Critical Review

Measure Twice: Promise of Liquid Biopsy in Pediatric High-Grade Gliomas

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Received 17 September 2019; revised 7 November 2019; accepted 17 December 2019

Abstract
Purpose: To review and critique the current state of liquid biopsy in pHGG.
Materials and Methods: Published literature was reviewed for articles related to liquid biopsy in pediatric glioma and adult glioma with a focus on high-grade gliomas.
Results: This review discusses the current state of liquid biomarkers of pHGG and their potential applications for liquid biopsy development.
Conclusions: While nascent, the progress toward identifying circulating analytes of pHGG primes the field of neuro-oncology for liquid biopsy development.

Introduction
Aggressive brain tumors, such as pediatric high-grade gliomas (pHGGs), are the number one cause of cancer-related deaths during childhood (defined as age 0-14 years in the United States).1 Despite decades of clinical trials with aggressive multimodal therapy, disease recurrence is...
near inevitable, and patient outcomes remain dismal with a combined 5-year survival rate of 17%. Clinical advances in central nervous system (CNS) tumors are hampered by inaccurate or incomplete measures of disease owing to sensitive neuroanatomical location, risk of serial biopsy, and limitations in imaging technologies. In addition, a definitive noninvasive biomarker for disease measurement is lacking. The development of liquid biopsies is critical to advance the clinical management of these tumors, with the hope of directing long-term disease control.

Although rare, pHGGS comprise 8% to 12% of childhood brain tumors with an annual incidence of 0.85 per 100,000 in the United States. These tumors are responsible for almost 13,000 years of potential life lost annually. Children typically present with headaches, seizures, vision changes, vomiting, or other neurologic deficits related to specific tumor location. Standard evaluation includes magnetic resonance imaging (MRI) and, in most cases, a neurosurgical intervention (i.e., biopsy or resection) for a histologic and molecular diagnosis.

Although pHGGS are distinct diseases from adult high-grade gliomas, treatment rationales in clinical trials are often shaped by the literature on adult gliomas. Childhood cancer consortia, such as the Children’s Oncology Group, have conducted numerous clinical trials (e.g., ACNS0126, ACNS0423) to improve outcomes for this population. However, current management consists of maximal surgical resection followed by radiation therapy with concurrent and adjuvant alkylator therapy. A notable exception is diffuse intrinsic pontine glioma (DIPG), which is an unresectable tumor with no known curative treatment. Radiation therapy is used to extend the symptom-free period, but offers minimal survival advantage.

Regardless of location and subgroup, patients with pHGGS are commonly monitored with regular clinical examinations and surveillance imaging. Despite the best available therapy, recurrence is near inevitable, and most cases recur locally within the radiation field and diffuse spread occurs in up to a third of patients. Clinical advances for this patient population are hampered by the rarity of the disease, sensitive tumor location, risks of serial biopsy, disease heterogeneity, limitations of drug delivery (blood-brain barrier), and inaccurate or incomplete measures of disease status. Over the past 50 years, despite hundreds of clinical trials, the dismal outcomes for children with pHGG have not significantly changed.

Although clinical advances are stagnant, remarkable advances have been made in the past decade in the understanding of the bimolecular basis of pHGG. Large cooperative working groups (eg, International Society of Pediatric Oncology Europe), patient registries, tissue banking and sharing, and open access data sets (eg, whole genome sequencing, whole exosome sequencing, methylation analysis) have yielded robust opportunities to study these rare childhood tumors. Interrogations of well-annotated large patient cohorts have revealed the diversity of pHGG with key genetic and epigenetic events associated with distinct age of onset, neuroanatomic locations, and prognosis, allowing for biologically and clinically relevant subgrouping.

The discovery of novel driver mutations in genes encoding histone 3 K27M (H3K27M) and G34V/R (H3G34R/V) in pHGG implicate chromatin remodeling, developmental signaling pathways, and gene expression mechanisms in disease pathogenesis. These unique recurrent mutations underscore fundamental differences between pediatric and adult high-grade gliomas. The World Health Organization classification of CNS tumors now includes some molecularly defined subsets (eg, H3K27M and isocitrate dehydrogenase 1 [IDH1] mutations, which are mutually exclusive and prognostically relevant). H3K27M-mutant pHGGS confer a worse prognosis compared with wild type, whereas IDH1 mutants (although rare in the pediatric setting) carry a better prognosis than IDH wild type.

