Prognostic Liquid Biopsy Biomarkers in Glioblastoma

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Background

Glioblastoma

- Glioblastoma (GBM): grade IV glioma and most common and aggressive form of malignant brain tumors with poor treatment response[1,2]
- Median overall survival: 14 months[3]
- Tissue biopsies currently gold-standard technique for GBM diagnosis and prognosis[4]
  - invasive, cannot be repeated over the course of treatment, and have a 5-10% morbidity associated[5-7]
- No approved circulating prognostic biomarkers for GBM[8] → problem for long-term decisions

Circulating Biomarkers

- Liquid biopsies: non-invasive and repeatable[7]
- Circulating biomarkers enable monitoring of tumor growth and response to treatment[4]
  - include circulating tumor cells, cell-free nucleic acids, proteins, and extracellular vesicles[7]

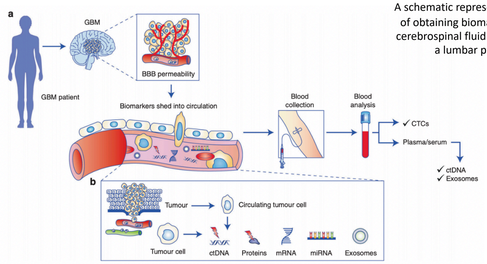
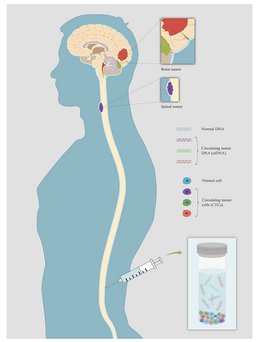


Figure 1.[7]
A schematic representation of biomarkers entering circulation through a leaky blood brain barrier (BBB) and analyzed through blood collection.

Figure 2.[20]
A schematic representation of obtaining biomarkers in cerebrospinal fluid through a lumbar puncture.



Blood-Brain Barrier and GBM

- Blood-brain barrier (BBB): protective barrier between blood vessels and the brain consisting of endothelial cells joined by tight junctions and other adjacent supporting cells[2,9]
  - function: regulate access and exchange of nutrients, vitamins, and other molecules into the brain[7]
- GBM-induced hypoxia → proangiogenic and inflamed microenvironment → damaged tight junctions and leaky BBB[2,7,10]
- However, intact BBB present in large parts of GBM → prevents efficient passage of therapeutics and limits detection of circulating biomarkers[9,11]
- Intact BBB → low yield of ctDNA in plasma[7]

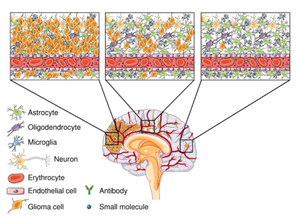


Figure 3.[9]
A visual representation of different degrees of BBB integrity and their effects on cancer therapeutics.

Methods

- Articles found via manual review using the PubMed and Google Scholar databases through search terms and sources referenced by other reviews
- Search terms: “circulating”, “biomarker”, “plasma”, “cerebrospinal fluid”, “glioblastoma”, “prognosis”, “ctDNA”, “exosomes”, “miRNA”, “microvesicles”, “proteins”

