Published in final edited form as:

Adv Biosyst. 2020 December ; 4(12): e2000029. doi:10.1002/adbi.202000029.

Liquid Biopsy Strategies to distinguish Progression from Pseudoprogression and Radiation Necrosis in Glioblastomas.

Anudeep Yekula[1], **Koushik Muralidharan**[1], **Zachary Rosh**[1], **Anna E. Youngkin**[1,2], **Keiko M. Kang**[1,3], **Leonora Balaj**[1,§], **Bob S. Carter**[1,§]

[1.]Department of Neurosurgery, Massachusetts General Hospital, Boston, MA, USA.

[2.]Trinity College of Arts and Sciences, Duke University, Durham, NC, USA.

[3.]School of Medicine, University of California, San Diego, La Jolla, CA, USA.

Abstract

Liquid biopsy for the detection and monitoring of central nervous system (CNS) tumors is of significant clinical interest. At initial diagnosis, the majority of patients with central nervous system tumors undergo magnetic resonance imaging (MRI), followed by invasive brain biopsy to determine the molecular diagnosis of the WHO 2016 classification paradigm. Despite the importance of MRI for long-term treatment monitoring, in the majority of patients who receive chemoradiation therapy for glioblastoma (GBM), it can be challenging to distinguish between radiation treatment effects including pseudoprogression, radiation necrosis (RN) and recurrent/progressive disease (PD) based on imaging alone. Tissue biopsy-based monitoring is high risk and not always feasible. However, distinguishing these entities is of critical importance for management of patients and can significantly affect survival. Liquid biopsy strategies including circulating tumor cells (CTCs), circulating free DNA (CfDNA) and extracellular vesicles (EVs) have the potential to afford significant useful molecular information at both the stage of diagnosis and monitoring for these tumors. We review current liquid biopsy-based approaches in the context of tumor monitoring to differentiate PD from pseudoprogression and RN.

Keywords

Glioblastoma; progression; pseudoprogression; liquid biopsy; radiation necrosis; extracellular vesicles; circulating tumor cells; circulating free DNA

To whom correspondence should be addressed: Bob S. Carter MD, PhD, Department of Neurosurgery, Massachusetts General Hospital, 55 Fruit St. Boston, MA. bcarter@mgh.harvard.edu, Leonora Balaj PhD, Department of Neurosurgery, Massachusetts General Hospital, 185 Cambridge St. Boston, MA. Balaj.Leonora@mgh.harvard.edu.

[§]Co-senior authors

Author Contributions

All Authors: Conceptualization, Writing, Reviewing and Editing.

Declaration of competing interests

None of the other authors declare any conflicts of interest.

Introduction

Glioblastoma is the most common malignant primary central nervous system tumor. GBM is highly aggressive and the median overall survival is only 15–23 months despite aggressive treatment [1]. Currently, maximal resection followed by radiation therapy with concurrent temozolomide (TMZ) and adjuvant TMZ treatment is the standard of care. Post treatment surveillance involves serial MRI. A challenge faced by clinicians is the diagnosis and management of a gadolinium enhancing lesion on a follow-up MRI post treatment. This suspicious lesion could be PD or a mere post treatment radiation effects such as pseudoprogression or radiation necrosis (RN). Pseudoprogression and RN are distinct clinical entities, which when identified and managed appropriately result in better outcomes, while PD of the tumor is often dismal. Patients with PD have a median survival of 3–6 months [2], and there is no standard of care. Systemic options include TMZ rechallenge, lomustine, and antiangiogenic therapy such as bevacizumab, but their effectiveness is limited. Re-radiation and re-resection can be considered depending on the location of the tumor and the condition of the patient [3]. Conversely, antiangiogenic drugs like bevacizumab or cediranib decrease contrast enhancement by altering permeability of tumor vasculature without actual reduction in tumor burden, referred to as pseudoresponse. Distinguishing these clinical entities from PD is crucial to avoid unnecessary reoperations, premature discontinuation of adjuvant TMZ or its substitution with second line agents.

MR imaging based monitoring is the current standard of care for post-surgical monitoring. Contrast enhancement on imaging is indicative of disrupted blood brain barrier (BBB), but not tumor presence [4]. Currently, MRI based Response Assessment in Neuro-Oncology (RANO) criteria is used to monitor treatment response in GBM patients.The criteria included T1 gadolinium enhancing disease, T2/FLAIR changes, new lesions, corticosteroid use, and clinical status [5]. Adoption of RANO criteria for monitoring response is not without limitations. There is ambiguity in identifying radiation effects, enrolling patients into clinical trials and monitoring immunotherapy response [6]. Advanced imaging modalities including diffusion-tensor imaging, perfusion imaging, MR spectroscopy (MRS), Positron Emission Tomography (PET) imaging have been used to identify true PD [7,8]. Although, MRS [9] and dynamic susceptibility contrast methods [10] show promise, imaging modalities cannot establish a definitive diagnosis nor capture the heterogeneous molecular landscape of the evolving tumor which is crucial in the setting of PD. Moreover, repeated biopsies cannot be performed to monitor tumor progression due to high risk, surgical inaccessibility and life threatening complications [11]. Furthermore, focal sampling cannot capture the true tumor heterogeneity.

As such, there is a great need for tools that can allow early diagnosis, molecular characterization, and assess response to therapy as well as distinguish PD from pseudoprogression and RN with higher sensitivity and specificity compared to current imaging-based technologies. Liquid biopsy refers to analysis of biofluids of patients to detect disease specific genomic or proteomic cargo for diagnostic and prognostic purposes. Liquid biopsy encompasses circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and extracellular vesicles (EVs).A longitudinal liquid biopsy based patient monitoring could provide better perspectives into the tumor presence, molecular status,

tumor evolution, response to therapy and also distinguish PD from post treatment radiation effects and ultimately strategize appropriate therapies to improve patient outcomes [12,13]. In this review, we briefly discuss the clinical entities of pseudoprogression and RN in the context of various liquid biopsy platforms to distinguish PD from pseudoprogression and RN.

Pseudoprogression

Pseudoprogression is a reversible subacute post treatment radiation effect identified as an increase in the size of the contrast enhancing lesion, with or without neurological deterioration following completion of RT alone or concomitant RT-TMZ, mimicking PD [14,15]. pseudoprogression most often occurs within the first 3 months following RT/RT-TMZ, but can present up to 6 months afterwards. Nearly half the patients with an enhancing lesion within 1 month post-RT have pseudoprogression [16]. Unlike patients with PD, patients with pseudoprogression remain asymptomatic. Some present with complications due to transient demyelination including worsening of pre-existing symptoms, transient cognitive decline, subacute rhombencephalitis or somnolence syndrome.

Pathologically, pseudoprogression corresponds to gliosis and reactive radiation-induced changes without evidence of viable tumor tissue[17]. This may represent an exaggerated response to therapy involving changes to the vascular endothelium and the blood brain barrier (BBB) as well as oligodendroglial injury leading to inflammation and increased vascular permeability [11]. Treatment-related cellular hypoxia could also contribute to this abnormal enhancement [11,18]. Some groups suggested pseudoprogression to be an active ‘inflammatory’ response against the tumor considering the association between pseudoprogression and increased survival [19]. Interestingly, patients with MGMT methylation show pseudoprogression twice as often [20]. Considering the fact that MGMT methylation status is associated with response to TMZ and thus favourable prognosis [21,22], identification of MGMT status is useful in predicting pseudoprogression and differentiating it from PD [15,16,23]. Conversely, patients without MGMT methylation have higher rates of PD, with rates of 60% occurrence [22]. Recent studies have demonstrated a correlation between P53 overexpression and pseudoprogression [24]. As such, P53 status could also be a potential biomarker for pseudoprogression. Emerging reports have suggested the association of higher expressions of interferon regulatory factor 9 (IRF9) and X ray repair cross-complementing 1 (XRCC1) in pseudoprogression [25].

Conventional MR imaging is unable to distinguish between pseudoprogression and early progression, and alternative techniques have not been validated in prospective trials [7,11,26]. The current method to distinguish the two is to perform follow-up examinations of patients comparing MR images at different points in time. Asymptomatic cases of suspected pseudoprogression are followed up by serial imaging, but when there is worsening of the symptoms due to transient cerebral edema, short course of corticosteroid treatment is initiated with close clinical surveillance and serial imaging [7].

Radiation Necrosis

Radiation necrosis is a permanent post treatment radiation effect characterized by an increase in the size of the contrast enhancing lesion occurring 3 months to years after RT [11]. Pseudoprogression and RN are often considered a spectrum of post treatment radiation changes. Unlike pseudoprogression, RN progresses without treatment, and has not been associated with better prognosis. With improvements in overall survival of patients with GBM, there is growing usage of reradiation, radiation surgery and hypofractionated radiotherapy adding to the cumulative dose of radiation received by a single patient contributing to the increasing incidence of RN of about 5 – 40% [27]. Patients with RN can be asymptomatic or present with symptoms and signs of necrosis including stroke-like migraine attacks after radiation therapy (SMART syndrome), radiation induced cavernous malformations or aneurysms, Moya-Moya syndrome, mineralizing microangiopathy, tissue calcification, atrophy, leukoencephalomyelopathy or rarely endocrine dysfunction [28–32].

