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Strategies, considerations, and recent advancements in the development of liquid biopsy for glioblastoma: a step towards individualized medicine in glioblastoma

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OBJECTIVE Glioblastoma (GBM) is a devasting primary brain tumor with less than a 5% 5-year survival. Treatment response assessment can be challenging because of inflammatory pseudoprogression that mimics true tumor progression clinically and on imaging. Developing additional noninvasive assays is critical. In this article, the authors review various biomarkers that could be used in developing liquid biopsies for GBM, along with strengths, limitations, and future applications. In addition, they present a potential liquid biopsy design based on the use of an extracellular vesicle–based liquid biopsy targeting nonneoplastic extracellular vesicles.

METHODS The authors conducted a current literature review of liquid biopsy in GBM by searching the PubMed, Scopus, and Google Scholar databases. Articles were assessed for type of biomarker, isolation methodology, analytical techniques, and clinical relevance.

RESULTS Recent work has shown that liquid biopsies of plasma, blood, and/or CSF hold promise as noninvasive clinical tools that can be used to diagnose recurrence, assess treatment response, and predict patient outcomes in GBM. Liquid biopsy in GBM has focused primarily on extracellular vesicles, cell-free tumor nucleic acids, and whole-cell isolates as focal biomarkers. GBM tumor signatures have been generated via analysis of tumor gene mutations, unique RNA expression, and metabolic and proteomic alterations. Liquid biopsies capture tumor heterogeneity, identifying alterations in GBM tumors that may be undetectable via surgical biopsy specimens. Finally, biomarker burden can be used to assess treatment response and recurrence in GBM.

CONCLUSIONS Liquid biopsy offers a promising avenue for monitoring treatment response and recurrence in GBM without invasive procedures. Although additional steps must be taken to bring liquid biopsy into the clinic, proof-of-principle studies and isolation methodologies are promising. Ultimately, CSF and/or plasma-based liquid biopsy is likely to be a powerful tool in the neurosurgeon's arsenal in the near future for the treatment and management of GBM patients.

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Glioblastoma (GBM) is the most common primary malignant brain tumor. Despite aggressive multimodal treatment with surgery, radiation therapy, and chemotherapy, the median overall survival for newly diagnosed GBM patients remains abysmal, at just 14–15 months. Care of GBM patients can be further complicated by the development of pseudoprogression—nonpathological, treatment-related changes that may occur in up to 45% of GBM patients within weeks to months following initiation of treatment.[1] Classically, pseudoprogression has been reported with the combined use of temozolomide therapy and radiotherapy, although it has also been observed with immunotherapy utilization (i.e., checkpoint inhibitors).[2] In addition, patients with MGMT promoter methylation and/or IDH mutations are particularly prone to the development of pseudoprogression.[1] Unfortunately, pseudoprogression causes increased contrast enhancement and cerebral edema that is clinically and radiographically

ABBREVIATIONS BBB = blood-brain barrier; circRNA = circular RNA; CTC = circulating tumor cell; ctDNA = circulating tumor DNA; EV = extracellular vesicle; GBM = glioblastoma; miRNA = microRNA; mtDNA = mitochondrial DNA; NGS = next-generation sequencing; PCR = polymerase chain reaction.

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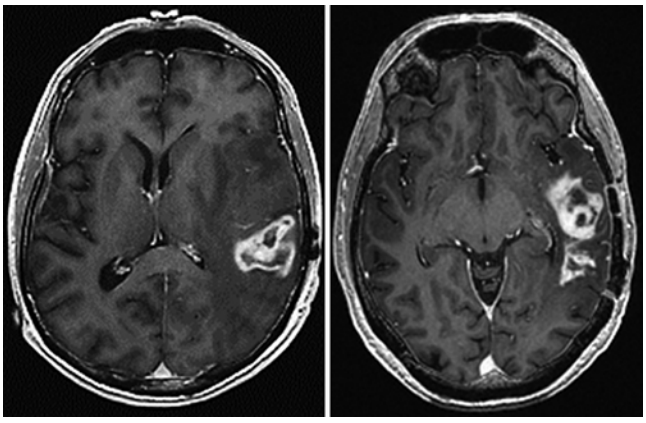

