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Cerebrospinal fluid liquid biopsy by low-pass whole genome sequencing for clinical disease monitoring in pediatric embryonal tumors

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Abstract

Background. Liquid biopsy assays that detect cell-free DNA (cfDNA) in cerebrospinal fluid (CSF) are a promising tool for disease monitoring in pediatric patients with primary central nervous system (CNS) tumors. As a compliment to tissue-derived molecular analyses, CSF liquid biopsy has the potential to transform risk stratification, prognostication, and precision medicine approaches.

Methods. In this pilot study, we evaluated a clinical pipeline to determine feasibility and sensitivity of low-pass whole genome sequencing (LP-WGS) of CSF-derived cfDNA from patients with CNS embryonal tumors. Thirty-two longitudinal CSF samples collected from 17 patients with molecularly characterized medulloblastoma (12), embryonal tumor with multilayered rosettes (2), CNS embryonal tumor, not elsewhere classified (NEC) (2), and atypical teratoid/rhabdoid tumor (1) were analyzed.

Results. Adequate CSF-derived cfDNA for LP-WGS analysis was obtained in 94% of samples (30/32). Copy number variants compatible with neoplasia were detected in 90% (27/30) and included key alterations, such as isodicentric ch17, monosomy 6, and *MYCN* amplification, among others. Compared to tissue specimens, LP-WGS detected additional aberrations in CSF not previously identified in corresponding primary tumor specimens, suggesting a more comprehensive profile of tumor heterogeneity or evolution of cfDNA profiles over time. Among the 12 CSF samples obtained at initial staging, only 2 (17%) were cytologically positive, compared to 11 (92%) that were copy number positive by LP-WGS.

Conclusions. LP-WGS of CSF-derived cfDNA is feasible using a clinical platform, with greater sensitivity for tumor detection compared to conventional CSF cytologic analysis at initial staging. Large prospective studies are needed to further evaluate LP-WGS as a predictive biomarker.

Key Points

- Low-pass whole genome sequencing (LP-WGS) can detect circulating tumor DNA in low-volume clinical cerebrospinal fluid (CSF) samples.
- LP-WGS on CSF-derived cell-free DNA is more sensitive than CSF cytology for tumor detection at initial diagnosis and staging.
- LP-WGS may detect tumor heterogeneity and genomic evolution over time better than currently available tools.

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Importance of Study

This report highlights the feasibility and utility of a clinical cerebrospinal fluid (CSF) liquid biopsy platform for real-time insight into tumor characteristics when used in tandem with currently available diagnostic tools, including MRI and CSF cytology. Copy number variants detected in CSF-derived cell-free DNA from patients with embryonal tumors can augment tissue-derived molecular analyses. CSF liquid biopsy has the potential to transform clinical practice for improved diagnosis, risk stratification, and disease surveillance.

Recent genomic and therapeutic advances have improved the overall survival for pediatric patients with embryonal tumors; however, outcomes after tumor recurrence remain poor.[1] Determining which patients are at high risk of recurrence requires reliable disease monitoring and accurate risk stratification. Contemporary methods used to risk stratify patients with central nervous system (CNS) tumors rely heavily on imperfect tools. Magnetic resonance imaging (MRI) is of limited utility in the absence of rather bulky disease and cytologic analysis of cerebrospinal fluid (CSF), while useful for initial staging, is rarely sufficient for detecting microscopic tumor cells remaining after surgery or standard therapy (ie, measurable residual disease, MRD).[2] Early detection of MRD in the peripheral blood has revolutionized risk stratification in other pediatric cancers, most notably pediatric leukemia, where it has significantly contributed to improved outcomes.[3,4] While molecular assays using CSF or other biofluids from children with primary CNS tumors have shown initial promise, they have yet to be clinically validated as surrogates for MRD.

Molecular classification of medulloblastoma and atypical teratoid rhabdoid tumor (ATRT) may be useful for future risk stratification, but currently relies on tumor tissue, limiting its utility for longitudinal monitoring.[5–8] Alternatively, minimally invasive CSF liquid biopsy can identify tumor-derived changes without the need for direct tumor sampling. To date, CSF liquid biopsy has been shown to reliably detect disease in medulloblastoma[9–12] and pediatric high-grade glioma (including diffuse midline glioma).[13–19] In addition, CSF liquid biopsy can predict the progression of disease prior to radiographic findings[9,12,14,16–18] and differentiate tumor versus treatment effect (pseudoprogression).[18] These applications could aid future determinations of how and when to intensify or de-escalate therapy for better outcomes. For example, liquid biopsy results could guide intensification of therapy prior to relapse of bulky or metastatic disease, or alternatively, minimize toxicity by eliminating radiation in patients with resolution of MRD at the end of chemotherapy.

CSF is an ideal biofluid for genomic characterization. Limited volume CSF specimens obtained via lumbar puncture (LP) can be analyzed by a range of methodologies to identify fragments of tumor DNA released from their cellular (tissue) compartment (ie, cell-free DNA, cfDNA) by mechanisms including cell death.[19–22] Since pediatric CNS tumors are typically isolated to the neuroaxis and plasma crossover is restricted by the blood–brain barrier, CSF has a paucity of non-tumor cfDNA and is therefore comparatively enriched for tumor-derived cfDNA. As such, the concentration of cell-free tumor DNA has been found to be significantly higher in CSF than in other biofluids, such as plasma and urine.[15–17,23]

Methods used for CSF-derived cfDNA analysis include droplet digital PCR (ddPCR), targeted next-generation sequencing (NGS), and whole exome sequencing (WES).[22,24,25] Limitations vary between assays; while ddPCR is relatively inexpensive and requires low DNA input (2–10 ng), it is mutation specific. More comprehensive assays, such as targeted NGS or WES, are more capable of detecting a wide range of molecular alterations but require significantly higher DNA input at greater depth of coverage, and are more expensive. By comparison, low-coverage or low-pass whole genome sequencing (LP-WGS) offers comprehensive genomic coverage using picogram to nanogram cfDNA yields, making it suitable for clinical applications.[9,10,15] While LP-WGS cannot detect single nucleotide variants, it is ideal for capturing the somatic copy number alterations (sCNA), which are present in abundance across many CNS tumors, particularly pediatric embryonal tumors.[13]

To assess the clinical utility, feasibility, and sensitivity of CSF liquid biopsy as a clinical monitoring tool, we evaluated CSF-derived cfDNA from patients with embryonal tumors treated at a single institution using LP-WGS performed in a CAP/CLIA environment. LP-WGS assessments were performed on CSF collected from patients at diagnosis, throughout therapy, and/or at recurrence, with results compared to somatic alterations found in the primary tumors and cytologic analysis of CSF.