Literature Review Table

Source	n	Type of Liquid Biopsy	Biomarker	Methods	Findings
Miller et al. 2019[12]	95 glioma patients (46 GBM)	CSF (lumbar puncture)	ctDNA	▪ QIAsymphony DSP Virus/Pathogen Midi Kit or QIAsymphony DSP Circulating DNA Kit ▪ MSK-IMPACT	▪ CSF-ctDNA positivity associated with tumor progression, tumor burden, and shorter survival and was an independent prognostic factor (all $p < .001$, GBM IDH WT median OS: 2.04 months v. 9.89 months)
Juratli et al. 2018[13]	38 GBM	CSF (intraoperative collection)	ctDNA	▪ Quick-ctDNA Serum and Plasma Kit ▪ Qiagen Multiplex Kit ▪ Bio-Rad QX200 TERT C228T_113 Assay	▪ TERTp-VAF correlated with tumor volume ($p < .01$) ▪ high TERTp-VAF associated with poor survival ($p = .008$) ▪ TERTp-VAF was an independent prognostic factor for OS ($p = .001$)
Shnaper et al. 2009[14]	33 GBM	CSF (lumbar puncture)	Proteins	▪ enzyme-linked immunosorbent assay tests	▪ high MIC-1/GDF15 levels associated with poor survival ($p = .007$)
Gállego et al. 2014[5]	111 GBM	Plasma	Proteins	▪ enzyme-linked immunosorbent assay tests (ELISA)	▪ IGFBP-2 and GFAP levels correlated with tumor volume (IGFBP-2: $p = .025$, GFAP: $p < .001$) ▪ high IGFBP-2 levels associated with increased risk of progression and death (PFS: $p = 0.02$, OS: $p = .001$, median OS: 12.8 months v. 29.4 months)
Lin et al. 2013[15]	305 glioma patients (145 GBM)	Plasma	Proteins	▪ RayBiotech protein antibody array ▪ enzyme-linked immunosorbent assay tests (ELISA)	▪ IL-15, MCP-1, GDNF, IL-1R4/ST2 associated with poor prognosis and IGFBP-6, MIP-1δ, ICAM-3, IL-7, MIP-3β, and sgp130 associated with good prognosis (all $p < .05$) ▪ score from 4-cytokine panel of IL-7, IL-1R4/ST2, sgp130, and MCP-1 was an independent prognostic factor of OS ($p < .001$) ▪ high risk score in GBM associated with poor survival ($p = .001$)
Manda et al. 2018[6]	96 high-grade glioma patients (73 GBM)	Serum	Extracellular Vesicles (Exosomes)	▪ total exosome isolation kit ▪ RNeasy lipid tissue kit ▪ RT-PCR	▪ EGFRvIII expression in exosomes associated with poor survival (OS: $p = .005$)
Evans et al. 2016[16]	16 GBM	Plasma	Extracellular Vesicles (Microvesicles)	▪ serial centrifugation and stained for phosphatidylserine using Annexin V ▪ flow cytometry analysis	▪ slope and trend in the number of Annexin V positive MV associated with recurrence and death (recurrence: $p < .01$, death: $p < .01$)
Lan et al. 2018[17]	60 glioma patients (27 GBM)	Serum	Exosomal miRNA	▪ ExoQuick Precipitation Solution ▪ micro BCA assay ▪ mirVana miRNA isolation kit ▪ qRT-PCR	▪ high exosomal miR-301a levels associated with poor survival ($p < .01$) ▪ exosomal miR-301 levels were an independent prognostic factor for OS ($p < .01$)
Zhi et al. 2015[18]	90 astrocytoma patients (24 GBM)	Serum	miRNA	▪ mirVana miRNA isolation kit ▪ qRT-PCR	▪ high miR-19a-3p, miR-106a-5p, and miR-181b-5p levels associated with poor survival (miR-19a-3p: $p = .023$, miR-106a-5p: $p < .0001$, miR-181b-5p: $p = .004$) ▪ combined 3-miRNAs panel was an independent prognostic factor associated with decreased survival ($p = .018$)
Hagemann et al. 2019[19]	45 GBM	Plasma	mRNA	▪ high pure viral RNA kit ▪ qRT-PCR	▪ high MACC1 transcript levels associated with poor survival ($p = .008$, median OS: 8.1 months v. 14.5 months) ▪ MACC1 levels associated with prognosis in conjunction with IDH mutation status and treatment regimen

Discussion

- Findings gathered from sources reveal numerous candidate circulating biomarkers that can help clinicians improve accuracy of outcome prediction and define more precise risk categories of GBM patients
- More studies with larger cohorts needed to validate findings and define clinical characteristics of biomarkers

