostic; (2) longitudinal monitoring of disease response in standard clinical care; (3) tracking tumor evolution throughout the course of disease to uncover new insights into tumor biology; (4) informing rational clinical trial enrollment; and (5) prognostication at various disease stages. As the neuro-oncology field more widely adopts CSF liquid biopsy, it is not a question of if, but rather when and how the field will tap into this vast liquid treasure to improve the day-to-day care of glioma patients and maximize clinical trial design to accelerate therapeutic drug development.

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