Methods

Patient Cohort and Sample Collection

Samples were collected prospectively, and/or retrospectively using banked specimens, from pediatric patients with primary embryonal tumors, including medulloblastoma, ATRT, and embryonal tumor with multilayered rosettes (ETMR), who received treatment at Seattle Children's Hospital over a 4-year period. Informed consent was obtained from all participants through the Seattle Children's Banking and Biology Study and approved by the local Institutional Review Board (#14449). Tumor and CSF samples were obtained at the time of clinical procedures. Demographic and clinical information were retrieved from the electronic medical record system. At the time of tumor resection, tumors were classified using the 2016 World Health Organization CNS tumor classification system; the tumors were later reclassified using the 2021 World Health Organization CNS tumor classification system.[26]

A clinical workflow to preserve CSF samples for potential future testing was developed for patients with primary CNS tumors undergoing clinical diagnostic testing. CSF was collected in sterile tubes during routine clinical procedures including diagnostic or therapeutic LP, at the time of neurosurgical tumor resection, and/or from external ventriculostomy devices (EVD). Staging samples were collected 10 or more days following tumor resection. CSF volume obtained ranged from 0.4 to 2 mL. "Oncology Hold" CSF processing included immediate CSF centrifugation and storage. This did not necessitate specialized collection procedures, Streck tubes, or other dedicated equipment for handling. Samples were processed within the clinical lab that operates continuously, 24 h a day. Staging LP specimens underwent standard clinical cytospin slide preparations, were stained with Wright-Giemsa, and evaluated for malignant cells under the microscope by a board-certified pathologist (BC). CSF leftover after clinical testing was stabilized within 1 h of reaching the core clinical lab by centrifugation at 1600×*g* for 10 min to remove cells followed by 16,000×*g* for 15 min to remove small cells and cellular debris. After processing, CSF was stored at –20 °C.

Cell-Free DNA Extraction

CSF samples were thawed and cfDNA was extracted using the automated QIAsymphony nucleic acid extractor with the DSP Circulating DNA Kit (Qiagen, Valencia, CA, Catalog 937556). Following quantification and characterization of the CSF-derived cfDNA by Qubit fluorometry and Agilent Tapestation, respectively, next-generation sequencing (NGS) library preparation was performed using the KAPA HyperPrep kit for adapter and index ligation. The library was subsequently purified using the Agencourt AMPureXP kit prior to amplification. The amplified library was then purified on the Agilent BRAVO workstation using AMPure beads. Sample pools were created using an equimolar strategy and diluted to 1 nM.

Low-Pass Whole Genome Sequencing

Sequencing was performed through a validated, laboratory-developed method used to screen for fetal aneuploidies and copy number alterations, as previously described,[27,28] using an Illumina NextSeq 500 High Output 75 cycle kit with a 37 bp paired-end read configuration to enable fragment length determination. On average, each cfDNA sample is sequenced to a depth of approximately 20 million paired-end reads, corresponding to a depth of <1 per nucleotide position. Reads were aligned to the human reference genome (hg19) with Bowtie (version 1.1.2), and run metrics were calculated with Picard (version 1.141). Reads were averaged over 1 megabase (Mb), with each Mb region composed of smaller 100kb bins that were staggered to overlap. The standard deviation of each Mb bin from the mean (*Z* score) was plotted by chromosome position.

LP-WGS results of CSF-derived cfDNA were compared with the results of molecular profiling performed on the primary tumor tissue. Samples were considered positive for tumor if copy number changes were detected in CSF that were compatible with the presence of circulating tumor DNA (ctDNA). Results were defined as insufficient if cfDNA was not present or extracted in sufficient quantity for successful sequencing, typically around 5 ng/mL. Samples were characterized as absent if the total cfDNA extracted was sufficient to perform sequencing and results did not reveal sCNA from a patient with CSF testing previously positive for sCNAs. LP-WGS results were considered falsely negative if clinical features confirmed the presence of tumor at the time of sample collection, including discernible tumor identified intra-operatively and/or positive CSF cytology plus radiographic findings consistent with tumor.

Next Generation Sequencing of Primary Tumors

Primary tumor tissue was profiled using a DNA-based targeted next generation sequencing panel, UW-OncoPlex™ (versions 5–7), as previously described.[29,30] In brief, DNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue using the Qiagen GeneRead DNA FFPE Kit (Qiagen, Valencia, CA) and sheared prior to library preparation using KAPA HyperPrep reagents (Roche, Wilmington, MA). Prepared libraries were subsequently hybridized to the UW-OncoPlex™ version 5 (OPXv5, customized Agilent SureSelect probes targeting 262 genes), UW-OncoPlex™ version 6 (OPXv6, customized IDT probes targeted 340 genes), or UW-OncoPlex™ version 7 (OPXv7, targeting 376 genes). Libraries were sequenced on Illumina NextSeq500 and HiSeq2500 platforms (Illumina, San Diego, CA), and processed through an automated, custom-designed bioinformatics pipeline developed by the University of Washington NGS Analytics Laboratory designed to identify clinically significant alterations in cancer including single nucleotide variants, insertions and deletions, copy number alterations, and select gene-fusions.