Pathologically, RN corresponds to white matter necrosis associated with calcification, fibrinoid deposition, vascular hyalinization and endothelial thickening which leads to chronic inflammatory state, oxidative stress and inhibition of neurogenesis [29,33–36]. Radiation induced vascular injury initiates the process of necrosis; subsequently, increased tumor necrosis factor alpha (TNF-α) drives endothelial cell apoptosis and increased vascular permeability, and increased vascular endothelial growth factor(VEGF) induces small vessel permeability and cerebral edema [7,37–40]. Conventional imaging tools cannot identify RN and alternative techniques have not yet been validated in prospective trials [7,11,26]. Suspected RN can be managed with corticosteroid treatment, hyperbaric oxygen therapy, anticoagulation, anti-angiogenic agents like bevacizumab, laser interstitial thermal therapy or even surgery [7]. Corticosteroids reduce radiation induced radiation induced inflammatory response, decrease BBB leakiness and reduce cerebral edema[41]. Hyperbaric oxygen therapy stimulates angiogenesis and restores blood supply after radiation induced vascular injury. It is even suggested as a prophylactic option in patients with high likelihood of developing RN [42,43]. Anticoagulants like heparin and warfarin inhibit cytokine release, prevent platelet aggregation and coagulation [44,45]. Anti-VEGF agents reduce small vessel permeability and BBB leakiness [46–49]. Laser interstitial thermal therapy focuses on thermal coagulation of peri-necrotic region of abnormal angiogenesis [50]. Surgery reduces mass effect, edema and decreases intracranial pressure in addition to providing true tissue diagnosis [51,52]. No controlled randomized clinical trials have been performed to establish the most beneficial regimen to manage RN.

Circulating tumor cells

Circulating tumor cells are cancer cells that leave the primary tumor and enter circulation. A fraction of these CTCs have the potential to invade distant sites and progress to metastasis [53]. Epithelial to mesenchymal transition (EMT) within the tumor enables some cells to gain a phenotype associated with increased motility and invasion [54,55]. CTCs are found either as single cells or in clusters, the latter of which have higher metastatic potential [56–58]. CTCs are hypothesized to be either randomly detached cells or metastatic tumor subclones. In either case, they contain the genomic, transcriptomic and proteomic characteristics of the primary tumor [12] and can be valuable tools to provide insight into the primary tumor [59,60].

Studies in multiple cancers have shown the possibility of CTC based diagnosis [61–63], monitoring [64–73] and prognosis [74–77]. These studies have also demonstrated that the presence, phenotype and the methylation status of markers within CTCs in peripheral circulation have prognostic significance [57,78,79].

GBMs rarely form clinically evident extracranial metastases [80]. This is attributed to the inability of glioma cells to survive in extracranial sites, and tolerate the immune system [60,81]. However, recent evidence of CTCs detected in blood of GBM patients (Table 1) [59,60,81,82] poses questions about the conventional theories of GBM dissemination, opening the field of CTC based liquid biopsy in brain tumors [59,60,82]. Although the capability of the detected GBM CTCs to metastasize has not been established, they can be used as tools to diagnose and monitor GBM [59,60]. Previous studies have used positive selection (surface marker based selection), negative selection (depletion of blood cells) or other novel platforms for CTC detection (Table 1).

GBM-CTCs were shown to contain tumor specific molecular characteristics and invasive mesenchymal signature [59]. Macarthur et al., showed an increase in CTC numbers post radiotherapy in a patient suggestive of PD, indicating the potential of CTCs in distinguishing PD from radiation effects [82]. Gao et al. identified CTCs in all grades of glioma patients, and showed that CTC detection can reliably identify PD from RN [83]. These studies provide a proof of principle that patients with GBM have CTCs in their peripheral blood. They demonstrate the potential of molecular characterization of these cells for minimally invasive tumor profiling and identification of PD from radiation effects. Recently, CTC clusters were also identified in the blood of GBM patients [81]. Interestingly, Lui et al., demonstrated the capacity of intravenously injected CTCs to ‘reseed’ the primary site using a xenograft model and showed that CTCs also demonstrated stemness phenotype more resistant to treatments [84]. This strengthens the notion that CTCs are a subset of aggressive primary GBM cells, with EMT and stemness characteristics.

Although current studies report a very high specificity, the sensitivity of CTC detection is variable, from 20.6% to 82% [59,60,82]. Higher sensitivities are required to establish CTCs as a potential diagnostic modality to diagnose and monitor brain tumors. Most studies inadequately characterize CTCs, are underpowered, use limited numbers of surface markers for CTC enrichment, have samples collected at variable time points along the disease course and lack long-term followup. Furthermore, CTCs were not detectable in each of multiple samples of a given patient at a given time point. This could indicate the lack of sensitivity of current techniques in detecting CTCs or the rarity CTCs in blood (1 cell per 10^9 blood cells). CTC analysis requires large volumes of fresh blood, and immediate sample processing. Also, detection is currently limited by technological constraints [12,85]. Several factors including localization of the primary tumor, circulation dynamics and entrapment in capillary beds limit CTC detection. Furthermore, EMT may alter the surface marker profiles, which may negatively affect CTC-assay performance [29,86]. Furthermore, the role of CTCs as diagnostic screening modalities is debatable as the disease would be in an advanced stage with CTC dissemination, but it can probably be a good monitoring tool for disease progression and prognosis. Nevertheless, CTCs can provide a distant insight into the primary tumor, and analysis using complementary technology could potentially indicate the presence

of a tumor, monitor disease progression, therapeutic responses, and reflect the genetic characteristics of the primary tumor.

Circulating Tumor DNA

Circulating tumor DNA (ctDNA) is a subtype of circulating, cell-free DNA (cfDNA) that originates from tumor cells and is composed of small fragments of DNA (180–200 base pairs in length) [85,87]. CtDNA is typically released during tumor cell death and rapidly cleared by phagocytic processes. As such, the concentration of cfDNA is about very low (10–100 ng/ml) in plasma in normal individuals and in early stage cancers. However, the levels could be almost 10-fold higher in patients with advanced cancers [87]. The challenge in ctDNA based liquid biopsy is two-fold, in extraction and in targeted detection. At the level of extraction, optimization of methodologies would increase the chance that these markers are detected. At the level of detection sensitive technologies, including droplet digital PCR, BEAMing (beads, emulsion, amplification, and magnetics) and next generation sequencing, allow identification of targeted mutations in various biofluids [85].

Studies in multiple cancers have demonstrated the utility of cfDNA based diagnosis [88–91], monitoring and assessing response to therapy [92–96]. Growing evidence also suggests that cfDNA concentration correlates with tumor burden, cancer stage, cellular turnover, and response to therapy [87,97]. However, the application of this strategy to gliomas has been hindered by the relatively low abundance of detectable molecular alterations in plasma (<10% of patients) as compared to other tumor types, likely due to the BBB [87].

However, emerging studies have reported the detection of tumor specific mutations in the cfDNA of patients with glioma (Table 2) [87,98–110]. Detection of glioma specific alterations such as TERT [105,111], EGFRvIII [102], IDH1[108] and histone mutations [112] has shown promise in minimally invasive diagnosis, molecular profiling and classification of tumors. EGFR gene is amplified in 30–40% of GBMs and nearly 50% of them express the in-frame deleted variant of EGFR receptor, EGFRvIII and represents an aggressive subtype of GBM [113–119]. IDH1 mutations occur in 10% GBMs [120]. TERT promoter mutations occur in 60% of GBMs, associated with poorer outcomes. Simultaneous presence of IDH1 and TERT promoter mutations confer survival benefit for GBM patients. H3K27M mutation status has both diagnostic and prognostic significance in diffuse midline glioma [121]. Furthermore, identification of prognostic markers such as MGMT can be valuable to guide therapy [98,101,107,109,110]. Considering the association of MGMT promoter methylation with pseudoprogression, a positive MGMT methylation status can suggest the likelihood that a contrast enhancing lesion indicates pseudoprogression. Emerging studies also suggest the possibility of using ctDNA analysis to pursue treatment alternatives [100] as well as assess response to immunotherapy [100,122]. Recent studies have shown the ability of ctDNA based longitudinal follow up in GBM patients. Miller et al. showed that CSF ctDNA based sequencing analysis can be used to track the evolution of tumors [104]. Arruda and Mourliere showed that the levels of tumor specific mutation status in ctDNA fraction of CSF parallels the disease status, correlating with progressive disease [103,123,124].