FIG. 1. Radiological comparison of pseudoprogression to true progression. **Left:** Axial T1-weighted Gd-enhanced MR image showing inflammatory pseudoprogression following completion of cycle 9 in a 63-year-old man. Biopsy showed necrosis with marked inflammatory infiltrate. **Right:** Axial T1-weighted Gd-enhanced MR image showing that true progression is indistinguishable from pseudoprogression at cycle 9 in a 46-year-old man. Biopsy showed viable tumor without inflammatory infiltrate.

indistinguishable from true progression, thereby complicating care in the GBM patient population (Fig. 1). Historically, pseudoprogression has been diagnosed most commonly by a combination of clinical and imaging findings, supported occasionally by histopathology from biopsy specimens. However, MRI findings are nonspecific and have low sensitivity and specificity in this setting. Furthermore, brain biopsy is invasive and has associated risks. Therefore, there is a vital need to develop and employ new noninvasive diagnostic assays to augment clinical and imaging findings.

Noninvasive diagnostic tools such as liquid biopsy have the potential to revolutionize GBM management (Fig. 2). Liquid biopsy uses tumor biomarkers such as circulating tumor cells (CTCs), exosomes and other extracellular vesicles, and cell-free nucleic acids found in patients' body fluids (e.g., blood, cerebrospinal fluid, and urine).[3,4] It is minimally invasive compared with brain biopsy and allows for real-time monitoring of disease progression. It has already been used routinely for disease monitoring and detection in a variety of cancers, including breast carcinoma and colon carcinoma.[5]

In GBM, recent work has suggested the clinical utility of liquid biopsyin distinguishing pseudoprogression from true progression.[6] In addition, it offers an accessible, affordable, and minimally invasive solution for monitoring the clinical course and treatment response in GBM. Liquid biopsy has the potential to detect early recurrence before a patient becomes symptomatic.[7] Furthermore, liquid biopsy could continuously monitor treatment response (via tumor shrinkage) or treatment resistance before any gross changes in tumor size are apparent on imaging.[7] Finally, liquid biopsy has been used to predict progression-free and overall survival of GBM patients in multiple studies.[8,9]

Importantly, liquid biopsy may prevent patients from receiving additional brain biopsies to determine whether imaging changes represent pseudoprogression versus true progression. It may also abrogate the need for additional advanced and expensive imaging modalities (i.e., PET). On an individual level, characterization of specific tumor biomarkers in a plasma or CSF sample could enable personalized treatment regimens and provide a means for detecting early recurrence.[10] Therefore, liquid biopsy could serve as a powerful tool to incorporate individualized medicine into GBM patient treatment.

Options for Liquid Biopsy

Two overarching strategies exist as the basis for liquid biopsy in GBM. The first strategy involves the detection of tumor-specific material in plasma or CSF. The viability of this strategy lies in the notion that the increased permeability of the blood-brain barrier (BBB) in GBM allows for extravasation of tumor-derived components that can then be detected within the bloodstream.[11] However, detecting small quantities of specific tumor components in biofluids containing components of many other cell types presents a significant challenge. A second strategy involves analyzing bulk components of biofluids to develop a signature specific to GBM patients versus normal healthy donors. For this strategy, the status of GBM would be indirectly measured through the effects that GBM exerts on other components of biofluids (e.g., circulating immune cells). In the following sections, we describe various biomarkers that could be utilized in the development of liquid biopsy for GBM, along with their strengths, limitations, and future applications.

Extracellular Vesicles

Extracellular vesicles (EVs) are membrane-encapsulated, 30-nm to 10-μm nanoparticles released by all cells.[4] EVs comprise several subgroups, including apoptotic bodies (500 nm–5 μm), large oncosomes (1–10 μm), microvesicles (typically 50–500 nm, up to 1 μm), and exosomes (30–150 nm).[4] Exosomes originate from the intraluminal vesicles manufactured in the multivesicular bodies of the late endosome, whereas microvesicles and large oncosomes bud directly off the plasma membrane and apoptotic bodies are formed via cell blebbing. In GBM, EVs have been shown to play a role in systemic immunosuppression,[12] induction of angiogenesis,[13] intercellular communication,[14] and promotion of tumor growth and invasion.[15] Furthermore, EVs have been identified in plasma, CSF, urine, saliva, tears, and other bodily fluids.[4] In addition, compared with cell-free nucleic acids, EVs are relatively structurally robust and readily cross the BBB.[11] Techniques used to isolate EVs for analysis currently include size exclusion chromatography, sequential filtration, differential ultracentrifugation, and density gradient ultracentrifugation, among others.[16] However, inconsistencies in EV nomenclature abound in the literature, and disparate isolation methods have not yet been reconciled or standardized across groups.[17] Furthermore, published studies on EVs are often limited by small sample sizes. Additional validation studies and well-designed prospective clinical trials will be vital to demonstrate robust outcomes correlations and confirm patient benefit.[18]