Results

Patient Demographics and Longitudinal LP-WGS Processing of cfDNA

Thirty-two samples from 17 patients were analyzed, including 12 patients with medulloblastoma, 2 with ETMR, 2 with CNS embryonal tumors not elsewhere classified, and 1 with ATRT. Nine patients were male and 8 were female, with a median age at diagnosis of 4.5 years (range 2 months—13 years). Ninety-four percent of samples (30/32) had adequate DNA for LP-WGS analysis. The median concentration of cfDNA extracted was 11.5 ng/mL (range 0–3,210 ng/mL). Twenty-seven of 30 samples (89%) were positive for copy number alterations compatible with tumor (Figure 1). Collection time points were delineated into 5 subcategories and included (1) diagnosis/surgery, (2) staging LP performed after surgery, (3) during therapy, (4) end of therapy, and (5) recurrence. All patients with samples negative for sCNA had no evidence of disease radiographically following complete resection or therapy, absence of leptomeningeal disease, and negative cytology

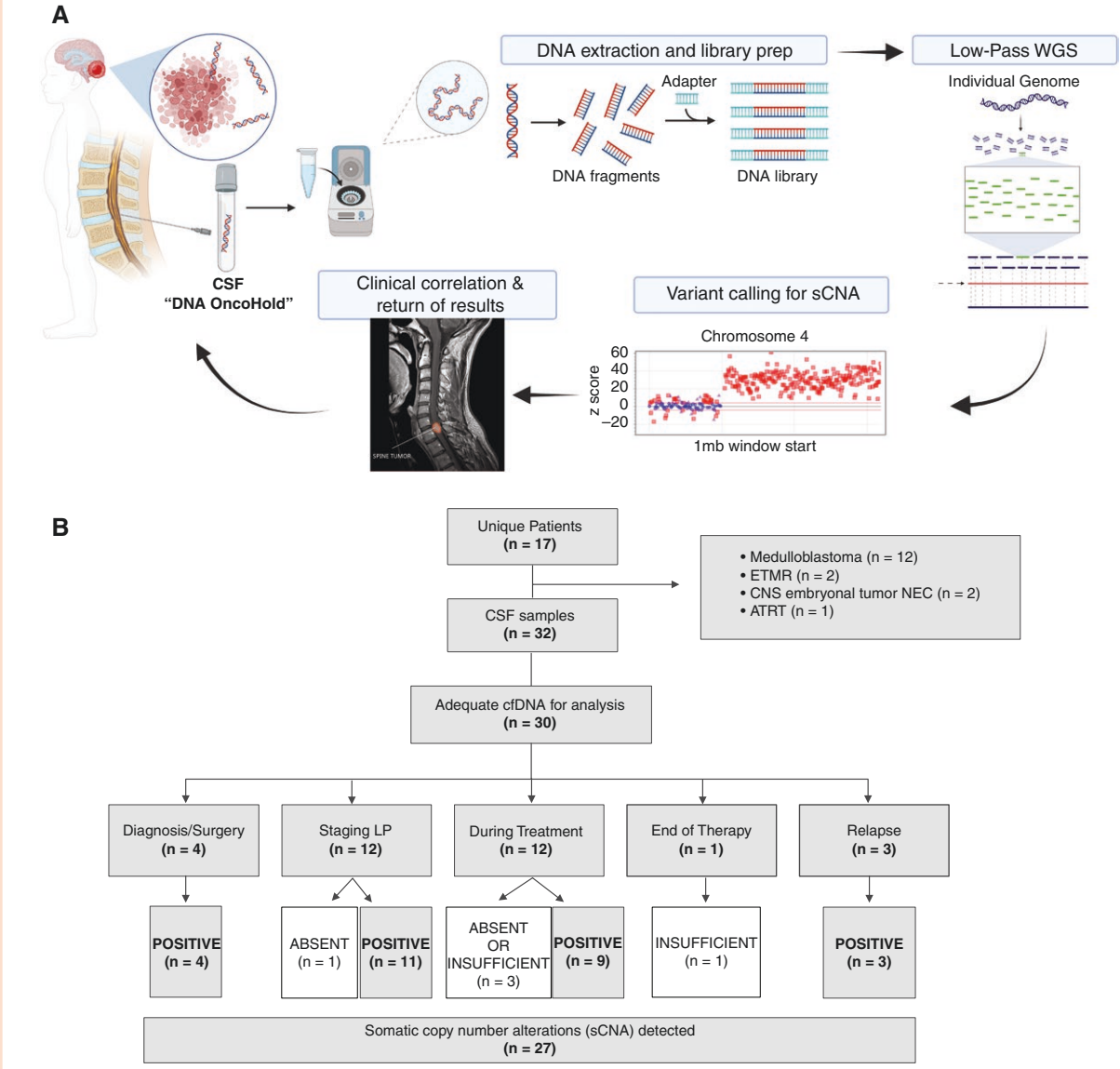

Figure 1. Low-pass whole genome sequencing (LP-WGS) sample collection and processing. (A) Graphical abstract of cerebrospinal fluid (CSF) processing including cell-free DNA (cfDNA) extraction, low-pass whole genome sequencing, and variant calling for somatic copy number alterations. (B) Experimental workflow included clinical correlation of end results with patient characteristics, CSF cytology, and known somatic tumor alterations by targeted next-generation sequencing. Created with BioRender.com.

at the time of sample collection, making false negative results unlikely based on clinical and radiographic features.

Figure 1 shows the distribution by collection timepoints, with 4/4 (100%) of the diagnostic samples obtained at surgery positive for sCNA. 11/12 (92%) of the samples collected post-operatively, either by EVD collection or staging LP, and 9/12 (75%) of samples collected during treatment, were positive for sCNA. One patient with a CSF sample collected at the end of therapy with insufficient DNA extraction has had no evidence of radiographic or cytologic recurrence 4 years since completion of therapy. Three patients were tested at radiographic recurrence and CSF was positive for tumor-derived cfDNA, consistent with relapse of the primary tumor, in all 3 cases.