These observations have ignited efforts to further molecularly subclassify this heterogeneous group of diseases into biologically and prognostically relevant groups through initiatives from the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy.

Ongoing efforts to dissect and stratify pHGGS continue to yield new insights into pediatric gliomagenesis and identify novel therapeutic opportunities. Leveraging the distinct molecular biology driving pHGG has led to the first study in human clinical trials in the pediatric setting (eg, NCT02960230). However, more than novel therapeutics are needed to improve patient outcomes (eg, inaccurate measures of disease threaten determination of therapeutic efficacy).

Currently few tools exist for the serial measurement of disease in pHGGS, which are predominately neuroimaging and clinical examination and have limited sensitivity and specificity. There is a critical need for minimally invasive biomarkers in pHGG. A real-time readout of tumor dynamics would fundamentally change the care of children with these aggressive tumors. In this review, we highlight the current efforts toward minimally invasive analytes of pHGG and evaluate their overall importance in improving the outcome for children with pHGGS.

**Imaging advances in pediatric high-grade gliomas**

Gadolinium-based contrast agent (GBCA)—enhanced MRI remains the standard of care for the diagnosis, operative planning, and assessment of the therapeutic...
response of pHGGs. The developing brain and biological differences in tumor biology are notable dissimilarities that could affect the imaging assessment of pHGG compared with adult tumors. Warren et al. recognized the potential limitations of applying adult-based response assessment in neuro-oncology criteria to pediatric brain tumors and proposed pediatric-centric imaging response criteria. The response assessment in pediatric neuro-oncology working group has identified the need for pediatric-centric imaging criteria owing to the heterogeneous group of pediatric brain tumors, relative paucity of clinical trials, and lack of standard metrics to establish radiographic tumor responses to therapy or survival.

To address this current gap in knowledge, the response assessment in pediatric neuro-oncology working group has set the goal of proposing optimal endpoints and study designs, develop a consensus on the radiologic assessment for clinical trials involving children with brain tumors, and better define response to reflect therapeutic activity in pHGG. Jaspan et al. published methodological perspectives on the use of morphologic and physiological MRI response metrics in a randomized phase 2 trial of newly diagnosed pHGG treated with temozolomide and irradiation with or without bevacizumab (HERBY).

The HERBY study was designed to assess treatment response to inform future clinical trials in pHGG from a biological and imaging perspective. Morphologic imaging response metrics included T2 and GBCA-enhanced T1-weighted sequences. In addition, diffusion- and perfusion-based metrics were assessed and included in the estimation of pHGG response assessments. The rationale behind this was that, compared with morphologic sequences alone, a multimodal MRI approach may provide a more informed biology-based approach to establishing treatment response. Despite initiatives to incorporate physiological sequences into pHGG clinical trial response assessments, GBCA-MRI remains the standard of care.

The use of T1-weighted GBCA-MRI as a metric of therapeutic response assessments in pHGGs is problematic. Functionally, GBCA enhancement is a measure of neurovascular unit disruption. As such, GBCA does not specifically localize regions of tumor regrowth. Biologically, tissue enhancement on T1-weighted MRI is a factor of the leakage and pooling of GBCA from the vascular space through the neurovascular unit into the brain tumor interstitial space. The limitations of GBCA-MRI in evaluating therapeutic responses in adult gliomas is well established (Fig 1). The nonspecificity of GBCA enhancement for the differentiation of tumor regrowth from treatment-related inflammation necessitates serial follow-up imaging or invasive surgical sampling to establish disease status.

Furthermore, in certain clinical contexts (e.g., bevacizumab therapy), the absence of GBCA enhancement does not preclude the presence of active tumor growth. In either context, this clinical dilemma may delay definitive therapy for disease recurrence and potentially decrease the potential for a response to that therapy. As such, the development of a blood-based biomarker for therapeutic responses to complement imaging would be advantageous in this population.