CSF studies have consistently shown higher sensitivities in ctDNA detection compared to blood based analysis [101,105–107], however, serial monitoring may not be practical

considering the invasiveness of CSF collection. While recent studies have explored the potential of alternative biofluids such as urine [123], blood based detection has shown promising sensitivity and is more practical for serial monitoring. cfDNA is shed by virtually all cells in the body; it is especially difficult to identify ctDNA within this background. Furthermore, ctDNA fragments have a very short half-life and require rapid processing [12]. Most cfDNA studies in glioma have small sample sizes and have used various methods of mutant detection to allow meaningful comparisons. Lack of standardized procedures for sample collection, isolation, and analysis has been another major hurdle for the field, making it challenging to compare sensitivities across various studies. Nevertheless, development of sensitive technologies for ctDNA capture and tumor specific mutant and methylation status can provide minimally invasive diagnosis and monitoring for GBMs, providing insights into the spatiotemporal heterogeneity over time and therapy.

Extracellular vesicles

Tumor cells actively release stable membrane bound nanobodies called EVs. They carry functional genomic and proteomic cargo from their parental cells and deliver that information to surrounding and distant recipient cells to modulate their behavior. EVs are identified to modulate and reprogram the tumor microenvironment to promote tumor proliferation, reprogram metabolic activity, induce angiogenesis, escape immune surveillance, acquire drug resistance and undergo invasion [125]. They can also be detected in biofluids including plasma, CSF, urine etc. Their stable configuration confers a protective niche for tumor derived mRNA, miRNA and proteins. EVs are classified according to size and biogenesis pathway: microvesicles (100–1000 nm) are formed by budding of the plasma membrane, exosomes (30–150 nm) are formed by the fusion of intracellular multivesicular bodies with the plasma membrane, apoptotic bodies (1000–5000 nm) are produced and released by dying cells, and large oncosomes (>1 μm) are formed by non-apoptotic blebs from plasma membrane [85,126,127]. Detection of tumor specific EVs amidst the vast background of normal EVs derived from every other cell of the body is challenging. Methodologies to allow for optimal EV isolation and sensitive technologies for EV cargo analysis are being developed.

Emerging reports have demonstrated the utility of EVs as biomarkers of cancer diagnosis [3,128–130] and prognosis [131–133]. Quantitative studies demonstrated that EV numbers in plasma were higher in patients with GBM patients compared to controls and the numbers dropped with therapy [134–137]. Higher numbers were noted in PD compared to patients with stable disease or pseudoprogression [134,135]. However, nanoparticle tracking analysis or flow cytometry based EV quantification methods are non-specific and non-representative of true tumor derived EV burden. Nevertheless, these studies indicate that the pattern of EV dynamics parallel the disease course in the broad sense.

Recent EV based mRNA studies have reported sensitivities between 28% and 82% for the detection of EGFRvIII in EVs extracted from serum of GBM patients [138,139]. In addition, analysis of CSF-derived EV mRNA has shown higher sensitivities in IDH mutant detection compared to blood based EV analysis [140,141]. Several protein based EV analysis methods have been used for tumor specific EV characterization [142,143]. Shao et al. used micro nuclear

magnetic resonance system chip based EV protein analysis and identified EGFRvIII, PDPN and IDH1 proteins in the plasma EVs of glioma patients. The sensitivities were higher for EGFRvIII and PDPN (68%) than they were for IDH1 (16%)[142]. Chandran et al. showed that detection of syndecan-1 in plasma (sensitivity, 71%) can differentiate high grade gliomas from low grade gliomas. Other groups have explored EV miRNAs including miR-301a[144], miR-182–5p, miR328–3p, miR-339–5p, miR-340–5p, miR-485–3p, miR-486–5p and miR-543[145] miR-21, miR-222, miR-124–3p[146], miR-320 and miR-574–3p, as well as a small noncoding RNA, RNU6[147] as diagnostic tools. Specifically, Lan et al. and Santangelo et al. showed that serum miR-301a levels [144], miR-21, miR-222 and miR-124–3p levels [146] in serum EVs were higher in GBM patients and paralleled the clinical disease course, with levels decreasing with surgical resection and increasing with recurrence [144].

Recent studies have explored the potential of fluorescent labelled EV quantification using imaging flow cytometry. Ricklefs et al. used imaging flow cytometry to show that EVs with double positive tetraspanin expression (CD63+/CD81+) are enriched in patient plasma samples [148]. Galbo et al. showed that CD9+/GFAP+/SVN+ EVs can predict response to therapy [149]. These studies highlight the possibility of monitoring GBM EVs using surface marker analysis. Jones et al. identified protoporphyrin positive EVs in plasma of patients with malignant glioma undergoing fluorescence guided surgery with 5-Amino levulinic acid (5-ALA) as a potential diagnostic strategy to identify and monitor malignant gliomas. As the drug is currently approved only for surgical resection, the potential of the drug in a longitudinal setting has not yet been evaluated [150].

The ability of GBM-EVs to cross the BBB has always been a topic of debate, which could be the reason for lower sensitivities of target detection in blood based EV analysis. Garcia-Romero et al. recently demonstrated that tumor specific EVs are capable of crossing intact BBB and navigating into plasma, using an orthotopic xenotransplant mouse model of human glioma-cancer stem cells featuring an intact BBB[141].

Although both CSF and plasma/serum based EV analysis is promising, the superiority of a biofluid for EV based monitoring is still unclear. However, plasma/serum based monitoring is more practical for the purposes of longitudinal monitoring as repeated CSF sampling is not feasible [151]. Biofluids such as urine and saliva need to be explored. Small sample sizes, variable technologies, lack of a gold standard method of EV characterization makes it difficult to make meaningful comparisons. However, these initial EV biomarker discovery studies show promise and their potential in longitudinal setting is yet to be explored.

Blood Brain Barrier

Although CSF is considered as the ideal biofluid for liquid biopsy based diagnosis and monitoring due to the anatomic proximity to the primary tumor, plasma and serum are easily accessible and minimally invasive. CSF collection is highly invasive, requires trained professionals and has several potential complications. The utility of blood based liquid biopsy depends on the inherent ability of the liquid biopsy substrates (CTCs, CtDNA, EVs) to cross the BBB and reach peripheral circulation. The BBB provides both physical and biochemical barriers with a continuous network of tight and adherens junctions between brain capillary endothelial cells preventing paracellular diffusion of hydrophilic

molecules[153]. The most obvious path for these substrates is circumnavigation of the BBB at the regions of BBB disruption. Despite the fact that GBM is a highly aggressive and invasive brain tumor with a disruption of BBB, large sections of BBB remain intact [152]. Wide scale disruptions of BBB usually occur with the progression of disease.

CTCs are large and require disrupted BBB to navigate their way into the bloodstream. This could be one of the reasons for their low abundance in blood. It is unlikely for the hydrophilic ctDNA to cross an intact BBB. CtDNA could enter the bloodstream via the sites of BBB disruption. Studies have shown higher ctDNA levels in blood in high grade gliomas than low-grade gliomas[87], which can partly be attributed to the BBB disruption in high grade gliomas. A positive correlation between the extent of BBB disruption and ctDNA levels in the blood was identified by Nabavizadeh and colleagues, indicating the ability to detect ctDNA as a function of BBB disruption strengthening this notion [154]. Morad et al demonstrated using *in vitro* and *in vivo* BBB models, the ability of native tumor derived EVs to breach the intact BBB and reach the circulation via transcytosis[155]. Kur et al showed a neuronal activity driven uptake of hematopoietic cell derived EVs by neurons across the BBB via transcytosis[156]. These studies provide a proof of principle that tumor specific EVs navigate through the intact BBB, and reach the peripheral circulation. However, further investigation is required to unveil the ability of liquid biopsy substrates to reach peripheral circulation as well as determine the optimal biofluid for monitoring disease progression.

Future directions

Promising developments in the field of liquid biopsy can aid clinicians making diagnostic and therapeutic decisions to manage GBMs. The potential of combining both liquid biopsy fractions, cfDNA from dying cells and actively secreted EVs from live cells might be a better representation of the ongoing tumor dynamics. Recent clinical application of liquid biopsy based diagnostics such as cobas EGFR Mutation Test version 2, which monitors T790M mutation status in plasma cfDNA in non-small cell lung cancer patients to aid the use of osimertinib [157,158], and ExoDx Prostate IntelliScore (EPI Test, Bio-Techne), a non-invasive EV based urine test measures three mRNAs considered to be important genomic RNA biomarkers that can guide urologists in determining the true need for a prostate biopsy [159,160] have shown promise of liquid biopsy for minimally invasive diagnostics and prognostics. However, there are several challenges along the pathway of blood-based biomarker development from discovery to clinical utility, and systematic approach to tackle these hurdles is critical to develop a blood based biomarker with clinical utility. These aspects are extensively reviewed elsewhere [151,161,162]. Ideally, an advanced machine learning model [163,164] integrating clinical, imaging and liquid biopsy based molecular characterization could help decision making during follow-up. With the advent of sensitive technologies, liquid biopsy could be the future of tumor diagnosis, monitoring and therapy response.