Concordance of Liquid Biopsy with sCNA in Primary Tumors

Copy number profiles by LP-WGS were largely concordant with the molecular profiles of the corresponding patient's primary tumor analyzed by next generation sequencing (UW-OncoPlex™). As shown in Figure 2, LP-WGS identified whole and partial chromosomal gains and losses, amplifications, and homozygous deletions, with many CSF samples found to harbor additional sCNA not identified in their primary tumors at diagnosis. While not depicted in Figure 2, tumor alterations from Patient 8 were identified by microarray and DNA methylation analysis rather than UW-OncoPlex™, but were also compatible

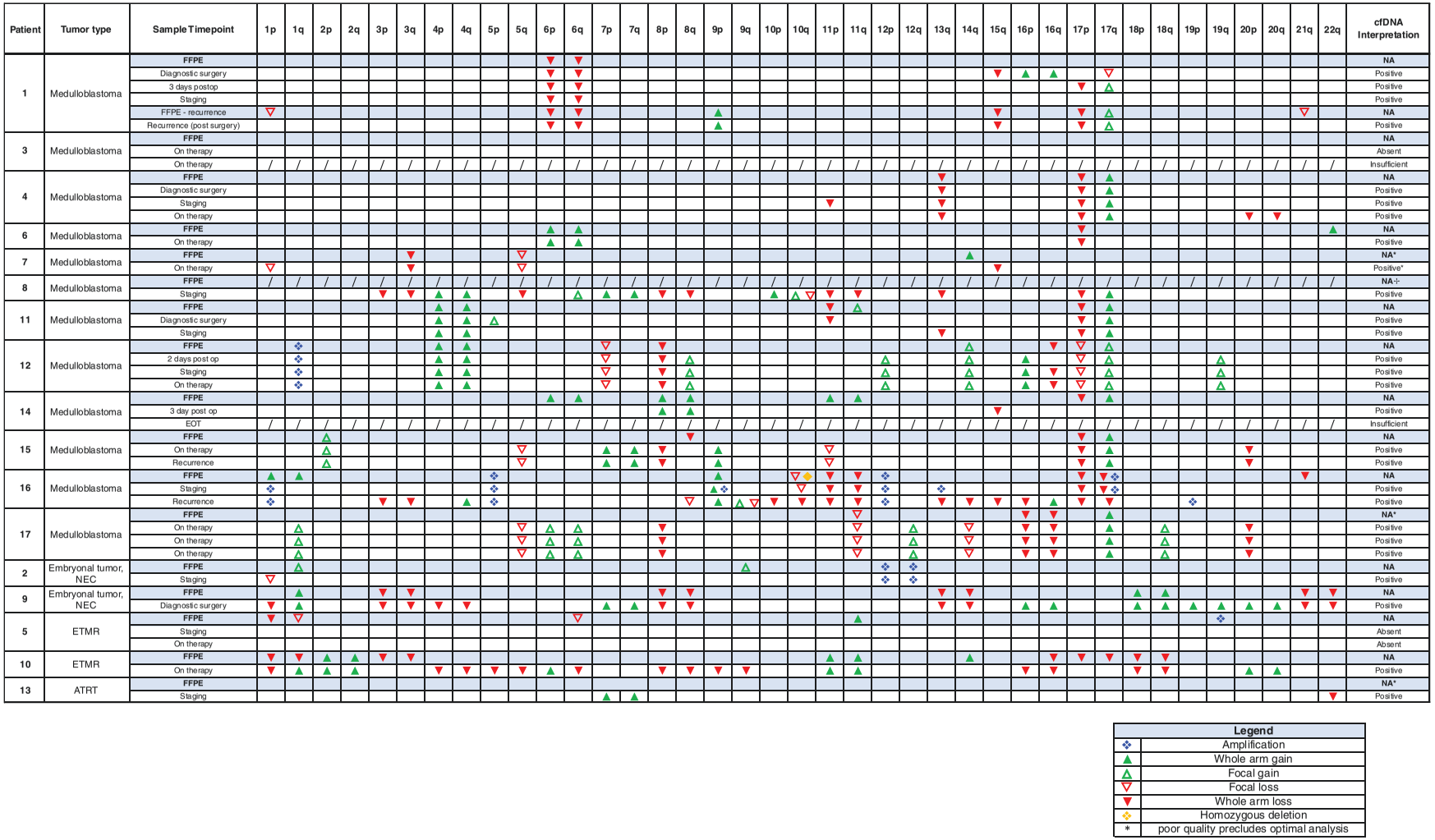

Figure 2. Copy number variants detected in cerebrospinal fluid (CSF) liquid biopsy by low-pass whole genome sequencing (LP-WGS) and tumor tissue by UW-OncoPlex™ Summary of all chromosomal copy number alterations detected in patient samples by genomic analysis. Individual chromosomes 1–22 listed in columns, with CSF samples marked in white rows and tumor tissue (formalin-fixed paraffin-embedded, FFPE) in blue rows. Samples with insufficient DNA for analysis marked with forward slashes.

with LP-WGS results. Two primary tumors were copy number neutral by sequencing of FFPE tumor tissue at diagnosis (Patient 3 and 13). Corresponding CSF cfDNA from Patient 3 (medulloblastoma *SHH*-activated, *TP53*-wildtype) was found to similarly lack sCNA in CSF while on therapy, whereby a subsequent surgical CSF sample had insufficient DNA for analysis. Patient 13 harbored a copy-neutral ATRT by primary tumor analysis and was found to be positive for sCNA in CSF at staging. While this finding may represent the ability of LP-WGS to detect tumor heterogeneity, evaluation of this particular patient's tumor sample was limited by poor quality, precluding optimal analysis for comparison.

Notably, 5/17 (29%) patients had evidence of tumor evolution or tumor heterogeneity by LP-WGS, with new alterations identified at recurrence or while on therapy, suggesting genomic progression over time (Figure 2: Patient 1, 4, 7, 10, and 16). Patient 16 with medulloblastoma (*SHH*-activated, *TP53*-mutated) had confirmation of recurrent, metastatic disease at the end of therapy by LP-WGS, despite negative cytology. Additional sCNAs detected at recurrence included copy loss of a portion of chromosome 3 and copy gain of a portion of chromosome 4, among many others (Figure 3).