Progress toward liquid biopsy

Measures of disease status must capture more than anatomic presence, and those capable of assessing pathophysiologic evolution of disease hold the potential to better inform the care of patients with pHGG. The liquid biome, including blood, cerebrospinal fluid (CSF), and urine, provides a rich, noninvasive biosource to interrogate for tumor-associated biomaterials. Biomaterials, such as tumor-associated proteins, nucleic acids, exosomes, and cells, hold the potential to act as analytes for disease progression.
Protein biomarkers in adult high-grade gliomas

Protein biomarkers are distinct intact peptides that can be differentially regulated in the setting of disease, including alterations in secretion, shedding, and loss from cells. They are detected in blood, urine, cerebral spinal fluid, and generally stable peptides that therefore can provide an objective, quantitative, and noninvasive measure of disease. The first biomarker was reported in 1848 with the discovery of the lambda light chain (Bence Jones proteins), which are detected with a urinalysis in 75% of patients with multiple myeloma.

From this initial discovery, the field of protein biomarkers has grown into the most clinically robust biomaterial for use in cancer monitoring and detection. CA-125, CA19-9, HER2/NEU, and Fibrin/FDP are all blood-based protein biomarkers approved by the US Food and Drug Administration for use in the detection, monitoring, or prognosis of ovarian, pancreatic, breast, and colorectal cancer, respectively. Although there are currently no FDA-approved biomarkers for high-grade gliomas, multiple candidate proteins are under exploration.

Protein biomarkers in pediatric high-grade gliomas

Proteins hold promise as biomarkers in pHGG, but their value remains largely to be realized. Investigations into proteins of interest in adult glioma have not been substantiated potential importance for pHGG, including vascular endothelial and basic fibroblast growth factors, where the levels between control, benign disease, and malignant disease were very similar. Tumor-associated proteins have been explored in a handful of pediatric CNS tumors, including cyclophilin A and DDAH1, which were associated with disease presence compared with healthy controls.

A proteomic analysis of CSF from pediatric CNS tumors, including pHGG, revealed 6 proteins able to discriminate metastatic cases from controls. Exploiting the unique drivers of pHGG holds promise for the detection of meaningful biomarkers. Investigations into the posttranslational modification of proteins in the liquid biomay prove a valuable strategy given the known pHGG driver mutations in epigenetic mechanisms, such as chromatin remodeling. Much opportunity exists to discover protein biomarkers that exploit unique features of pHGG and ultimately support the development and utility of a liquid biopsy.

Cell-free tumor DNA in circulation and cerebrospinal fluid

Cell-free DNA (cfDNA) are short (approximately 160 base pair fragments of genetic material) and when derived from a tumor are known as circulating tumor DNA (ctDNA), which can be detected in the blood and CSF from patients with cancer. These DNA contain both genetic and epigenetic information revealing the tumor site of origin and, importantly, specific DNA alterations (i.e., point mutations, specific methylation patterns). However, ctDNA has a high turnover rate because of its rapid clearance from the bloodstream, which could make detecting it difficult in cases of low tumor burden.

Beyond diagnostic utility, longitudinal ctDNA has the potential to inform treatment monitoring and, in the era of precision medicine, aid therapeutic predication. Currently, in some diseases, such as melanoma, gastrointestinal, or breast cancers, ctDNA is used as a metric for disease progression, with higher levels correlating to an increased disease burden or recurrence.
Cell-free DNA in adult high-grade glioma

In adult high-grade glioma, ctDNA has been detected in the blood and CSF. Studies investigating ctDNA have focused on the detection of molecular aberrations from known diagnostic, predictive, prognostic, or monitoring values from the literature on adult tissue biomarkers. These ctDNA targets include IDH1 mutations; EGFRvIII mutation; loss of heterozygosity for 1p, 10q, 19q; and the abnormal methylation of promoters (eg, O-6-methylguanine-DNA methyltransferase) but are currently limited to exploratory investigations and prospective clinical utility testing.