Conclusion

Liquid biopsy strategies offer minimally invasive tools for diagnosis as well as monitoring brain tumors for response to therapy and for predicting treatment related changes. Despite

recent advances in liquid biopsy based biomarking brain tumors, the sensitivity of detection in brain tumors have been low. As of now, there are no clinically applicable circulating biomarkers for the diagnosis and monitoring of GBMs, but promising developments in the field with complimentary sensitive technologies have moved the needle closer to a clinical assay. Biobanking and appropriate sample collection and handling protocols are needed to allow the field to harvest and save biofluids for development and validation of biomarkers and technologies. Ideally, a three-pronged monitoring approach correlating clinical status, imaging characteristics and liquid biopsy based molecular characterization, to provide a comprehensive clinical and molecular snapshot of the tumor in space and time, to assess the evolution of the tumor, and identify true PD from radiation effects could be a potential solution to the current challenge.

Acknowledgments

Funding sources

This work is supported by grants U01 CA230697 (BSC, LB), UH3 TR000931 (BSC), P01 CA069246 (BSC). The funding sources had no role in the writing the manuscript or decision to submit the manuscript for publication. The authors have not been paid to write this article by any entity. The corresponding author has full access to the manuscript and assumes final responsibility for the decision to submit for publication.

References

1. Ostrom QT et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2009–2013. Neuro-Oncology vol. 18 v1–v75 (2016). [PubMed: 28475809]
2. Gruber ML & Buster WP Temozolomide in Combination With Irinotecan for Treatment of Recurrent Malignant Glioma. Am. J. Clin. Oncol 27, 33–38 (2004). [PubMed: 14758131]
3. van Linde ME et al. Treatment outcome of patients with recurrent glioblastoma multiforme: a retrospective multicenter analysis. J. Neurooncol 135, 183–192 (2017). [PubMed: 28730289]
4. Ambady P, Bettegowda C. & Holdhoff M. Emerging methods for disease monitoring in malignant gliomas. CNS Oncol 2, 511–522 (2013). [PubMed: 25054821]
5. Wen PY et al. Updated Response Assessment Criteria for High-Grade Gliomas: Response Assessment in Neuro-Oncology Working Group. J. Clin. Oncol 28, 1963–1972 (2010). [PubMed: 20231676]
6. Chukwueke UN & Wen PY Use of the Response Assessment in Neuro-Oncology (RANO) criteria in clinical trials and clinical practice. CNS Oncol 8, CNS28 (2019).
7. Parvez K, Parvez A. & Zadeh G. The diagnosis and treatment of pseudoprogression, radiation necrosis and brain tumor recurrence. Int. J. Mol. Sci 15, 11832–11846 (2014). [PubMed: 24995696]
8. Thust SC, van den Bent MJ & Smits M. Pseudoprogression of brain tumors. Journal of Magnetic Resonance Imaging vol. 48 571–589 (2018).
9. Chiang GC et al. Magnetic Resonance Spectroscopy, Positron Emission Tomography and Radiogenomics-Relevance to Glioma. Front. Neurol 9, 33 (2018). [PubMed: 29459844]
10. Larsen VA, Simonsen HJ, Law I, Larsson HBW & Hansen AE Evaluation of dynamic contrast-enhanced T1-weighted perfusion MRI in the differentiation of tumor recurrence from radiation necrosis. Neuroradiology 55, 361–369 (2013). [PubMed: 23262559]
11. Brandsma D, Stalpers L, Taal W, Sminia P. & van den Bent MJ Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. Lancet Oncol. 9, 453–461 (2008). [PubMed: 18452856]
12. Shankar GM, Balaj L, Stott SL, Nahed B. & Carter BS Liquid biopsy for brain tumors. Expert Review of Molecular Diagnostics vol. 17 943–947 (2017). [PubMed: 28875730]

13. Bark JM, Kulasinghe A, Chua B, Day BW & Punyadeera C. Circulating biomarkers in patients with glioblastoma. British Journal of Cancer vol. 122 295–305 (2020). [PubMed: 31666668]
14. Cruz LCH da et al. Pseudoprogression and Pseudoresponse: Imaging Challenges in the Assessment of Posttreatment Glioma. American Journal of Neuroradiology vol. 32 1978–1985 (2011). [PubMed: 21393407]
15. Brandes AA et al. Disease progression or pseudoprogression after concomitant radiochemotherapy treatment: Pitfalls in neurooncology. Neuro. Oncol 10, 361–367 (2008). [PubMed: 18401015]
16. Ellingson BM, Wen PY, van den Bent MJ & Cloughesy TF Pros and cons of current brain tumor imaging. Neuro. Oncol 16 Suppl 7, vii2–11 (2014).
17. Tsien C. et al. Parametric Response Map As an Imaging Biomarker to Distinguish Progression From Pseudoprogression in High-Grade Glioma. J. Clin. Oncol 28, 2293–2299 (2010). [PubMed: 20368564]
18. Gahramanov S. et al. Potential for differentiation of pseudoprogression from true tumor progression with dynamic susceptibility-weighted contrast-enhanced magnetic resonance imaging using ferumoxytol vs. gadoteridol: a pilot study. Int. J. Radiat. Oncol. Biol. Phys 79, 514–523 (2011).
19. Yaman E. et al. Radiation induced early necrosis in patients with malignant gliomas receiving temozolomide. Clin. Neurol. Neurosurg 112, 662–667 (2010). [PubMed: 20627551]
20. Brandes AA et al. Recurrence pattern after temozolomide concomitant with and adjuvant to radiotherapy in newly diagnosed patients with glioblastoma: correlation With MGMT promoter methylation status. J. Clin. Oncol 27, 1275–1279 (2009). [PubMed: 19188675]
21. Esteller M. et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N. Engl. J. Med 343, 1350–1354 (2000). [PubMed: 11070098]
22. Hegi ME et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N. Engl. J. Med 352, 997–1003 (2005). [PubMed: 15758010]
23. Zikou A. et al. Radiation Necrosis, Pseudoprogression, Pseudoresponse, and Tumor Recurrence: Imaging Challenges for the Evaluation of Treated Gliomas. Contrast Media Mol. Imaging 2018, 6828396 (2018).
24. Kang H-C et al. Pseudoprogression in patients with malignant gliomas treated with concurrent temozolomide and radiotherapy: potential role of p53. Journal of Neuro-Oncology vol. 102 157–162 (2011). [PubMed: 20632071]
25. Qian X. et al. Identification of biomarkers for pseudo and true progression of GBM based on radiogenomics study. Oncotarget 7, 55377–55394 (2016). [PubMed: 27421136]
26. Brandes AA et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J. Clin. Oncol 26, 2192–2197 (2008). [PubMed: 18445844]
27. Langleben DD & Segall GM PET in differentiation of recurrent brain tumor from radiation injury. J. Nucl. Med 41, 1861–1867 (2000). [PubMed: 11079496]
28. Fink J, Born D. & Chamberlain MC Radiation necrosis: relevance with respect to treatment of primary and secondary brain tumors. Curr. Neurol. Neurosci. Rep 12, 276–285 (2012). [PubMed: 22350279]
29. Belka C, Budach W, Kortmann RD & Bamberg M. Radiation induced CNS toxicity-molecular and cellular mechanisms. Br. J. Cancer 85, 1233–1239 (2001). [PubMed: 11720454]
30. Sheline GE, Wara WM & Smith V. Therapeutic irradiation and brain injury. Int. J. Radiat. Oncol. Biol. Phys 6, 1215–1228 (1980).
31. Giglio P. & Gilbert MR Cerebral radiation necrosis. Neurologist 9, 180–188 (2003). [PubMed: 12864928]
32. Johannesen TB, Lien HH, Hole KH & Lote K. Radiological and clinical assessment of long-term brain tumour survivors after radiotherapy. Radiother. Oncol 69, 169–176 (2003). [PubMed: 14643954]
33. Reinhold HS, Calvo W, Hopewell JW & van der Berg AP Development of blood vessel-related radiation damage in the fimbria of the central nervous system. Int. J. Radiat. Oncol. Biol. Phys 18, 37–42 (1990).