Key Molecular Alterations Identified by LP-WGS

LP-WGS identified key molecular alterations that were subgroup defining or potentially targetable in multiple CSF samples. Patients with medulloblastoma were found to harbor the canonical monosomy 6 alteration in WNT subgroup medulloblastoma (Figure 4), along with high-risk alterations including isodicentric chromosome 17 (6 patients) and *MYCN* amplification (1 patient) (Figures 2 and 5). *MYCN* amplification was readily apparent in 1 sample from patient 12, resulting in a signal >12,000–14,000× above background (Figure 5). Other key sCNA included amplifications of *TERT*, *CCND2*, *MDM2,* and *CDK4*.

Comparison of LP-WGS to Clinical Testing Using Cytology and MR Imaging

To explore the correlation between LP-WGS and clinical testing routinely obtained for disease monitoring, we compared LP-WGS results to MRI findings and CSF cytologic analysis at the time of sample collection (Table 1). MRI evidence of tumor was present at the time of collection in 21 of 32 samples. All 21 samples with corresponding radiographic evidence of tumor and 6 without tumor had detectable sCNA in CSF.

While not all LP-WGS samples had a corresponding cytology specimen for comparison, 24 timepoints had both cytology and LP-WGS performed, with cytologic evidence of tumor present in 6/23 samples. All cytology-positive CSF samples from patients with tumors harboring copy number changes were also positive by LP-WGS (6/6; 100%). Among the 12 CSF samples obtained at staging, only two (17%) were cytologically positive for malignant cells, compared to 11 (92%) copy number positive by LP-WGS.

Chang staging for medulloblastoma incorporates both cytologic and MR imaging findings to define the presence of metastatic disease.[31,32] The rate of tumor detection by LP-WGS in patients with embryonal tumors classified as Chang stage M1–M3 was 15/15 (100%). All but one of the 15 patients with metastatic disease at diagnosis had detectable disease by MR imaging at the time of sample collection. The radiographically negative case represented Patient 6 with M3 medulloblastoma (non-WNT, non-SHH), who had resolution of spinal drop metastasis on MRI at time of CSF sample collection after induction chemotherapy.

While the rate of false positive LP-WGS results is unknown, there were 6 samples positive for sCNA in the CSF without MRI or cytologic evidence of disease at sample collection. Of these, 2/6 samples were obtained at "on therapy" timepoints, between induction and consolidation chemotherapy, in patients with spinal drop metastases or a near total resection at diagnosis. The remainder ($n = 4$) were obtained at staging shortly after surgery. While a precise definition of a false positive LP-WGS result is challenging, a putative definition could be a CSF sample positive for detectable sCNA in a patient who does not develop tumor progression or recurrence. In this limited cohort, staging and on therapy samples would not be considered falsely positive prior to completion of standard therapy.

Discussion

We describe the performance of a clinical liquid biopsy test for disease monitoring of pediatric embryonal tumors. While prior reports have highlighted the utility of LP-WGS using research platforms on banked specimens,[9] we report the feasibility of a clinical LP-WGS pipeline, with CAP/CLIA laboratory analysis of CSF-derived cfDNA obtained during routine clinical procedures and using low-volume samples. We found LP-WGS liquid biopsy had greater sensitivity for tumor detection compared to traditional cytologic analysis of CSF, and in this limited cohort, a greater sensitivity than previously published. For example, compared to other reports which have found sCNA in 62% of cytology-negative CSF samples collected at baseline, we report a 92% positivity rate for LP-WGS in staging LP samples.[9] Irrespective of therapy timepoint, LP-WGS was a sensitive tool for detection of tumor sCNA and results were concordant with molecular profiling of the primary tumors analyzed by next generation sequencing.

Compared to tissue specimens, cfDNA may offer a more comprehensive profile of tumor heterogeneity, which might otherwise be underrepresented due to the constraints of obtaining all tumor components in biopsy specimens.[24,33–37] Our report highlights both the ability of cfDNA to detect additional sCNA not identified in corresponding primary tumor specimens and potential evolution of cfDNA profiles over time. Due to the limited number of recurrent samples in this pilot cohort ($n = 3$), direct comparison of CSF LP-WGS at diagnosis and recurrence (mean sCNA 7.3 vs. 12.7, respectively; $P = .2$) warrants further prospective investigation.

A key technical factor in the success of this platform was immediate CSF processing to optimize cfDNA extraction. Collection during clinical procedures did not necessitate Streck tubes or other specialized equipment. Immediate