Tumor hemorrhage, biopsy, and neuroanatomical location may challenge the sensitivity of ctDNA as a diagnostic biomarker. For example, Wang et al. highlight that proximity to CSF reservoirs was associated with increased detection ctDNA in CSF.45 However, as the field of precision medicine grows, the stage may be set for the use of ctDNA as a companion diagnostic and predictive and monitoring biomarker. For example, the detection of EGFRvIII mutation (therapeutic target of interest) can be missed on tissue resection owing to heterogeneous expression but has been detected in the blood as ctDNA.46

Furthermore, the authors report that 2 patients with detectable preresection EGFRvIII ctDNA had undetectable levels after a gross total resection.46 Pathologic variants of EGFR are more common in adult HGG than in pHGG; however, EGFR mutations are present in pHGG and efforts to leverage a molecularly defined lesion not just for therapeutic proposes but also as a minimally invasive biomarker of disease provide a valuable example.

Cell-free DNA in pediatric high-grade glioma

The rapidly advancing molecular subgrouping of pHGG provides a rich landscape of candidate mutations for the development of ctDNA biomarkers, and the detection of ctDNA in the blood and CSF of pediatric patients with HGG has been reported. Multiple reports of H3K27M mutant ctDNA in CSF exist, this prognostic biomarker is known to portend a poor prognosis and to be common in midline gliomas, such as DIPG.47 Diagnostic noninvasive biomarkers for DIPG are highly desirable given that the disease is sensitive to neuroanatomical location and concern for harm with biopsy, and most patients are diagnosed based on imaging features and history. However, a growing number of centers are now safely performing biopsies of these tumors,18 allowing for access to molecularly defined clinical trials.

Recently, the detection of H3K27M has been reported in the plasma of patients with DIPG and when correlated with CSF ctDNA and followed longitudinally, the detection correlated with disease progression. The authors suggested that the measurement of H3K27M ctDNA could have diagnostic, prognostic, predictive, and monitoring biomarker potential; however, this has not been prospectively studied. Oncogenic BRAFv600E mutations are seen in pHGG, although more commonly in low-grade gliomas, and hold the potential to be predictive, prognostic, and monitoring biomarkers.

Although not yet reported in the blood of patients with gliomas, the assessment of BRAFv600E ctDNA has been used as a monitoring biomarker in pediatric patients with Langerhans Cell Histiocytosis (LCH).49 In this small study, the measurement of BRAFv600E ctDNA correlated with disease stage and treatment response regardless of the treatment agents used.50 The oncogenic neurotrophic tyrosine receptor kinase (NTRK) fusion is present in pHGG and although rare, NTRK is more commonly found in children age <3 years.51 The recent tissue agnostic approval of the pan-tropomyosin receptor kinase inhibitor (TRK), loratrectinib, an inhibitor of the NTRK fusion product, makes the noninvasive detection of NTRK fusions an attractive biomarker.

The literature on adult gliomas measured NTRK ctDNA as a monitoring biomarker of treatment response and predictive biomarker of resistance mechanisms for agents aimed at overcoming the mutational resistance mechanism.52 Pediatric studies, including CNS tumors, with next generation NTRK inhibitors are ongoing (e.g., NCT02650401) and include the measurement of ctDNA as biologic correlates of disease. The continued prospective collection of ctDNA in clinical trials is needed to fully appreciate the utility of ctDNA in pHGG. The clinical development of ctDNA is an exciting tool for pHGG and precision medicine; however, this biomaterial currently falls short of capturing the robust dynamic tumor biology present at the cellular level.

Meaningful messengers: MicroRNA in adult high-grade glioma

MicroRNAs (miRNAs) are small, 18 to 22 nucleotide segments of single-stranded noncoding RNA. MiRNAs function as regulators of posttranscriptional and translational processes by targeting specific messenger RNAs.53,54 MiRNAs were first noted in 1993 with the discovery of a novel gene regulation pathway.55 In 2004, Takamizawa et al demonstrated that the let-7 family of miRNAs held prognostic value in lung cancer, with higher expression correlated to a more aggressive disease state.56 In cancer, miRNAs are dysregulated and can act as tumor suppressors or oncogenes depending on their prescribed function. Now, unique miRNA expression profiles have been linked to specific cancers, making miRNAs not only an important effector of disease but also...
a useful and specific diagnostic tool. The presence of miRNAs has been documented in both pediatric and adult HGG. Birks et al. analyzed the miRNA profiles of 24 pediatric CNS tumors and noted a distinct deregulation of miRNA-129, miR-142-5p, and miR-25. Although promising, the utility of miRNA in cancer therapeutics and detection needs to be further validated before becoming a common-place cancer detection strategy.