34. Schultheiss TE, Kun LE, Ang KK & Stephens LC Radiation response of the central nervous system. Int. J. Radiat. Oncol. Biol. Phys 31, 1093–1112 (1995).
35. Yoshii Y. Pathological review of late cerebral radionecrosis. Brain Tumor Pathol. 25, 51–58 (2008). [PubMed: 18987829]
36. Zhao W. & Robbins MEC Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Curr. Med. Chem 16, 130–143 (2009). [PubMed: 19149566]
37. Ansari R. et al. Anti-TNFA (TNF-α) Treatment Abrogates Radiation-Induced Changes in Vascular Density and Tissue Oxygenation. Radiation Research vol. 167 80–86 (2007). [PubMed: 17243316]
38. Mayhan WG Cellular mechanisms by which tumor necrosis factor-alpha produces disruption of the blood-brain barrier. Brain Res. 927, 144–152 (2002). [PubMed: 11821008]
39. Wilson CM, Gaber MW, Sabek OM, Zawaski JA & Merchant TE Radiation-induced astrogliosis and blood-brain barrier damage can be abrogated using anti-TNF treatment. Int. J. Radiat. Oncol. Biol. Phys 74, 934–941 (2009).
40. van Bruggen N. et al. VEGF antagonism reduces edema formation and tissue damage after ischemia/reperfusion injury in the mouse brain. J. Clin. Invest 104, 1613–1620 (1999). [PubMed: 10587525]
41. Shaw PJ & Bates D. Conservative treatment of delayed cerebral radiation necrosis. J. Neurol. Neurosurg. Psychiatry 47, 1338–1341 (1984). [PubMed: 6512555]
42. Kohshi K. et al. Successful treatment of radiation-induced brain necrosis by hyperbaric oxygen therapy. J. Neurol. Sci 209, 115–117 (2003). [PubMed: 12686413]
43. Leber KA, Eder HG, Kovac H, Anegg U. & Pendl G. Treatment of cerebral radionecrosis by hyperbaric oxygen therapy. Stereotact. Funct. Neurosurg 70 Suppl 1, 229–236 (1998). [PubMed: 9782255]
44. Glantz MJ et al. Treatment of radiation-induced nervous system injury with heparin and warfarin. Neurology 44, 2020–2027 (1994). [PubMed: 7969953]
45. Rizzoli HV & Pagnanelli DM Treatment of delayed radiation necrosis of the brain. Journal of Neurosurgery vol. 60 589–594 (1984). [PubMed: 6699703]
46. Levin VA et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. Int. J. Radiat. Oncol. Biol. Phys 79, 1487–1495 (2011).
47. Wong ET, Huberman M, Lu X-Q & Mahadevan A. Bevacizumab reverses cerebral radiation necrosis. J. Clin. Oncol 26, 5649–5650 (2008). [PubMed: 18981459]
48. Gonzalez J, Kumar AJ, Conrad CA & Levin VA Effect of bevacizumab on radiation necrosis of the brain. Int. J. Radiat. Oncol. Biol. Phys 67, 323–326 (2007).
49. Furuse M, Kawabata S, Kuroiwa T. & Miyatake S-I Repeated treatments with bevacizumab for recurrent radiation necrosis in patients with malignant brain tumors: a report of 2 cases. J. Neurooncol 102, 471–475 (2011). [PubMed: 20694573]
50. Rahmathulla G, Recinos PF, Valerio JE, Chao S. & Barnett GH Laser interstitial thermal therapy for focal cerebral radiation necrosis: a case report and literature review. Stereotact. Funct. Neurosurg 90, 192–200 (2012). [PubMed: 22678505]
51. Siu A. et al. Radiation necrosis following treatment of high grade glioma--a review of the literature and current understanding. Acta Neurochir. 154, 191–201; discussion 201 (2012). [PubMed: 22130634]
52. Mou Y-G et al. Surgical management of radiation-induced temporal lobe necrosis in patients with nasopharyngeal carcinoma: report of 14 cases. Head Neck 33, 1493–1500 (2011). [PubMed: 21928423]
53. Dasgupta A, Lim AR & Ghajar CM Circulating and disseminated tumor cells: harbingers or initiators of metastasis? Mol. Oncol 11, 40–61 (2017). [PubMed: 28085223]
54. Joosse SA, Gorges TM & Pantel K. Biology, detection, and clinical implications of circulating tumor cells. EMBO Molecular Medicine vol. 7 1–11 (2015). [PubMed: 25398926]
55. Yang J. & Weinberg RA Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev. Cell 14, 818–829 (2008). [PubMed: 18539112]

56. Aceto N. et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 158, 1110–1122 (2014). [PubMed: 25171411]
57. Gkountela S. et al. Circulating Tumor Cell Clustering Shapes DNA Methylation to Enable Metastasis Seeding. Cell 176, 98–112.e14 (2019). [PubMed: 30633912]
58. Ignatiadis M, Lee M. & Jeffrey SS Circulating Tumor Cells and Circulating Tumor DNA: Challenges and Opportunities on the Path to Clinical Utility. Clin. Cancer Res 21, 4786–4800 (2015). [PubMed: 26527805]
59. Sullivan JP et al. Brain Tumor Cells in Circulation Are Enriched for Mesenchymal Gene Expression. Cancer Discovery vol. 4 1299–1309 (2014). [PubMed: 25139148]
60. Müller C. et al. Hematogenous dissemination of glioblastoma multiforme. Sci. Transl. Med 6, 247ra101 (2014).
61. Cristofanilli M. et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N. Engl. J. Med 351, 781–791 (2004). [PubMed: 15317891]
62. Krebs MG et al. Molecular analysis of circulating tumour cells-biology and biomarkers. Nat. Rev. Clin. Oncol 11, 129–144 (2014). [PubMed: 24445517]
63. Ilie M. et al. 'Sentinel' Circulating Tumor Cells Allow Early Diagnosis of Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. PLoS ONE vol. 9 e111597 (2014).
64. Yan W-T et al. Circulating tumor cell status monitors the treatment responses in breast cancer patients: a meta-analysis. Sci. Rep 7, 43464 (2017). [PubMed: 28337998]
65. Paoletti C. et al. Development of Circulating Tumor Cell-Endocrine Therapy Index in Patients with Hormone Receptor–Positive Breast Cancer. Clinical Cancer Research vol. 21 2487–2498 (2015). [PubMed: 25381338]
66. Smerage JB et al. Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500. Journal of Clinical Oncology vol. 32 3483–3489 (2014). [PubMed: 24888818]
67. Miyamoto DT et al. Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. Cancer Discov. 2, 995–1003 (2012). [PubMed: 23093251]
68. Antonarakis ES et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N. Engl. J. Med 371, 1028–1038 (2014). [PubMed: 25184630]
69. Luo X. et al. Isolation and molecular characterization of circulating melanoma cells. Cell Rep. 7, 645–653 (2014). [PubMed: 24746818]
70. Sakaizawa K. et al. Mutation analysis of BRAF and KIT in circulating melanoma cells at the single cell level. British Journal of Cancer vol. 106 939–946 (2012). [PubMed: 22281663]
71. Maheswaran S. et al. Detection of mutations in EGFR in circulating lung-cancer cells. N. Engl. J. Med 359, 366–377 (2008). [PubMed: 18596266]
72. Gasch C. et al. Heterogeneity of epidermal growth factor receptor status and mutations of KRAS/PIK3CA in circulating tumor cells of patients with colorectal cancer. Clin. Chem 59, 252–260 (2013). [PubMed: 23136247]
73. Kondo Y. et al. KRAS mutation analysis of single circulating tumor cells from patients with metastatic colorectal cancer. BMC Cancer 17, 311 (2017). [PubMed: 28468669]
74. Bono J. S. de et al. Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer. Clinical Cancer Research vol. 14 6302–6309 (2008). [PubMed: 18829513]
75. Giuliano M. et al. Circulating tumor cells as early predictors of metastatic spread in breast cancer patients with limited metastatic dissemination. Breast Cancer Research vol. 16 (2014).
76. Normanno N, De Luca A, Gallo M, Chicchinelli N. & Rossi A. The prognostic role of circulating tumor cells in lung cancer. Expert Rev. Anticancer Ther 16, 859–867 (2016). [PubMed: 27337696]
77. Tsai W-S et al. Circulating Tumor Cell Count Correlates with Colorectal Neoplasm Progression and Is a Prognostic Marker for Distant Metastasis in Non-Metastatic Patients. Sci. Rep 6, 24517 (2016).
78. Lorente D. et al. Circulating tumour cell increase as a biomarker of disease progression in metastatic castration-resistant prostate cancer patients with low baseline CTC counts. Annals of Oncology vol. 29 1554–1560 (2018). [PubMed: 29741566]