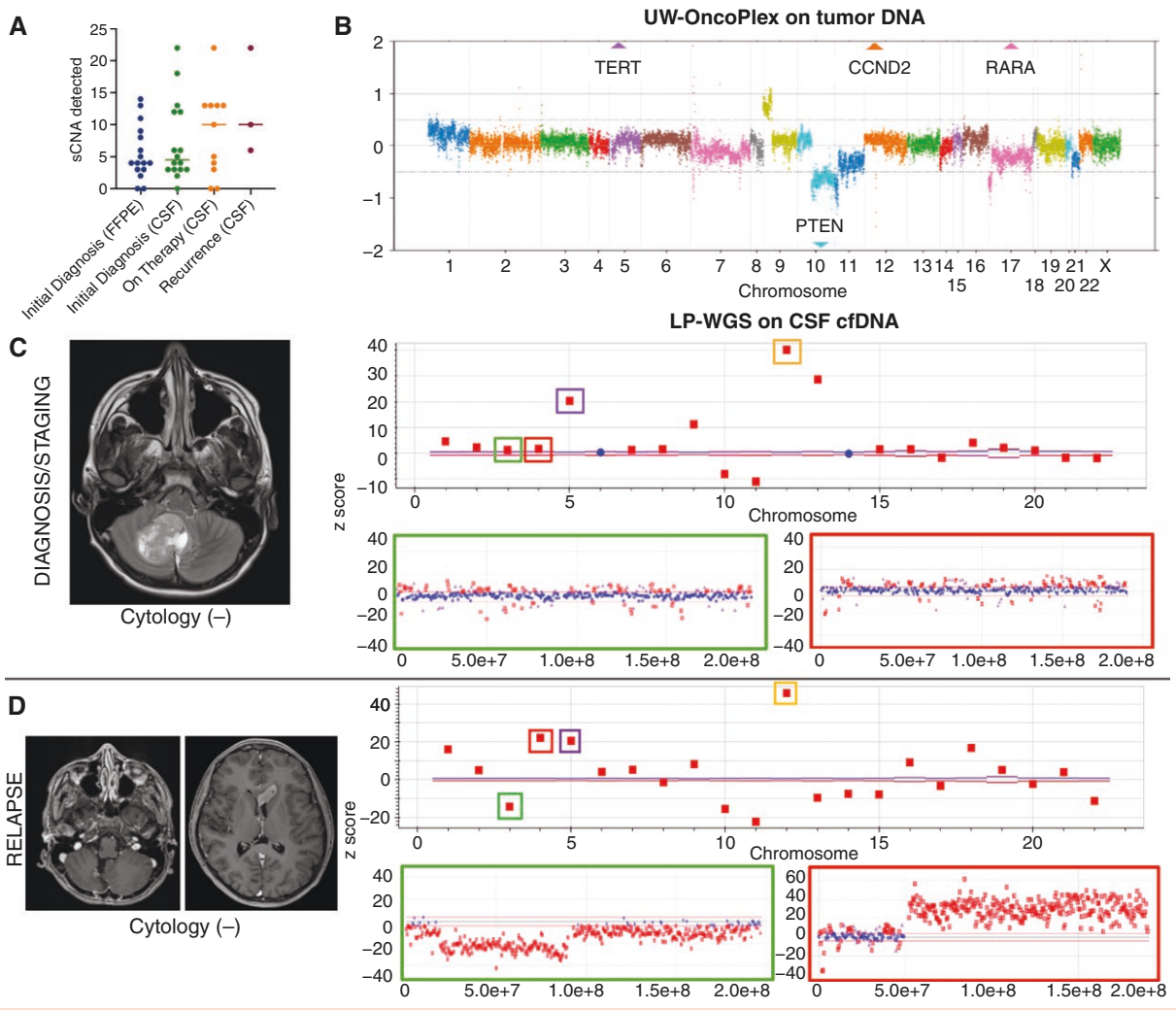

Figure 3. Low-pass whole genome sequencing (LP-WGS) confirms recurrence at the end of therapy with additional acquired alterations. (A) Comparison of somatic copy number alterations (sCNA) detected by tumor tissue (formalin-fixed paraffin-embedded, FFPE; UW-OncoPlex™) and cerebrospinal fluid (CSF) samples (LP-WGS) distributed by timepoint. Initial diagnostic samples from CSF included samples obtained during surgery, from external ventriculostomy devices (EVDs), and staging lumbar punctures (LPs). Kruskal–Wallis test, $P = .347$. B–D) Case vignette shows the clinical course and molecular findings in a patient with SHH medulloblastoma and a germline *TP53* mutation. (B) Testing of tumor tissue by UW-OncoPlex™ identified biallelic inactivation of *TP53*, as well as copy alterations including amplification of *TERT* (chr 5) and *CCND2* (chr 12). (C) LP-WGS copy number plot demonstrated concordant alterations in CSF at diagnosis including *TERT* and *CCND2* amplification (note the absence of findings on chromosomes 3 and 4). CSF cytology was negative. (D) Brain MRI images at the completion of therapy, following gross total resection, craniospinal radiation, and chemotherapy, shows nodularity along the resection bed in the right cerebellar hemisphere and the left frontal horn with surrounding T2 FLAIR hyperintensity. LP-WGS on CSF confirmed radiographic recurrence despite negative cytology. Additional sCNA acquired at recurrence included copy loss of a portion of chromosome 3 and copy gain of a portion of chromosome 4.

centrifugation and freezer storage was likely the most significant variable compared to other LP-WGS platforms and we attribute our 94% DNA extraction rate to this streamlined process, reducing pre-analytic sample variability. Our LP-WGS platform is routinely used for non-invasive prenatal screening of cfDNA extracted from peripheral blood in pregnant patients, providing a methodological backbone for success.[21] LP-WGS has several advantages over other methodologies for CSF liquid biopsy testing. LP-WGS is faster and less expensive than targeted NGS, and PCR amplification errors are mitigated. Most importantly, LP-WGS requires substantially less DNA input compared to conventional NGS panels, making it an attractive option for diagnostic considerations in children, where CSF volume may be limited.

Limitations

Testing limitations highlighted by this report include constraints on the patient population that may ultimately benefit from LP-WGS disease monitoring. Somatic alterations are generally less abundant in pediatric tumors when compared to adult CNS tumors, and point mutations, fusions,