**Small but mighty: Exosomes in adult high-grade glioma**

Exosomes are small membrane-bound vesicles ranging from 50 to 140 nm and were first discovered in the late 1980s. Exosomes have come of age as promising effectors of the tumor microenvironment and liquid biomarkers of disease diagnosis and progression. Once thought to be a byproduct underlying a mechanism for transferrin receptor elimination by red blood cells, we now know that many different cell types extrude exosomes in normal physiological conditions. Intravesicular cargo includes proteins, DNA, RNA (including noncoding RNAs), and various lipid species. The wide variety of exosome cargo point to the functional relevance of exosomes in intercellular communication. For example, exosomes contain both cytosolic and membrane-bound proteins (cytoplasmic proteins that support vesicular biogenesis) and membrane-bound proteins (eg, CD9 and CD63) that imply an immune function. Much of the lipid and protein cargo is thought to reflect the metabolic state of the cell of origin.

Furthermore, exosomes are thought to mediate intercellular communication within a microenvironment. In cancer settings, exosomes are described to mediate angiogenesis, establishment of the premetastatic niche, and tumor progression. As a liquid biomarker, exosomes remain a promising prognostic or diagnostic analyte, carrying myriad miRNAs, with significant differences seen between healthy controls and various cancers, including glioblastoma, colorectal, pancreatic, colorectal, lung, and breast cancer.

**Exosomes function in high-grade glioma**

Advances in understanding how exosomes shape HGG are predominantly conducted in adult glioblastoma and shed important insights into the exosome role in tumor progression and its role as a biomarker for disease. Currently, studies focus on 3 primary areas: impact of exosomes in shaping the tumor microenvironment, characterization of exosome cargo, and use of exosomes as a liquid biomarker.

Exosomes extruded from irradiated glioblastoma cells alter migratory behaviors of recipient cells in culture, indicating a role for exosomes in shaping the tumor microenvironment. These exosomes carry cargo, including connective tissue growth factor mRNA and insulin-like growth factor binding protein, and support the potential to affect tumor cell phenotypes or behaviors. Untreated tumor cells extrude cargo with cell-signaling components, notably farnesylated Ras found in glioblastoma-derived exosomes.

Furthermore, recent studies from an MD Anderson group led by Dr Heimburger revealed that glioblastoma-derived exosomes affect immunosuppression by targeting tumor-associated monocytes driving differentiation toward an immune-suppressive M2-macrophage fate, specifically elucidating the activation of the STAT3 pathway in this population. Not only do exosomes harbor effector molecules, they also harbor tumor-associated proteins such as mutant forms of EGFR, IDH1, and miR21. These studies, and other exosome-based biomarkers of glioblastoma, are highlighted in a review by Rennert et al focusing on adult glioma. A dearth of studies have extended into pediatric brain tumors, although miRNA that contain exosomes have been documented. Exosome detection in CSF and blood highlight the potential impact of exosomes as biomarkers of disease.

In adult disease, exosomes derived from different subtypes of glioma contain a differential expression of exosome biogenesis mRNA genes, which indicates that a potential inequality in exosome extrusion may exist across subtypes. Discrete omics are identified in adult versus pediatric tumors, which further calls into question how much of the glioblastoma-derived exosome knowledge from adult disease will translate into pediatric disease. A small handful of studies investigating exosomes in pediatric disease are beginning to provide insights. Recently, miRNA-containing exosomes have been documented in pediatric brain tumors, opening the field for potential clinical trials with exosome analytes or the development of exosomes as diagnostic agents for disease progression.