79. Tong B. et al. Prognostic significance of circulating tumor cells in non-small cell lung cancer patients undergoing chemotherapy. Oncotarget vol. 8 (2017).
80. Smith DR, Hardman JM & Earle KM Contiguous glioblastoma multiforme and fibrosarcoma with extracranial metastasis. Cancer 24, 270–276 (1969). [PubMed: 4307749]
81. Krol I. et al. Detection of circulating tumour cell clusters in human glioblastoma. British Journal of Cancer vol. 119 487–491 (2018). [PubMed: 30065256]
82. Macarthur KM et al. Detection of brain tumor cells in the peripheral blood by a telomerase promoter-based assay. Cancer Res. 74, 2152–2159 (2014). [PubMed: 24525740]
83. Gao F. et al. Circulating tumor cell is a common property of brain glioma and promotes the monitoring system. Oncotarget 7, 71330–71340 (2016). [PubMed: 27517490]
84. Liu T. et al. Circulating Glioma Cells Exhibit Stem Cell-like Properties. Cancer Res. 78, 6632–6642 (2018). [PubMed: 30322863]
85. Zachariah MA, Oliveira-Costa JP, Carter BS, Stott SL & Nahed BV Blood-based biomarkers for the diagnosis and monitoring of gliomas. Neuro. Oncol 20, 1155–1161 (2018). [PubMed: 29746665]
86. Aktas B. et al. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Research vol. 11 (2009).
87. Bettegowda C. et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci. Transl. Med 6, 224ra24 (2014).
88. Forshew T. et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci. Transl. Med 4, 136ra68 (2012).
89. Kinde I, Wu J, Papadopoulos N, Kinzler KW & Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc. Natl. Acad. Sci. U. S. A 108, 9530–9535 (2011). [PubMed: 21586637]
90. Wen L. et al. Erratum: Genome-scale detection of hypermethylated CpG islands in circulating cell-free DNA of hepatocellular carcinoma patients. Cell Research vol. 25 1376–1376 (2015). [PubMed: 26620315]
91. Mao L, Hruban RH, Boyle JO, Tockman M. & Sidransky D. Detection of oncogene mutations in sputum precedes diagnosis of lung cancer. Cancer Res. 54, 1634–1637 (1994). [PubMed: 8137272]
92. Gormally E. et al. TP53 and KRAS2 mutations in plasma DNA of healthy subjects and subsequent cancer occurrence: a prospective study. Cancer Res. 66, 6871–6876 (2006). [PubMed: 16818665]
93. Fernandez-Cuesta L. et al. Identification of Circulating Tumor DNA for the Early Detection of Small-cell Lung Cancer. EBioMedicine vol. 10 117–123 (2016). [PubMed: 27377626]
94. Fackler MJ et al. Novel Methylated Biomarkers and a Robust Assay to Detect Circulating Tumor DNA in Metastatic Breast Cancer. Cancer Research vol. 74 2160–2170 (2014). [PubMed: 24737128]
95. Genovese G. et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N. Engl. J. Med 371, 2477–2487 (2014). [PubMed: 25426838]
96. Sozzi G. et al. O-297 Quantification of free circulating DNA as a diagnostic marker in lung cancer. Lung Cancer vol. 41 S86–S87 (2003).
97. Palmirotta R. et al. Liquid biopsy of cancer: a multimodal diagnostic tool in clinical oncology. Therapeutic Advances in Medical Oncology vol. 10 175883591879463 (2018).
98. Balaña C. et al. O6-methyl-guanine-DNA methyltransferase methylation in serum and tumor DNA predicts response to 1,3-bis(2-chloroethyl)-1-nitrosourea but not to temozolamide plus cisplatin in glioblastoma multiforme. Clin. Cancer Res 9, 1461–1468 (2003). [PubMed: 12684420]
99. Schwaederle M. et al. Detection rate of actionable mutations in diverse cancers using a biopsy-free (blood) circulating tumor cell DNA assay. Oncotarget vol. 7 (2016).
100. Piccioni DE et al. Analysis of cell-free circulating tumor DNA in 419 patients with glioblastoma and other primary brain tumors. CNS Oncol 8, CNS34 (2019).
101. Wang Z. et al. promoter methylation in serum and cerebrospinal fluid as a tumor-specific biomarker of glioma. Biomed Rep 3, 543–548 (2015). [PubMed: 26171163]

102. Salkeni MA et al. Detection of EGFRvIII mutant DNA in the peripheral blood of brain tumor patients. J. Neurooncol 115, 27–35 (2013). [PubMed: 23877363]
103. De Mattos-Arruda L. et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. Nat. Commun 6, 8839 (2015). [PubMed: 26554728]
104. Miller AM et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature 565, 654–658 (2019). [PubMed: 30675060]
105. Juratli TA et al. TERT Promoter Mutation Detection in Cell-Free Tumor-Derived DNA in Patients with IDH Wild-Type Glioblastomas: A Pilot Prospective Study. Clinical Cancer Research vol. 24 5282–5291 (2018). [PubMed: 29941484]
106. Mouliere F. et al. Detection of cell-free DNA fragmentation and copy number alterations in cerebrospinal fluid from glioma patients. EMBO Mol. Med 10, (2018).
107. Liu B-L et al. Quantitative detection of multiple gene promoter hypermethylation in tumor tissue, serum, and cerebrospinal fluid predicts prognosis of malignant gliomas. Neuro. Oncol 12, 540–548 (2010). [PubMed: 20154338]
108. Boisselier B. et al. Detection of IDH1 mutation in the plasma of patients with glioma. Neurology 79, 1693–1698 (2012). [PubMed: 23035067]
109. Lavon I, Refael M, Zelikovitch B, Shalom E. & Siegal T. Serum DNA can define tumor-specific genetic and epigenetic markers in gliomas of various grades. Neuro. Oncol 12, 173–180 (2010). [PubMed: 20150384]
110. Majchrzak-Celińska A. et al. Detection of MGMT, RASSF1A, p15INK4B, and p14ARF promoter methylation in circulating tumor-derived DNA of central nervous system cancer patients. J. Appl. Genet 54, 335–344 (2013). [PubMed: 23661397]
111. Cordova C. et al. Plasma cell-free circulating tumor DNA (ctDNA) detection in longitudinally followed glioblastoma patients using TERT promoter mutation-specific droplet digital PCR assays. Journal of Clinical Oncology vol. 37 2026–2026 (2019).
112. Azad TD, Jin MC, Bernhardt LJ & Bettegowda C. Liquid biopsy for pediatric diffuse midline glioma: a review of circulating tumor DNA and cerebrospinal fluid tumor DNA. Neurosurg. Focus 48, E9 (2020).
113. Sugawa N, Ekstrand AJ, James CD & Collins VP Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. Proc. Natl. Acad. Sci. U. S. A 87, 8602–8606 (1990). [PubMed: 2236070]
114. Ekstrand AJ, Sugawa N, James CD & Collins VP Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proceedings of the National Academy of Sciences 89, 4309–4313 (1992).
115. Frederick L, Wang XY, Eley G. & James CD Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res. 60, 1383–1387 (2000). [PubMed: 10728703]
116. Aldape KD et al. Immunohistochemical detection of EGFRvIII in high malignancy grade astrocytomas and evaluation of prognostic significance. J. Neuropathol. Exp. Neurol 63, 700–707 (2004). [PubMed: 15290895]
117. Ohgaki H. & Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J. Neuropathol. Exp. Neurol 64, 479–489 (2005). [PubMed: 15977639]
118. Parsons DW et al. An integrated genomic analysis of human glioblastoma multiforme. Science 321, 1807–1812 (2008). [PubMed: 18772396]
119. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455, 1061–1068 (2008). [PubMed: 18772890]
120. Louis DN et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 131, 803–820 (2016). [PubMed: 27157931]

121. Huang TY et al. Detection of Histone H3 mutations in cerebrospinal fluid-derived tumor DNA from children with diffuse midline glioma. Acta Neuropathol Commun 5, 28 (2017). [PubMed: 28416018]
122. Zhao J. et al. Author Correction: Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma. Nat. Med 25, 1022 (2019).
123. Mouliere F. et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci. Transl. Med 10, (2018).
124. Cell-free DNA in newly diagnosed patients with glioblastoma – a clinical prospective feasibility study. Oncotarget vol. 10 (2019).
125. Yekula A. et al. Extracellular Vesicles in Glioblastoma Tumor Microenvironment. Frontiers in Immunology vol. 10 (2020).
126. Roy S. et al. Navigating the Landscape of Tumor Extracellular Vesicle Heterogeneity. Int. J. Mol. Sci 20, (2019).
127. Yekula A. et al. Large and small extracellular vesicles released by glioma cells in vitro and in vivo. Journal of Extracellular Vesicles vol. 9 1689784 (2020).
128. Park YH et al. Author Correction: Prostate-specific extracellular vesicles as a novel biomarker in human prostate cancer. Sci. Rep 9, 6051 (2019). [PubMed: 30988318]
129. García-Silva S. et al. Correction: Use of extracellular vesicles from lymphatic drainage as surrogate markers of melanoma progression and BRAFV600E mutation. The Journal of Experimental Medicine vol. 216 1230–1230 (2019). [PubMed: 31010898]
130. van der Mijn JC et al. Analysis of AKT and ERK1/2 protein kinases in extracellular vesicles isolated from blood of patients with cancer. J Extracell Vesicles 3, 25657 (2014). [PubMed: 25491250]
131. Goto T. et al. An elevated expression of serum exosomal microRNA-191, - 21, −451a of pancreatic neoplasm is considered to be efficient diagnostic marker. BMC Cancer 18, 116 (2018). [PubMed: 29385987]
132. Fu F, Jiang W, Zhou L. & Chen Z. Circulating Exosomal miR-17–5p and miR-92a-3p Predict Pathologic Stage and Grade of Colorectal Cancer. Transl. Oncol 11, 221–232 (2018). [PubMed: 29367070]
133. Liu Q. et al. Circulating exosomal microRNAs as prognostic biomarkers for non-small-cell lung cancer. Oncotarget 8, 13048–13058 (2017). [PubMed: 28055956]
134. Koch CJ et al. Microvesicles as a Biomarker for Tumor Progression versus Treatment Effect in Radiation/Temozolomide-Treated Glioblastoma Patients. Transl. Oncol 7, 752–758 (2014). [PubMed: 25500085]
135. Evans SM et al. Initial evidence that blood-borne microvesicles are biomarkers for recurrence and survival in newly diagnosed glioblastoma patients. J. Neurooncol 127, 391–400 (2016). [PubMed: 26746692]
136. Osti D. et al. Clinical Significance of Extracellular Vesicles in Plasma from Glioblastoma Patients. Clin. Cancer Res 25, 266–276 (2019). [PubMed: 30287549]
137. André-Grégoire G, Bidère N. & Gavard J. Temozolomide affects Extracellular Vesicles Released by Glioblastoma Cells. Biochimie vol. 155 11–15 (2018). [PubMed: 29454008]
138. Skog J. et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat. Cell Biol 10, 1470–1476 (2008). [PubMed: 19011622]
139. Manda SV et al. Exosomes as a biomarker platform for detecting epidermal growth factor receptor-positive high-grade gliomas. J. Neurosurg 128, 1091–1101 (2018). [PubMed: 28574310]
140. Chen WW et al. BEAMing and Droplet Digital PCR Analysis of Mutant IDH1 mRNA in Glioma Patient Serum and Cerebrospinal Fluid Extracellular Vesicles. Mol. Ther. Nucleic Acids 2, e109 (2013). [PubMed: 23881452]
141. García-Romero N. et al. DNA sequences within glioma-derived extracellular vesicles can cross the intact blood-brain barrier and be detected in peripheral blood of patients. Oncotarget vol. 8 (2017).
142. Shao H. et al. Protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy. Nat. Med 18, 1835–1840 (2012). [PubMed: 23142818]