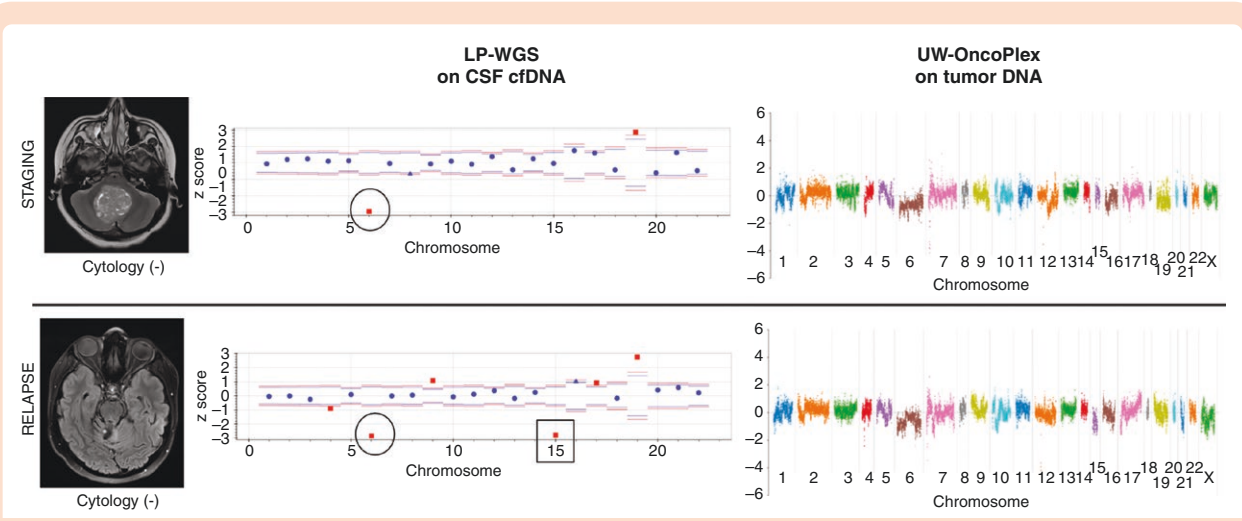

Figure 4. Low-pass whole genome sequencing (LP-WGS) reveals WNT subgroup medulloblastoma with evolution from serial sampling of cerebrospinal fluid (CSF). **Top**: Brain MRI obtained at initial diagnosis shows a large, heterogeneous posterior fossa mass; CSF cytology was negative for malignant cells. LP-WGS on CSF-derived cell-free DNA (cfDNA) detected monosomy of chromosome 6 (black circle). Following near total resection, tumor characterization by UW-OncoPlex™ demonstrated an activating mutation in *CTNNB1* and copy loss of monosomy 6, concordant with LP-WGS results. **Bottom**: 3 years off therapy, the patient presented with recurrent tumor in the resection bed. CSF cfDNA confirmed radiographic recurrence by LP-WGS, again detecting monosomy 6, along with newly acquired copy loss of a portion of chromosome 15 (black square). CSF cytology remained negative at recurrence.

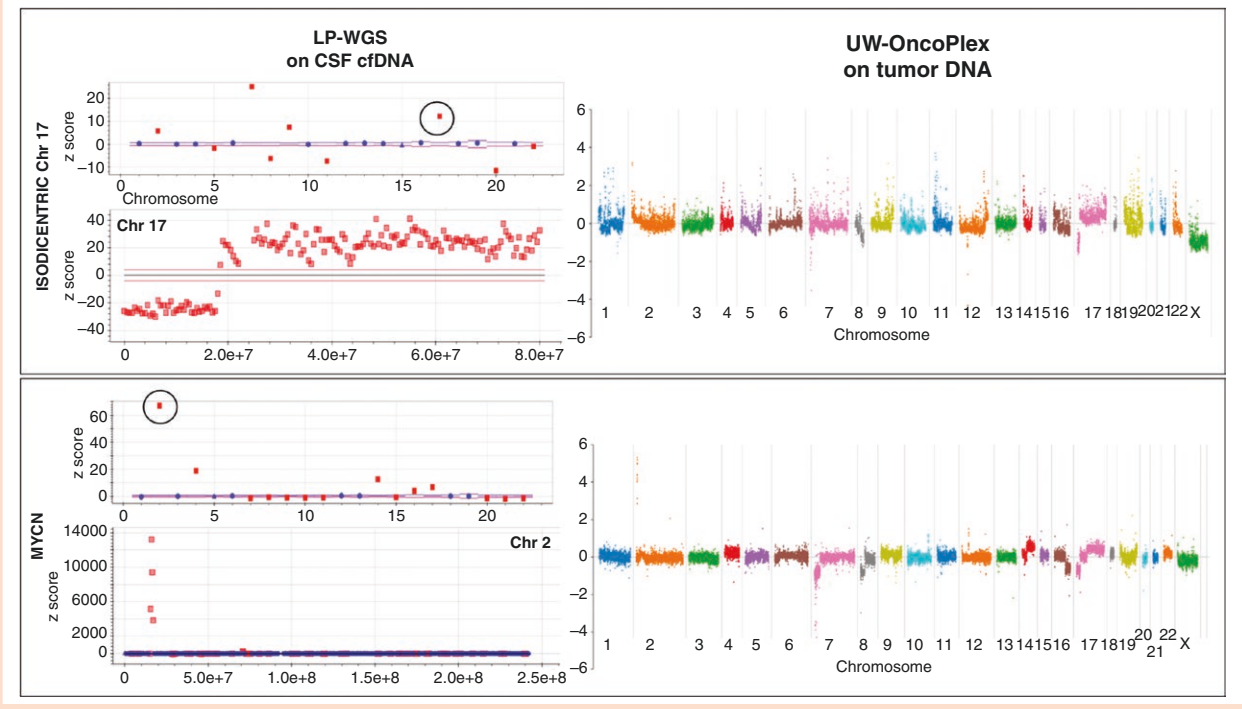

Figure 5. Case vignettes highlight key molecular drivers for non-WNT/non-SHH medulloblastoma. **Top**: Copy number plots showing isodicentric chromosome 17 by cerebrospinal fluid (CSF) low-pass whole genome sequencing (LP-WGS) (left) and concordance with somatic copy number alterations (sCNA) found in tumor tissue by UW-OncoPlex™ targeted next generation sequencing (right). **Bottom**: High-level amplification of *MYCN* is readily detected by CSF LP-WGS (left) and is concordant with copy number variation found on chromosome 2 in tumor tissue by UW-OncoPlex™ (right).