**Circulating cells: Circulating tumor cells, circulating hybrid cells, clusters, and tumor-associated immune cells**

Circulating cells with the potential to provide information on tumor state, including its pathology, heterogeneity, mutational burden, and predictive risk for disease progression, remain a holy grail of liquid biopsies. To date, a number of tumor-associated cell populations are detectible in the peripheral blood of patients with cancer, with the most noted being circulating tumor cells (CTCs), which were first detected in 1869 in a patient with metastatic cancer. CTCs hold the promise of providing temporal information of the evolving tumor in the form of a noninvasive, liquid biopsy.
The utilization of CTCs have provided prognostic value, genetic material, tumor phenotype (e.g., HER2 for breast cancer), and a measure of tumor cell heterogeneity. Once a barrier, new advances in the isolation of CTCs has led to approval by the U.S. Food and Drug Administration for the use of CTC enumeration for clinical testing. Notably, the commercially available CellSearch system relies on cytokeratin expression and lack of CD45 expression and magnetic-based separation, microfluidics systems that separate based on size, and the Gilupi Cell Collector that extracts CTCs directly from the bloodstream.

Although promising, the use of CTCs for reliable tumor monitoring has fallen short of expectations, largely owing to the rarity of CTCs in peripheral blood. Reported detectable ranges from 0.1 to 1 cell/mL are dependent on tumor stage and tumor site. In addition, the use of common epithelial proteins to identify CTCs may hamper the ability to definitively enumerate tumor-associated cells distinct from circulating epithelial cells. Finally, inconsistency among reports on the efficacy and reproducibility of CTCs as a biomarker for treatment response, prognosis, and risk of relapse hamper strong reliance on this biomarker.

**Circulating tumor cells in adult high-grade glioma**

The use of CTCs for prognosis in glioblastoma is in its infancy. The fact that patients with glioblastoma rarely experience metastatic spread led to the concept that glioblastoma is restricted to the brain. Subsequently, reports of detectable glioblastoma metastatic growth in organ-transplant recipients received from glioblastoma donors challenged this notion. CTCs were first identified in patients with glioblastoma in 2014. Glial fibrillary acid protein-positive CTCs were identified in 20.6% of patients with glioblastoma and found to harbor specific aberrations of the primary tumor, including EGFR gene amplification or chromosome insertions or deletions.

Consistent with CTCs identified in other organ systems, glioblastoma CTCs were rare and only detectible as 1 to 22 CTCs in $2.1 \times 10^6$ peripheral blood mononuclear cells. Using a microfluidic approach to deplete blood cells from peripheral blood specimens and thus isolating CTCs, Sullivan et al reported that glioblastoma CTCs have mesenchymal properties. In these studies 13 of 33 adult patients with glioblastoma harbored CTCs. Furthermore, Gao et al. enhanced the detection of CTCs using a novel fluorescence in situ hybridization-based approach that allowed for the detection of CTCs in patients with all 7 subtypes of glioblastoma in 77% of patients studied.

Most recently, in the *British Journal of Cancer*, temporal analyses of 13 adult patients with glioblastoma undergoing treatment were studied for the presence of CTCs, identifying CTC clusters throughout the disease progression, which suggests that CTCs may not travel the bloodstream as solitary entities. This small handful of reports highlights that the study of glioblastoma CTCs lags far behind that of other organ systems. Although glioblastoma CTC is nascent, currently all reported studies of CTCs are in adult cancers. Pediatric glioblastoma provides a needed setting for the development of noninvasive biomarkers for a treatment-resistant disease with unappreciated evolution behaviors and unknown susceptibilities.

**Developing the landscape of circulating cellular biomarkers**

Although CTCs are the most well-studied circulating cells in all cancers, including adult glioblastoma, other circulating cells exist and hold value. A novel cancer cell population derived from the fusion between tumor and immune cells, circulating hybrid cells, were recently identified in melanoma and pancreatic cancer. Circulating hybrid cells outnumbered conventionally defined CTCs in murine models of cancer and in human patients with pancreatic cancer, which indicates a larger population of tumor burden in peripheral blood than previously appreciated. This revelation provides an opportunity to develop a high-impact liquid biomarker.