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References

[1]Zachariah MA, Oliveira-Costa JP, Carter BS, Stott SL, Nahed BV. Blood-based biomarkers for the diagnosis and monitoring of gliomas. *Neuro Oncol*. 2018;20(9):1155-1161. doi:10.1093/neuonc/noy074
[2]Dubois LG, Campanati L, Righy C, et al. Gliomas and the vascular fragility of the blood brain barrier. *Front Cell Neurosci*. 2014;8:418. Published 2014 Dec 12. doi:10.3389/fncel.2014.00418
[3]Johnson DR, O'Neill BP. Glioblastoma survival in the United States before and during the temozolomide era. J Neurooncol 2012;107:359–64
[4]Westphal M, Lamszus K. Circulating biomarkers for gliomas. *Nat Rev Neurol*. 2015;11(10):556-566. doi:10.1038/nrneurol.2015.171
[5]Gállego Pérez-Larraya J, Paris S, Idbaih A, et al. Diagnostic and prognostic value of preoperative combined GFAP, IGFBP-2, and YKL-40 plasma levels in patients with glioblastoma. *Cancer*. 2014;120(24):3972-3980. doi:10.1002/cncr.28949
[6]Manda SV, Kataria Y, Tatireddy BR, et al. Exosomes as a biomarker platform for detecting epidermal growth factor receptor-positive high-grade gliomas. J Neurosurg. 2018;128(4):1091-1101. doi:10.3171/2016.11.JNS161187
[7]Müller Bark J, Kulasinghe A, Chua B, Day BW, Punyadeera C. Circulating biomarkers in patients with glioblastoma. *Br J Cancer*. 2020;122(3):295-305. doi:10.1038/s41416-019-0603-6
[8]Petrescu GED, Sabo AA, Torsin LI, Calin GA, Dragomir MP. MicroRNA based theranostics for brain cancer: basic principles. *J Exp Clin Cancer Res*. 2019;38(1):231. Published 2019 May 29. doi:10.1186/s13046-019-1180-5
[9]van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. *Drug Resist Updat*. 2015;19:1-12. doi:10.1016/j.drup.2015.02.002
[10]Zhao C, Wang H, Xiong C, Liu Y. Hypoxic glioblastoma release exosomal VEGF-A induce the permeability of blood-brain barrier. *Biochem Biophys Res Commun*. 2018;502(3):324-331. doi:10.1016/j.bbrc.2018.05.140
[11]Hafeez U, Cher LM. Biomarkers and smart intracranial devices for the diagnosis, treatment, and monitoring of high-grade gliomas: a review of the literature and future prospects. *Neurooncol Adv*. 2019;1(1):vdz013. Published 2019 Jul 8. doi:10.1093/noajnl/vdz013
[12]Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature. 2019;565(7741):654-658. doi:10.1038/s41586-019-0882-3
[13]Juratli TA, Stasik S, Zolal A, et al. *TERT* Promoter Mutation Detection in Cell-Free Tumor-Derived DNA in Patients with *IDH* Wild-Type Glioblastomas: A Pilot Prospective Study. *Clin Cancer Res*. 2018;24(21):5282-5291. doi:10.1158/1078-0432.CCR-17-3717
[14]Shnaper S, Desbaillets I, Brown DA, et al. Elevated levels of MIC-1/GDF15 in the cerebrospinal fluid of patients are associated with glioblastoma and worse outcome. *Int J Cancer*. 2009;125(11):2624-2630. doi:10.1002/ijc.24639
[15]Lin Y, Zhang G, Zhang J, et al. A panel of four cytokines predicts the prognosis of patients with malignant gliomas. *J Neurooncol*. 2013;114(2):199-208. doi:10.1007/s11060-013-1171-x
[16]Evans SM, Putt M, Yang XY, et al. Initial evidence that blood-borne microvesicles are biomarkers for recurrence and survival in newly diagnosed glioblastoma patients. *J Neurooncol*. 2016;127(2):391-400. doi:10.1007/s11060-015-2051-3
[17]Lan F, Qing Q, Pan Q, Hu M, Yu H, Yue X. Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma. *Cell Oncol (Dordr)*. 2018;41(1):25-33. doi:10.1007/s13402-017-0355-3
[18]Zhi F, Shao N, Wang R, et al. Identification of 9 serum microRNAs as potential noninvasive biomarkers of human astrocytoma. *Neuro Oncol*. 2015;17(3):383-391. doi:10.1093/neuonc/nou169
[19]Hagemann C, Neuhaus N, Dahlmann M, et al. Circulating MACC1 Transcripts in Glioblastoma Patients Predict Prognosis and Treatment Response. *Cancers (Basel)*. 2019;11(6):825. Published 2019 Jun 13. doi:10.3390/cancers11060825
[20]Yan W, Xu T, Zhu H, Yu J. Clinical Applications of Cerebrospinal Fluid Circulating Tumor DNA as a Liquid Biopsy for Central Nervous System Tumors. *Onco Targets Ther*. 2020;13:719-731. Published 2020 Jan 23. doi:10.2147/OTT.S229562