EV biomolecular cargo is composed of a mixture of

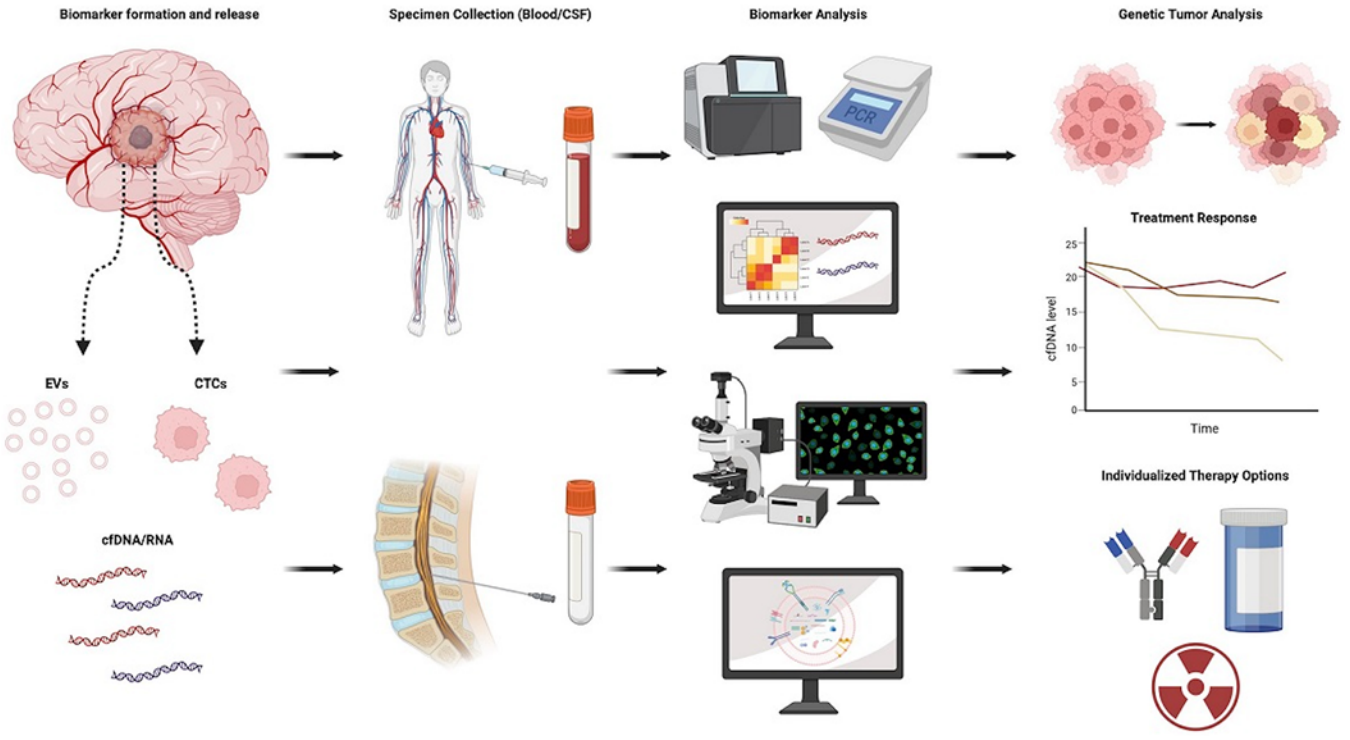

FIG. 2. Overview of liquid biopsy in GBM. GBM tumor cells produce EVs, CTCs, and cell-free DNA and RNA, which cross the BBB to varying degrees and enter the bloodstream. A substantial portion of each biomarker remains in the CSF. Liquid biopsy samples either CSF or blood/plasma for these biomarkers, which are analyzed using a variety of methods (e.g., droplet digital PCR, whole genome sequencing, NGS, and proteomics). Resultant data can be used to characterize the genetic tumor landscape, quantify treatment response, and guide individualized therapy options. Created with BioRender.com.