143. Chandran VI et al. Ultrasensitive Immunoprofiling of Plasma Extracellular Vesicles Identifies Syndecan-1 as a Potential Tool for Minimally Invasive Diagnosis of Glioma. Clinical Cancer Research vol. 25 3115–3127 (2019). [PubMed: 30679164]
144. Lan F. et al. Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma. Cell. Oncol 41, 25–33 (2018).
145. Ebrahimkhani S. et al. Deep sequencing of circulating exosomal microRNA allows non-invasive glioblastoma diagnosis. NPJ Precis Oncol 2, 28 (2018). [PubMed: 30564636]
146. Santangelo A. et al. A microRNA signature from serum exosomes of patients with glioma as complementary diagnostic biomarker. J. Neurooncol 136, 51–62 (2018). [PubMed: 29076001]
147. Manterola L. et al. A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool. Neuro. Oncol 16, 520–527 (2014). [PubMed: 24435880]
148. Ricklefs FL et al. Imaging flow cytometry facilitates multiparametric characterization of extracellular vesicles in malignant brain tumours. J Extracell Vesicles 8, 1588555 (2019).
149. Galbo PM et al. Circulating CD9 /GFAP /survivin exosomes in malignant glioma patients following survivin vaccination. Oncotarget vol. 8 (2017).
150. Jones PS et al. Characterization of plasma-derived protoporphyrin-IX-positive extracellular vesicles following 5-ALA use in patients with malignant glioma. EBioMedicine 48, 23–35 (2019). [PubMed: 31628025]
151. Yekula A. et al. From laboratory to clinic: Translation of extracellular vesicle based cancer biomarkers. Methods (2020) doi:10.1016/j.ymeth.2020.02.003.
152. Sarkaria JN et al. Is the blood–brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. Neuro. Oncol 20, 184 (2018). [PubMed: 29016900]
153. Cardoso FL, Brites D. & Brito MA Looking at the blood-brain barrier: molecular anatomy and possible investigation approaches. Brain Res. Rev 64, 328–363 (2010). [PubMed: 20685221]
154. Mair R. et al. Measurement of Plasma Cell-Free Mitochondrial Tumor DNA Improves Detection of Glioblastoma in Patient-Derived Orthotopic Xenograft Models. Cancer Res. 79, 220–230 (2019). [PubMed: 30389699]
155. Morad G. et al. Tumor-Derived Extracellular Vesicles Breach the Intact Blood–Brain Barrier via Transcytosis. ACS Nano vol. 13 13853–13865 (2019). [PubMed: 31479239]
156. Kur I-M et al. Neuronal activity triggers uptake of hematopoietic extracellular vesicles in vivo. PLoS Biol. 18, e3000643 (2020).
157. Jenkins S, Cross D. & Scudder SA Osimertinib (TAGRISSOTM) and the cobas® EGFR Mutation Test v2. Companion and Complementary Diagnostics 429–443 (2019) doi:10.1016/b978-0-12-813539-6.00023-7.
158. Brown P. The Cobas® EGFR Mutation Test v2 assay. Future Oncology vol. 12 451–452 (2016). [PubMed: 26838018]
159. McKiernan J. et al. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol 2, 882–889 (2016). [PubMed: 27032035]
160. McKiernan J. et al. A Prospective Adaptive Utility Trial to Validate Performance of a Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer in Patients with Prostate-specific Antigen 2–10 ng/ml at Initial Biopsy. European Urology vol. 74 731–738 (2018). [PubMed: 30237023]
161. Ioannidis JPA & Bossuyt PMM Waste, Leaks, and Failures in the Biomarker Pipeline. Clin. Chem 63, 963–972 (2017). [PubMed: 28270433]
162. Zhao Z. et al. Extracellular vesicles as cancer liquid biopsies: from discovery, validation, to clinical application. Lab Chip 19, 1114–1140 (2019). [PubMed: 30882822]
163. Jang B-S, Jeon SH, Kim IH & Kim IA Prediction of Pseudoprogression versus Progression using Machine Learning Algorithm in Glioblastoma. Sci. Rep 8, 12516 (2018). [PubMed: 30131513]
164. Akbari H. et al. NIMG-70. QUANTITATIVE IMAGE ANALYSIS AND MACHINE LEARNING TECHNIQUES FOR DISTINGUISHING TRUE PROGRESSION FROM PSEUDOPROGRESSION IN PATIENTS WITH GLIOBLASTOMA. Neuro-Oncology vol. 20 vi191–vi192 (2018).

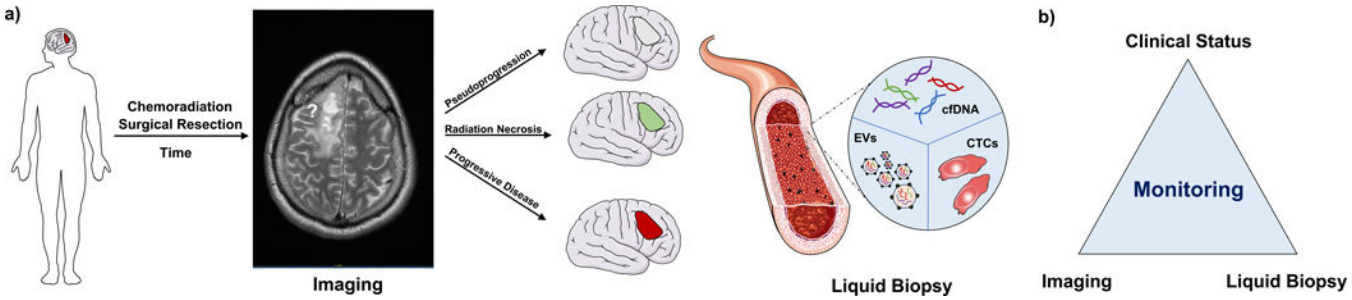

Figure 1.

a. Patients with glioblastoma, post-surgical resection and chemoradiation, are monitored using serial MR imaging. A gadolinium enhancing lesion on contrast enhanced magnetic resonance imaging (MRI) could either be true progressive disease (PD), or radiation effects such as pseudoprogression or radiation necrosis (RN). Liquid biopsy strategies including circulating tumor cells (CTCs), circulating free DNA (CfDNA), extracellular vesicles (EVs) can provide minimally invasive modalities of monitoring brain tumors. **b.** Three-pronged monitoring approach using integrating clinical status, imaging modalities and liquid biopsy strategies could be a potential solution for tracking the tumor evolution over time and therapy. *Blood vessel image from Smart Servier.*

Table 1.

Summary of studies using circulating tumor cell-based analysis for GBM.