In addition, cancer-associated macrophage-like cells or tumor epitope detection in monocytes, which are immune cells with detectable apoptotic neoplastic cells within their cytoplasm, have been reported in a variety of cancers. Tumor-extrinsic biomarkers, such as circulating immune signatures, may provide an immunomonitoring opportunity that is important to the growing number of immunooncology clinical trials. Taken together, the myriad of cells in circulation offer a fertile ground for temporal information of the evolving tumor and its immunogenicity in the form of a noninvasive, liquid biopsy.

**Conclusions**

Major advances in technology to analyze circulating cancer biomarkers primes the neuro-oncology field to interrogate the liquid biome and develop noninvasive analyses of disease for pHGG. The rarity of pHGG and invasive nature of tumor biopsies hamper major advances in understanding the disease, including disease initiation, progression, and evolution. The development of the liquid biome in pHGG (Table 1) holds promise to improve patient care through the identification of diagnostic, prognostic, predictive, and monitoring biomarkers. The discovery of circulating analytes capable of distinguishing the MRI inaccuracy of pseudo- from true progression can...
preventing subjecting patients to unnecessary neurosurgical intervention.

Biomarkers capable of delineating pseudo- from true responses will facilitate offering patient’s investigational agents through clinical trials, perhaps earlier in the disease progression course. Although many agents have failed to show activity in early phase clinical trials, the deep and rapid development of the biomolecular landscape of pHGG is unearthing new potential targets.

Furthermore, cutting-edge technology for nucleic acid isolation and sequencing, including ctDNA, RNA, and microRNAs, makes the monitoring of mutations, cellular behaviors, and evolution during treatment of pHGG feasible and ultimately promises biologically relevant insights in the mechanism of disease progression, treatment resistance, and recurrence, as well as associated therapeutic opportunities. The multidisciplinary care of pHGG provides a robust opportunity for each disciple to leverage access to noninvasive patient biosources to advance biomarker development in childhood CNS tumors.

Pediatric neuro-oncology as a field is poised to capitalize on the major advances in liquid biopsy. The development of noninvasive disease analytes in pHGG cannot be sidelined owing to the highly resistant, recurrent, and lethal course of these tumors. However, given that the disease course is abbreviated and treatment responses are poor, coordinated efforts across institutions must be engaged and consortiums must maintain focus in collecting the necessary biomaterial to facilitate advancing the treatment and management of pHGG. This type of concerted effort, bringing advances in biomarker knowledge in multianalytes, has the best chance to develop new measures of disease that can affect disease progression and change pHGG from a death sentence to a managed disease.

### Table 1  Summary of circulating biomaterials with examples

| Biomaterial          | Biosources | Serum | Plasma | CSF | Urine | Adult examples                                      | Pediatric examples      |
|----------------------|------------|-------|--------|-----|-------|----------------------------------------------------|-------------------------|
| Protein              | ctDNA      |       |        |     |       | VEGF[^30^], MMPs/TIMPs[^31^]                       | VEGF[^33^], bFGF[^33^], CypA[^44^], DDAH1[^34^] |
|                      | miRNA      |       |        |     |       | IDH1[^59^], EGFRvIII[^59^], MGMT[^41^], 1p/10q/19q codeletion[^42^] | H3K27M[^48^], BRAFV600E[^48^] |
| Exosomes             | CTCs       |       |        |     |       | Ras[^75^], EGFR[^78^], IDH1[^70^], miR21[^100^]     |                         |
| Circulating cells    | CHCs       |       |        |     |       | GFAP[^100^]                                        |                         |

Abbreviations: bFGF = basic fibroblast growth factor; CHCs = circulating hybrid cells; CSF = cerebrospinal fluid; CTCs = circulating tumor cells; ctDNA = circulating tumor DNA; CypA = cyclophilin A; DDAH1 = dimethylarginine dimethylaminohydrolase 1; GFAP = glial fibrillary acidic protein; IDH1 = isocitrate dehydrogenase 1; MGMT = O-6-methylguanine-DNA methyltransferase; miRNA = micro RNA; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; VEGF = vascular endothelial growth factor.

Biosources, such as serum, plasma, CSF, and urine, can contain multiple biomaterials, including proteins, ctDNA, miRNA, exosomes, CTCs, and CHCs.

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