nucleic acids, metabolites, and proteins reflective of their cell of origin.[17] In GBM, genetic heterogeneity gives rise to variable cargo in tumor-derived EVs.[19] Thus, tumor-derived EV cargo has been examined as a potential focal point for liquid biopsy. Putative molecular signatures have been established for GBM-derived EVs via analysis of EV proteomes,[19,20] RNA contents,[19–21] genome methylation/genetic mutations,[22] and surface markers.[19,23] Important molecular pathways identified by these methods include those involved in complement activation/immune response,[20] tissue remodeling/regeneration,[24] invasion,[25] and metabolism.[26] Studies focused on more specific biomarkers have identified EGFRvIII,[27] PD-L1,[12] and vWF[20] as potentially important markers, among others. Many of these experimental findings will require validation in the clinical setting. Because of the complexity and heterogeneity of EV cargo, a comprehensive signature that incorporates these findings must be developed for consistent and accurate diagnosis. Interestingly, this may be an advantage of EV-based liquid biopsy, as needle biopsy is often limited in its ability to detect heterogeneity.[28]

Alternatively, bulk plasma EV analyzed without specifically separating and concentrating tumor-derived EVs also provides valuable diagnostic information in designing a liquid biopsy. For instance, Cilibrasi et al. demonstrated differences in proteomic signatures of complement, inflammatory, and coagulation regulators in plasma EVs of GBM patients compared with healthy donors.[20] In addition, the overall plasma EV concentration is higher in GBM patients than in that taken from healthy donors; this change is specific to GBM versus brain metastases and extra-axial brain tumors.[29] Importantly, multiple groups have demonstrated that EV levels decline after tumor resection and rise again when the tumor relapses, thereby demonstrating its utility in clinical monitoring of GBM patients.[6,29] Furthermore, increased EV levels during chemotherapy or radiation therapy have been demonstrated to be associated with shorter overall survival and earlier recurrence.[30] Finally, these changes in plasma EV concentration may also be used to distinguish between true progression and pseudoprogression during chemotherapy and radiation therapy.[6]

Spectral signatures, such as those obtained from Raman spectroscopy and flow cytometry, may allow rapid and straightforward detection of EVs. Maas et al. reported that orally administering 5-aminolevulinic acid prior to tumor resection allowed for detection of GBM EVs via flow cytometry in patient plasma.[31] However, GBM-derived EVs constitute a minority of EVs found in plasma samples.[23] Furthermore, important differences in surface markers and content have been found in subpopulations of EVs, raising the issue of whether EV biopsy would be better performed on an individual or bulk basis.[19,23] Characterizing bulk populations of plasma-derived EVs via flow cytometry immunophenotyping may eliminate the need to isolate GBM-specific EVs, thus streamlining liquid biopsy in GBM. For example, our group has recently shown that t-distributed stochastic neighbor embedding reveals