Author, Year	Biofluid	Methodology of CTC enrichment	Genetic cargo evaluated	Diagnostic sensitivity	Potential role
Sullivan, 2014	Blood	CTC-iChip microfluidic technology; characterization using antibody cocktail, STEAM: SOX2, tubulin-3, EGFR, A2B5 and cMET.	SERPINE1, TGFB1,TGFBR2,VIM; EGFR amplification	39%	Diagnosis/ Prognosis
Muller, 2014	Blood	Density gradient centrifugation followed by fluorescence immunocytochemistry using anti- GFAP antibody	EGFR amplification	20%	Diagnosis/ Prognosis
Macarthur, 2014	Blood	Density gradient centrifugation followed TERT promotor-based CTC detection assay	TERT	72%: pre-radiotherapy 8% post-radiotherapy	Prognosis/ Monitoring
Malara, 2016	Blood	Vimentin positive cell sorting and short time expansion	-	2/2	Prognosis/ Monitoring
Gao, 2018 *	Blood	CTCs detection based on the aneuploidy of chromosome 8 examination by CEP8-FISH	Chromosome 8 aneuploidy	24 of 31 (77%) GBM (82%)	Diagnosis/ Prognosis/ Monitoring
Krol, 2018 λ	Blood	Parsortix microfluidic system	SOX2	7/13(53.8%)	Diagnosis/ Prognosis/ Monitoring

Abbreviations.CEP8, Centromere Probe (CEP) 8; CTC, circulating tumor cells; EGFR, epidermal growth factor receptor; FISH, Fluorescence in situ Hybridization; GBM, Glioblastoma; GFAP, glial fibrillary acidic protein; SERPINE1, Serpin Family E Member 1; SOX2, SRY (sex determining region Y)-box 2; TERT, Telomerase reverse transcriptase; TGFB1, Transforming Growth Factor Beta 1; TGFBR2, Transforming Growth Factor Beta Receptor 2;

*These described cases in this series are not limited to GBM

λThe study evaluates for CTC clusters

Table 2.

Summary of studies using circulating free DNA-based analysis for GBM.

Author, Year	Biofluid	Methodology of cfDNA analysis	Genetic cargo evaluated	Diagnostic sensitivity	Potential role
Balana, 2003	Plasma	Methylation Specific PCR assay	MGMT methylation status	81%	Prognosis; Treatment selection
Liu, 2010 *	CSF, serum	Methylated DNA immunoprecipitation RT-PCR analysis	MGMT, p16INK4a, TIMP3, THBS1 promoter hypermethylation	CSF, 50% Serum 50%	prognosis
Lavon, 2010 *	Serum	Methylation Specific PCR assay	MGMT promoter methylation status	51%	Diagnosis
Boisselier. 2012 *	Plasma	DNA amplification by COLD PCR and further characterization by digital PCR	IDH1 mutation	60%	Diagnosis
Salkeni, 2013	Plasma	Long range PCR amplification	EGFRvIII deletion variant	23%	Monitoring
Majchrzak-Celińska, 2013 *	Serum	Methylation Specific PCR assay	MGMT, RASSF1A, p15INK4B, p14ARF promoter methylation	81%	Diagnosis
Bettegowda 2014 *	Plasma	Droplet digital PCR	TP53, EGFR, PTEN	<10%	Diagnosis
Wang, 2015 *	Serum CSF	Methylation Specific PCR assay	MGMT promotor methylation	Serum, 21% CSF, 43%	Prognosis
De Mattos-Arruda, 2015 *	CSF, Plasma	Targeted capture massively parallel sequencing	IDH1, TP53, PTEN, EGFR, FGFR2, ERBB2 mutations	-	Monitoring
Schwaederle, 2016 *	Plasma	Next generation sequencing	TP53, NOTCH1	27%	Molecular profiling, Prognosis
Juratli, 2018 *	CSF, Plasma	Nested PCR	TERT promoter mutations	CSF, 92% Plasma, 8%	Diagnosis
Piccioni. 2019 *	Plasma	Guardant360® cfDNA digital next generation sequencing assay	TP53, NF1, MET, APC, PDGFRA mutations MET, EGFR, ERBB2 amplifications	55%	Molecular profiling, treatment selection
Miller, 2019 *	CSF	Next generation sequencing	IDH1, IDH2, TP53 mutations; CDKN2A, CDKN2B deletions; EGFR amplification	49% (posttherapy)	Prognosis, Monitoring
Mouliere, 2019 *	CSF, Plasma Urine	Tumor-guided capture sequencing	Matched clonal and private mutations	CSF, 50% Plasma, 50% Urine, 13%	Diagnosis
Cordova, 2019	Plasma	Droplet digital PCR	TERT promoter mutations	46%	Monitoring

Abbreviations.APC, adenomatous polyposis coli; CDKN2A, Cyclin Dependent Kinase Inhibitor 2A, CDKN2B, Cyclin Dependent Kinase Inhibitor 2B; CfDNA, circulating free DNA; CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 Receptor Tyrosine Kinase 2; FGFR2, Fibroblast growth factor receptor 2; IDH, isocitrate dehydrogenase; MGMT, O(6)-Methylguanine-DNA methyltransferase; NF1, neurofibromatosis type 1; PDGFRA, platelet-derived growth factor receptor alpha; PTEN, Phosphatase and tensin homolog; RASSF1A, Ras association domain family 1 isoform A; RT-PCR, real time polymerase chain reaction; TERT, Telomerase reverse transcriptase; THBS1, Thrombospondin 1; TIMP3, TIMP Metallopeptidase Inhibitor 3.

* These described cases in this series are not limited to GBM.

Table 3.

Summary of studies using extracellular vesicle EV-based analysis for GBM.

Author, Year	Biofluid	Methodology of EVanalysis	Genetic cargo evaluated	Diagnostic sensitivity	Potential role
Skog, 2008	Serum	Nested RT-PCR EGFRvIII mRNA	EGFRvIII	28%	Diagnosis
Shao, 2012	Plasma	Micro nuclear magnetic resonance system chip based EV protein analysis	EGFRvIII, IDH1, PDPN proteins	68% (EGFRvIII, PDPN) 16% IDH1	Diagnosis
Chen & Balaj, 2013	CSF, Serum	BEAMing (beads, emulsion, amplification, magnetics) RT-PCR and ddPCR	IDH1 mutation	62.5% 0%	Diagnosis
Akers, 2013	CSF	RT-PCR	miR-21	85% initial cohort, 87% validation cohort	Diagnosis/ Monitoring
Manterola, 2014	Serum	RT-PCR	miR-320, miR-574–3p, RNU6–1 expression	miR-320, 65%, miR-574–3p, 59%, RNU6–1, 73%	Diagnosis
Koch, 2014	Plasma	Flow cytometry: size of 300 nm or greater and Annexin V positivity	-	-	Monitoring
Evans, 2016	Plasma	Flow cytometry: Annexin V positivity	-	-	Monitoring/ Prognosis
Garcia-Romero, 2017 *	Plasma	Fast Cold-PCR	IDH1 mutation	48%	Diagnosis
Galbo, 2017 *	Serum	Imaging flow cytometry-fluorescent labelled antibodies	CD9+/GFAP+/SVN+ EVs	-	Monitoring
Andre-Gregoire, 2018	Plasma	Tunable resistive pulse sensing analysis (TRPS)	-	-	-
Ricklefs, 2018 *	Plasma	Droplet PCR	PD-L1 DNA	67%	Monitoring
Manda, 2018 *	Serum	Semi-nested RT-PCR	EGFRvIII mRNA	82%	Diagnosis
Lan, 2018 *	Serum	RT-PCR	miR-301A	_	Prognosis/ Monitoring
Ebrahimkhani, 2018 *	Serum	Deep sequencing	miR-182–5p, miR-328–3p, miR-339–5p, miR-340–5p, miR-485–3p, miR-486–5p and miR-543	92% λ	Diagnosis
Santangelo, 2018 *	Serum	RT-PCR	miR-21, miR-222, miR-124–3p	miR-21, 84%, miR-222, 80% miR-124–3p 78%	Diagnosis/ Monitoring
Osti, 2019 *	Plasma	Nanoparticle tracking analysis, Mass spectrometry	Proteins: vWF, APCS, C4B, AMBP, APOD, AZGP1, C4BPB, Serpin3, FTL, C3, and APOE	-	Monitoring
Jones & Yekula, 2019	Plasma	Imaging flow cytometry based monitoring of PpIX positive EVs pre and post 5-ALA based fluorescent guided surgery	PpIX positive EVs	4 out of 4	Diagnosis/ Monitoring
Chandran, 2019	Plasma	Mass spectrometry, Nanoparticle tracking analysis, Electron microscopy	Levels of Syndecan 1	71%	Diagnosis/ Classification

Author, Year	Biofluid	Methodology of EVanalysis	Genetic cargo evaluated	Diagnostic sensitivity	Potential role
Ricklefs, 2019 *	Plasma	Imaging flow cytometry-fluorescent labelled antibodies	CD63+/CD81+ EVs	-	-

Abbreviations.AMBP, Alpha-1-Microglobulin/ Bikunin Precursor; APCS, Serum amyloid P component; APOD, Apolipoprotein D; APOE, Apolipoprotein E; AZGP1, Alpha-2-Glycoprotein 1; C3, complement C3; C4B, Complement C4B; C4BPB, Complement Component 4 Binding Protein Beta; CSF, cerebrospinal fluid; ddPCT, droplet digital PCR; EGFR, epidermal growth factor receptor; FTL, Ferritin Light Chain; IDH, isocitrate dehydrogenase; PDPN, podoplanin; PpIX, Protoporphyrin; RT-PCR, reverse transcriptase polymerase chain reaction; vWF, von Willebrand factor; 5-ALA, 5 Aminolaevulinic acid.

* These described cases in this series are not limited to GBM.