Table 1. Disease Detection by Modality

Patient	Tumor type	Sample timepoint	Collection route	MRI	Cytology	LP-WGS
1	Medulloblastoma, WNT	Diagnostic surgery	Surgical	Pos	N/a	Pos
		3 d post-op	EVD	Pos	N/a	Pos
		Staging	LP	Pos	Neg	Pos
		Recurrence (post-surgery)	LP	Neg	Neg	Pos
3	Medulloblastoma, SHH-TP53 wt	On therapy	LP	Neg	N/a	Absent
		On therapy	Surgical	Neg	N/a	Insufficient
4	Medulloblastoma, G4	Diagnostic surgery	Surgical	Pos	N/a	Pos
		Staging	LP	Pos	Neg	Pos
		On therapy	LP	Pos	Neg	Pos
6	Medulloblastoma, non-WNT, non-SHH	On therapy	LP	Neg	Neg	Pos
7	Medulloblastoma, non-WNT, non-SHH	On therapy	LP	Pos	Neg	Pos
8	Medulloblastoma, G4	Staging	LP	Pos	Neg	Pos
11	Medulloblastoma, G4	Diagnostic surgery	Surgical	Pos	N/a	Pos
		Staging	LP	Neg	Neg	Pos
12	Medulloblastoma, G3	2 d post-op	EVD	Pos	N/a	Pos
		Staging	LP	Pos	Pos	Pos
14	Medulloblastoma, non-WNT, non-SHH	3 d post-op	EVD	Neg	N/a	Pos
		EOT	LP	Neg	Neg	Insufficient
15	Medulloblastoma, non-WNT, non-SHH	On therapy	LP	Pos	Neg	Pos
		Recurrence	LP	Pos	Neg	Pos
16	Medulloblastoma, SHH-TP53	Staging	LP	Neg	Neg	Pos
		Recurrence	LP	Pos	Neg	Pos
17	Medulloblastoma, non-WNT, non-SHH	On therapy	LP	Pos	Pos	Pos
		On therapy	LP	Pos	Pos	Pos
		On therapy	LP	Pos	Pos	Pos
2	Embryonal tumor, NEC	Staging	LP	Pos	Neg	Pos
9	Embryonal tumor, NEC	Diagnostic surgery	Surgical	Pos	N/a	Pos
5	ETMR	Staging	LP	Neg	Neg	Absent
		On therapy	LP	Neg	Neg	Absent
10	ETMR	On therapy	LP	Neg	Neg	Pos
13	ATRT	Staging	LP	Pos	Pos	Pos

Abbreviations: ATRT = atypical teratoid rhabdoid tumor; ETMR = embryonal tumor with multilayered rosettes; EVD = external ventricular device; LP = lumbar puncture; LP-WGS = low-pass whole genome sequencing; NEC = not elsewhere classified; neg = negative; pos = positive; post-op = post-operatively; wt = wild-type.

small deletions, and other subtle molecular drivers will not be detected by this method. Tumors lacking sCNA will not be amenable to disease monitoring through LP-WGS, underscoring the importance of correlating the molecular profiling of tumors at the time of original diagnosis with CSF data for interpretation.

False negative liquid biopsy results can occur when tumor DNA exists in the collected sample, but in quantities below the limit of detection of the assay, that is, low DNA yield. In LP-WGS processing, these samples would fail DNA extraction or have low-quality DNA that fails sequencing. Both scenarios could produce the same absent LP-WGS result as a sample collected from CSF without tumor present. Two samples from this cohort had insufficient DNA extracted from end of therapy and on therapy timepoints, and these insufficient results cannot be distinguished from true negative results. Pre-analytic factors, such as time to processing and dilutional effect if the sample is obtained in the operating room, can result in low DNA yield, a well-established factor that can impede interpretation.[15] Our experience to date highlights the ongoing challenges and a cautionary note for broadly implementing LP-WGS without substantial efforts to minimize artifact and interpretation error.

Future Directions

The introduction of CSF liquid biopsy as a minimally invasive diagnostic tool for CNS tumors has compelled researchers to start applying this technology towards its next

logical application, as a surrogate marker of MRD.[38,39] Liu et al. have demonstrated this in medulloblastoma, wherein CSF samples analyzed by LP-WGS to detect end of therapy MRD showed higher rates of recurrence and decreased progression-free survival.[9] Moreover, MRD preceded MRI findings and cytologic evidence of recurrent disease by an average of 3 months. These data provide strong evidence that LP-WGS liquid biopsy may be prognostic in pediatric medulloblastoma and can reliably detect MRD in samples obtained clinically. The field has embraced a near-term goal of incorporating LP-WGS platforms upfront into therapeutic trials for patients with high-grade tumors, including embryonal tumors, and trial designs are already underway.

Further prospective study is needed to establish how best to incorporate CSF liquid biopsy monitoring and surveillance into patient care in tandem with our current tools, namely MRI and CSF cytology. To this aim, a handful of centers offer clinical CSF LP-WGS platforms with results returned to providers for clinical decision-making.[10,11,40] Testing is agnostic to tumor histopathology or subtype, including diagnoses ranging from low-grade glioma to diffuse midline glioma. The full range of clinical benefit has yet to be fully elucidated, but a few examples of actionable results in embryonal tumors include (1) aiding interpretation of equivocal MRI findings at the end of therapy to reveal active tumor vs. treatment effect, and (2) tailoring maintenance therapy duration for persistent cfDNA positivity by CSF liquid biopsy. These ongoing efforts may provide evidence to develop clinical practice guidelines for inclusion of CSF liquid biopsy as a routine diagnostic and surveillance tool.

Broader LP-WGS liquid biopsy applications are more preliminary and in various stages of implementation. LP-WGS is being investigated as a diagnostic tool that would obviate the need for tumor biopsy in patients with brainstem or midline lesions where tissue sampling carries a higher risk of complications. Non-invasive molecular characterization could potentially guide the choice of targeted therapy or clinical trial inclusion. The ability of CSF liquid biopsy to detect acquired structural changes at recurrence may also aid therapeutic decisions or planned sequencing in immunotherapy and targeted therapy trials. For example, antigen evolution on tumors following immunotherapy may expose opportunity for precision medicine approaches where chimeric antigen receptor (CAR) T cells are sequentially modified for better targeting.

Overall, the success of any CSF liquid biopsy platform requires standardization of sample processing, analytic methodology, quality control measures, and clinical interpretation.[41] As our genomic technology improves, and access to that technology becomes more widely available, research collaboration and integration of concurrent tumor testing will maximize the benefit of CSF liquid biopsy for patients.

Keywords

cell-free DNA | cerebrospinal fluid liquid biopsy | embryonal tumors | low-pass whole genome sequencing | measurable residual disease

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Conflict of interest statement

None declared.

Authorship statement

Experimental design: S.E.S.L., T.L., V.P., C.L., B.C., and E.C. Experimental implementation: All authors. Manuscript preparation and critical review: All authors.

Data availability

De-identified data will be made available upon reasonable request.

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