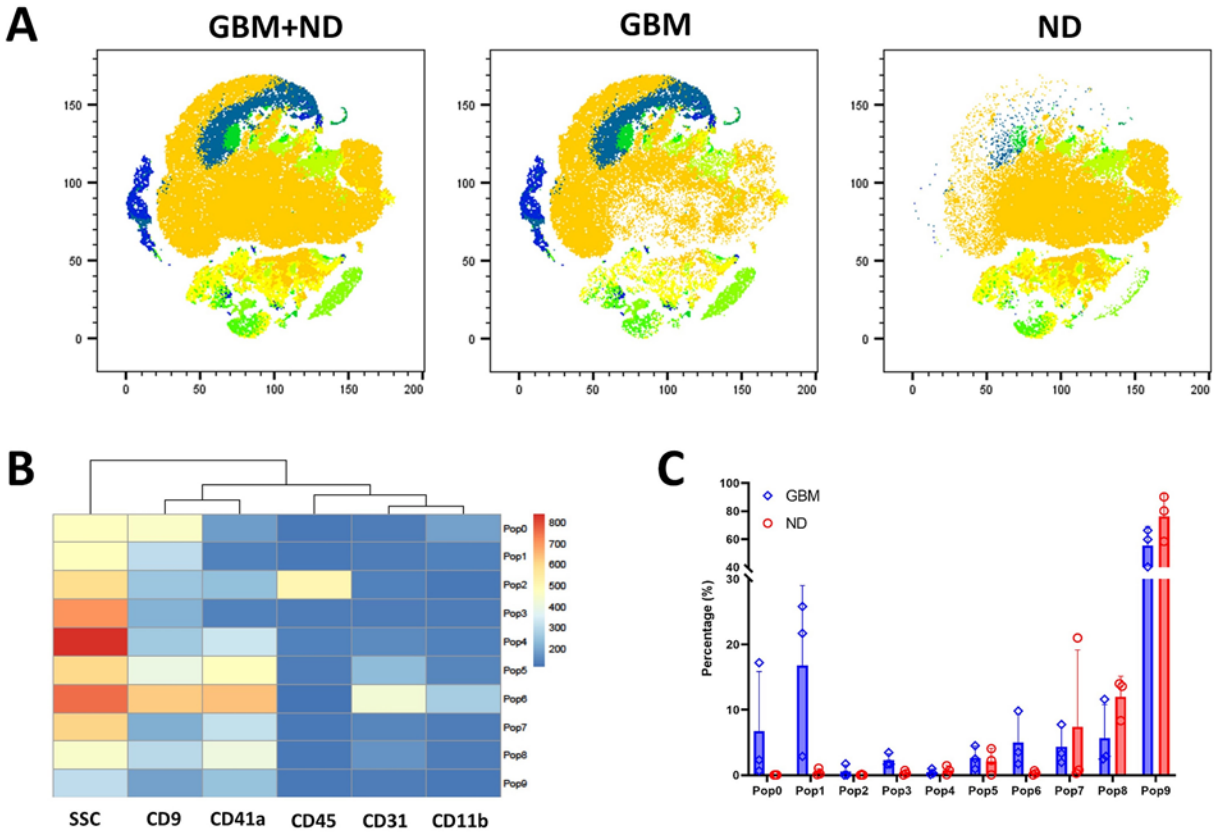

FIG. 3. Nonneoplastic plasma EV phenotype in GBM patients differs from normal donors (ND). **A:** Multiparametric analysis of several nonneoplastic EV surface markers (CD9, CD11b, CD31, CD41a, and CD45) and a measure of EV size (side scatter; SSC) by t-distributed stochastic neighbor embedding, showing markedly different clustering features of GBM plasma EVs compared with normal donors. Self-organizing map of flow cytometry data analysis revealing 10 diverse EV populations, represented by different colors. **B:** Heatmap illustrating the relative size (side scatter) and surface marker expression level of each population. **C:** Self-organizing map of flow cytometry data showing three unique EV populations (Pop0, Pop1, and Pop6) enriched in GBM patient plasma compared with normal donors.

unique clustering features of EVs derived from the plasma of GBM patients versus healthy donors (Fig. 3). We have also used flow cytometry data to define unique EV subpopulations in GBM. Thus, utilizing spectral signatures of bulk plasma EVs may make liquid biopsy more accessible and efficient as the technique moves into the clinic.

RNA

RNAs hold much promise as surrogate biomarkers for cancer progression and therapeutic responses. A variety of tumor-associated RNAs have been detected in peripheral blood, CSF, saliva, and urine.[32,33] In GBM, RNA markers can be harvested in circulating cell-free form RNA, as well as extracted from circulating exosomes, platelets, and circulating tumor cells (CTCs).[19,34,35] For such GBM-associated RNAs, the leading candidate liquid biopsy samples are peripheral blood—with serum having a higher concentration than plasma[36]—and CSF because excreted biofluids are subjected to additional filtration and RNase degradation.[32,37] Focused ultrasound (FUS) has been shown to facilitate the release of a wide variety of brain tumor biomarkers in animal models and MRI-guided FUS has been proposed as a modality to enhance the export of GBM-associated RNAs across the human BBB.[38] Alternatively, a recent study by Ita et al. found that the differentially expressed immune genes *GZMB* and *HLA-A* have a positive correlation between plasma- and glioma-derived messenger RNA (mRNA).[39] This suggests that another mechanism for RNA biomarkers is from the immunological response to the GBM, thereby circumventing the BBB.

Beyond protein-coding mRNA, posttranscriptional regulatory noncoding RNAs such as microRNA (miRNA) and circular RNA (circRNA) have been shown to be useful markers of GBM burden because of their relative abundance, low molecular weight, and exosomal packaging, which may ease their egress from the CNS.[40,41] Some oncogenic miRNAs such as miR-10b and miR-106a-5p are found in higher concentrations in the peripheral blood of GBM patients.[42,43] Tumor suppressor miRNAs, including miR-29a and miR-485-3p, decrease in circulation when GBM progresses.[44,45] Other miRNAs and circRNAs found in liquid biopsies are correlated with response to chemotherapy and radiation therapy.[46,47] Early studies indicate that miRNA signatures found in liquid biopsies may have similar utility in immunotherapy response predication and

monitoring.[48] Notably, the door is open to elucidate RNA markers prognostic of GBM resection outcomes.

Thus far, the poor sensitivity and specificity of individual RNA markers of GBM have hampered its clinical adoption. Signatures consisting of multiple RNAs, particularly from CSF liquid biopsies, are likely the solution to this problem.[49] Simply combining miR-21 with miR-15b expression yields a diagnostic assay that can differentiate GBM from primary CNS lymphoma with 90% sensitivity and 100% specificity.[50] Akers et al. found a 9-miRNA signature that correlates with GBM tumor volume, offering CSF detection sensitivity and specificity of 67% and 80%, respectively.[51] With larger transcriptomics profiles—RNA-seq of CTCs, for example—network analysis can add interactome contexts to generate more robust signatures.[52] Building more complex signatures of RNAs combined with other biomolecules discussed elsewhere in this review may offer even better liquid biopsy assays for GBM.

Cell-Free Circulating Tumor DNA

Cell-free circulating tumor DNA (ctDNA) is released from GBM cells and has garnered interest as a potential substrate for liquid biopsy in recent years. Circulating tumor DNA is shed largely by apoptotic and necrotic cells via the action of DNAseIL3 and caspase-activated DNase, although some groups have argued that phagocytosis of tumor cells by macrophages may also contribute.[53] Circulating tumor DNA is made primarily of fragments approximately 140–180 base pairs in size,[54] which approximates the 147-bp size of the nucleosome. Circulating tumor DNA has been previously explored as a biomarker in cancers outside of the CNS. In a study of 640 patients with various tumor types, Bettegowda et al. reported that ctDNA was detectable in the blood in greater than 75% of patients with advanced breast, bladder, melanoma, and hepatocellular malignancies, versus less than 50% of primary brain tumors.[55] Whether this low level of plasma ctDNA is due to the BBB remains a point of controversy, as one study found that disruption of the BBB has no impact on the ability of GBM cells to shed ctDNA,[54] while other groups have shown that disruption of the BBB increases ctDNA in CSF/plasma and may increase detection specificity.[53,56] Circulating tumor DNA also possesses a half-life of less than 2 hours, necessitating rapid sample processing for analysis.[57]

Despite these technical limitations, ctDNA is a potentially robust source of diagnostic and prognostic information in the setting of GBM. GBM patients have higher ctDNA concentrations in plasma and CSF compared with healthy controls.[3] A high preoperative ctDNA concentration is associated with less progression-free survival and worse overall survival outcomes in GBM.[58] A recent meta-analysis by Kang et al. found that the total diagnostic sensitivity and specificity of ctDNA assays for GBM were 0.69 and 0.98, respectively.[59] Furthermore, several studies have found that ctDNA levels correlate with features of tumor pathology (e.g., macrophage density and tumor vessel size) along with tumor size.[53] Therefore, ctDNA levels may serve as an early detection for recurrence,[60–62] a means for tracking treatment response,[57] and a way to differentiate pseudoprogression from true progression/recurrence.[3,57]

Importantly, ctDNA analysis may reveal tumor-specific mutations, enabling specific and minimally invasive study of the mutational topography of GBM tumors. Mutation types include point mutations, chromosomal and microsatellite changes, mutation/hypermethylation of promoter sequences, and gene-gene fusions. Commonly affected genes include the *TERT* promoter,[61,63] *EGFRvIII*,[64] *TP53*,[60] *MGMT*,[65] *PDGFRA*,[65,66] *PTEN*,[60,62,65] *IDH*,[65,67] *PIK3CA*,[55,60,65] and *BRAF*,[55,65] among others. Palande et al. identified gene-gene fusions identifiable in ctDNA that incorporate tyrosine kinases and thus may be targeted by kinase inhibitors such as imatinib and sorafenib.[66] Whereas invasive needle biopsy may fail to capture the genetic heterogeneity of GBM tumors, ctDNA has been shown to detect mutations that are not found in biopsy samples.[3,62]

Circulating tumor DNA is consistently more easily identified in CSF than in blood,[67] and diagnostic accuracy of ctDNA obtained from CSF samples is higher.[59] Although CSF collection via lumbar puncture is more invasive than a blood draw, it remains less invasive and prone to complications than surgical excision or biopsy. Interestingly, Mair et al. identified mitochondrial DNA (mtDNA) as a potential alternative DNA source in liquid biopsy; mtDNA is detectable in urine as well as serum and CSF.[54] After receiving a sample, a variety of methods are used to analyze mutations in ctDNA samples[64,65] mainly using methylation-based polymerase chain reaction (PCR), digital droplet PCR, and next-generation sequencing (NGS), the adoption of which have respectively increased sensitivities of liquid biopsy in GBM.[18] However, methods of isolating ctDNA vary between institutions, which may contribute to variations in findings and diagnostic accuracy.[59] Thus, standardization of ctDNA isolation methodology will be crucial if its use in liquid biopsy is to be successfully introduced to the clinic.

Cell-Based Strategies

Alongside plasma biomarkers, the detection and quantification of circulating cells have been explored as a basis for liquid biopsy. Because of the compromise of the BBB in GBM, CTCs may enter into the bloodstream and be found in the periphery of GBM patients.[11,68] Therefore, the isolation of CTCs serves as a direct means to obtain information regarding the GBM genome on analysis with NGS.[69] However, isolation of CTCs is a difficult technique that can further be confounded by a low yield of CTCs after completion of isolation.[68] Furthermore, available research involving CTCs is limited by small sample sizes and the use of different isolation strategies, thereby precluding accurate comparisons to be drawn between studies.[18]

Alternatively, the investigation of global cell populations within the peripheral blood avoids the need to rely on detecting a small population of CTCs or other individual biomarkers. The principle of this methodology rests on the notion that GBMs produce systemic immunosuppressive effects despite never leaving the CNS.[70] GBM itself is enriched in monocytes, which differentiate into myeloid-derived suppressor cells, nonclassical monocytes, and M2

TABLE 1. Options for biomarkers in the development of liquid biopsy for GBM

Biomarker/ Strategy Type	Brief Description	Strengths	Limitations	Future Applications
Extracellular vesicles	Membrane-encapsulated; 30-nm to 10-μm nanoparticles; released by all cells	Found in many biofluids; slow to degrade in peripheral circulation	Inconsistencies in EV nomenclature, isolation techniques; tumor-derived EVs make up minority of plasma EVs	Flow cytometry signatures; combination of biomarkers in EV signatures; characterizing bulk EV populations
Cell-free RNA	Cell-free, circulating RNA; multiple subtypes (e.g., miRNA, circRNA); RNA found in cell-free form as well as circulating exosome, platelets, and CTCs	Found in many biofluids; up- & downregulation of various RNAs in response to treatment	Poor sensitivity, specificity of individual RNA markers; RNase degradation in peripheral blood	Focused ultrasound to facilitate release of RNA, other biomarkers into blood; immunotherapy response prediction, monitoring; miRNA biopsy signatures
Cell-free DNA	Cell-free, circulating tumor DNA; 140- to 180-bp fragment	Mutations reflective of GBM heterogeneity; high overall specificity (>95%) & sensitivity depending on method of isolation (>60–90%)	Degrades quickly in peripheral circulation (<2 hrs); higher concentration in CSF vs blood	Use of mtDNA; lumbar puncture–based biopsies in the hospital setting
Circulating tumor cells	Tumor-derived cells present in peripheral circulation; enter bloodstream following compromise of the BBB	Direct samples for whole-cell sequencing from the periphery	Complicated, time-consuming isolation technique; low yield of isolation	Improving isolation techniques; whole-cell sequencing
Circulating nontumor cells	Non–tumor-derived cells present in peripheral circulation (e.g., monocytes); properties changed by the presence of GBM	Possible to target multiple cell types; analysis of whole blood vs isolation of particular components	Analysis techniques still under development; specific targets/cell types not yet established	Simple whole-blood biopsies of cell populations; possible GBM blood signature w/ multiple cell types

macrophages.[70,71] These cells can then reenter circulation to exert their global immunosuppressive effects. Importantly, Giordano et al. demonstrated that there is an increase in CD163+ monocytes in GBM patients compared with healthy donors, which become CD163/FKBP51s+ in cases of residual tumor.[71] Furthermore, CD163/FKBP51s+ monocytes were significantly decreased in individuals with pseudoprogression compared with those with true progression. Similar to monocytes, platelets also infiltrate the tumor microenvironment and are capable of providing angiogenic factors for GBM growth. These platelets differentiate into tumor-educated platelets that express higher levels VEGFR1/2 and vWF, which could further serve as a basis of liquid biopsy detection.[72]

Discussion

A variety of promising options exist for liquid biopsy in GBM, including approaches based on analysis of EVs, nucleic acids, tumor-derived cells, and circulating nontumor cells (Table 1). In comparing the various liquid biopsy modalities, EVs are better equipped to cross the BBB than nucleic acids and remain in the peripheral circulation.[11] DNA and RNA are present in higher levels in CSF and degrade quickly in the peripheral circulation, necessitating rapid transfer and analysis of patient samples.[57] Thus, biopsies focused on cell-free tumor DNA and RNA are typically more successful using CSF, whereas EV-based biopsies are successful using a simple blood draw. Thus, EVs hold promise for blood-, plasma-, or serum-based liquid biopsy, which is significantly less invasive than a lumbar puncture or tumor biopsy. Furthermore, EVs contain a multitude of biomarkers, including nucleic acids, metabolites, and proteins, that can be used to create a "tumor signature" for each patient. Because of the heterogeneity of GBM tumors, this signature is likely to include multiple mutations that are undetectable by needle biopsy alone. EVs are also present in higher concentrations than CTCs, which are difficult to isolate and rare in the peripheral blood. It may be prudent to utilize CSF-based biopsies of nucleic acids or CTCs, for example, if an institution does not have access to EV isolation equipment or CSF collection can be accomplished during a requisite operation. Regardless of the method used, if liquid biopsy is to successfully transition to the clinic, standard isolation and analysis techniques are required. Validating experimental findings via well-designed prospective clinical trials will further demonstrate patient benefit of EV-based liquid biopsy.

Although biomarker-specific liquid biopsy shows great promise in providing detailed and specific information regarding the genotype of individual GBM patients, the subsequent isolation and analysis required for these techniques may be quite time intensive and costly. Therefore, future research has instead looked to develop overall signatures that can be used to detect GBM, rather than relying on the identification of individual biomarkers. As demonstrated by our group, the immunophenotype characterization of plasma EVs shows differences in EV populations between normal healthy donors and GBM patients (Fig. 3). Similarly, immunophenotyping of white blood cells in GBM patients not only serves as a strategy to detect and monitor tumor size but also provides information re-

garding individual responses to imunnotherapy.[73] Other research has developed techniques to analyze serum without the need for further isolation or identification of blood components. Theakstone et al. demonstrated the use of spectroscopy in characterizing signatures of GBM patients with sensitivities and specificities greater than 88% for detection of GBM.[74] In particular, this strategy may serve as an effective first screening tool for GBM given that it does not rely on the detection of a small population of specific biomarkers.

While these strategies of rapid detection may serve a more prominent role in tumor detection and evaluation of tumor burden, alternative liquid biopsy strategies that detect changes in DNA, RNA, and tumor-derived EV cargo may be beneficial when designing individualized treatment regimens and evaluating treatment response. Indeed, cell-free tumor DNA displays excellent sensitivity in GBM.[18,50] Therefore, the future of GBM patient care likely consists of a combination of various liquid biopsy options that can be employed depending on the question at hand. This variety of diagnostic modalities will ultimately allow for more discrete characterization of patient disease, use of more effective strategies, and improve patient outcomes and quality of life.

Conclusions

Liquid biopsy offers a promising avenue for minimally-invasive monitoring of treatment response and recurrence in GBM. Although additional steps must be taken to bring liquid biopsy into the clinic, proof-of-principle studies and isolation methodologies are promising. Ultimately, CSF and/or plasma-based liquid biopsy is likely to be a powerful tool in the neurosurgeon's arsenal in the near future for the treatment and management of GBM patients.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Parney, Bauman, Bouchal. Acquisition of data: Bauman, Aibaidula. Analysis and interpretation of data: Bouchal, Aibaidula. Drafting the article: Bauman, Bouchal, Monie, Aibaidula, Singh. Critically revising the article: Parney, Bauman, Bouchal, Monie, Singh. Reviewed submitted version of manuscript: Parney, Bauman, Bouchal, Monie, Singh. Approved the final version of the manuscript on behalf of all authors: Parney. Statistical analysis: Aibaidula.

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