Gliomas are the most common type of primary brain tumor and have an invariable fatal outcome and dismal prognosis. Each year, ∼200 000 patients are diagnosed with a glioma worldwide.1 Gliomas are subdivided into astrocytoma, oligodendroglioma, and oligoastrocytoma based on immunophenotypical similarity to a cell of putative origin. The tumors are assigned malignancy grades according to WHO criteria.2 Gliomas usually recur and tend to increase in malignancy grade over time (Fig. 1). Glioma progression is accompanied by extensive neovascularization, and the newly formed blood vessels are leaky, which is reflected by tumor enhancement on MRI. Glioblastomas (GBMs) represent astrocytomas of the highest malignancy grade (WHO grade IV) and are the most common gliomas and the most aggressive primary brain tumors in adults, with a median survival of only 14.6 months.3,4 Various molecular aberrations of gliomas (eg, the combined loss of chromosome arms 1p and 19q, the presence of isocitrate dehydrogenase 1 (IDH1) mutation, epidermal growth factor receptor (EGFR) amplification, copy number aberrations of chromosomes 7 and 10, and MGMT promoter hypermethylation) harbor diagnostic, prognostic, or predictive information.5–13 Various molecular aberrations of gliomas (eg, the combined loss of chromosome arms 1p and 19q, the presence of isocitrate dehydrogenase 1 (IDH1) mutation, epidermal growth factor receptor (EGFR) amplification, copy number aberrations of chromosomes 7 and 10, and MGMT promoter hypermethylation) harbor diagnostic, prognostic, or predictive information.5–13 The molecular tests are carried out on tumor biopsies or resection specimens. Mutant IDH1, MGMT promoter methylation, and loss of 1p and 19q can also be detected in serum and cerebrospinal fluid (CSF) of glioma patients, and efforts to trace these aberrations in circulating tumor cells or circulating DNA are ongoing.9–13

Therapeutic modalities for gliomas include surgical resection, radiotherapy, and chemotherapy. The gold standard for measuring the effects of treatment in patients with gliomas is the application of the Response Assessment in Neuro-Oncology (RANO) criteria to the radiological appearance of the tumor on MRI.14,15 There is considerable variation in the radiological presentation of gliomas and their recurrences.16 A notorious problem in measuring the effects of treatment is so-called pseudoprogression, a treatment-related response of brain tissue to chemotherapy and radiation. Glioma pseudoprogression causes an increase in enhancement and edema on MRI that mimics true tumor progression.17,18 This condition is probably induced by treatment-related local inflammation, resulting in edema and increased abnormal vessel permeability. There is a need for diagnostic discrimination because combined chemotherapy and radiation (the standard of care for GBM) may induce pseudoprogression in ∼30% of cases.19,20 Unfortunately, there are no reliable radiological techniques to distinguish between pseudoprogression and tumor recurrence or progression.21,22 The identification of proliferating tumor cells in tissue biopsies taken in situations of pseudoprogression may be troublesome, and the significance of the presence of scattered cells with morphological or molecular characteristics of the original lesion is disputable (Fig. 2).

Validated biomarkers for patients suffering from gliomas are urgently needed for standardizing measurements of the effects of treatment in daily clinical practice and trials. Circulating body fluids offer easily accessible sources for such markers. This review highlights various categories of tumor-associated circulating biomarkers identified in blood and cerebrospinal fluid of glioma patients, including circulating tumor cells, exosomes, nucleic acids, proteins, and oncometabolites. The validation and potential clinical utility of these biomarkers is briefly discussed. Although many candidate circulating protein biomarkers were reported, none of these have reached the required validation to be introduced for clinical practice. Recent developments in tracing circulating tumor cells and their derivatives as exosomes and circulating nucleic acids may become more successful in providing useful biomarkers. It is to be expected that current technical developments will contribute to the finding and validation of circulating biomarkers.

**Keywords:** biomarker, blood, cerebrospinal fluid, circulating tumor cell, nucleic acid, exosome, glioma, Omics.
Currently, there are no biomarkers or radiographic or clinical modalities to reliably distinguish glioma recurrence from radiation necrosis or to monitor tumor response to therapy. Objective measurable parameters for the presence of tumor, tumor activity, and response to treatment would be a welcome addition to the currently available diagnostic arsenal.

Recently, advances in “omics” based technologies, including genomics, transcriptomics, proteomics, and metabolomics, have led to an explosion of activity in the field of biomarker research, particularly related to cancer. The general definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”.23,24 Cancer biomarkers include a broad range and level of biochemical entities such as nucleic acids, proteins, sugars, lipids, and small metabolites, and cytogenetic and cytokinetic parameters as well as whole tumor cells and exosomes (microparticles). The advantage of biomarkers present in blood or CSF is their relatively easy accessibility, which facilitates repetitive measurements with obviously better monitoring of disease.

In order to evaluate and compare tumor biomarkers, the “Tumor Marker Utility Grading System” has been proposed by the National Comprehensive Cancer Network (NCCN).25,26 In this system, potential tumor markers are evaluated for their diagnostic, prognostic, or predictive performance as reflected by overall survival, disease-free survival, quality of life, or cost of care.25–27 In the NCCN system, levels of evidence have been applied to several potential glioma biomarkers including IDH1 mutation, MGMT methylation, loss of 1p/19q, BRAF fusion, and CpG island methylator phenotype (CIMP).26,27 Among these biomarkers, only 1p/19q testing has been credited for the highest level of evidence because of its ability to improve clinical decision-making and predict patient outcome (IA level).26,27

Because of its anatomical proximity to the CNS, CSF is a promising source of biomarker discovery for diseases of the...
CNS. CSF samples are used for traditional cytology and have also been recently used for detecting brain metastasis by employing sensitive techniques such as flow cytometry, fluorescence in-situ hybridization (FISH), and PCR/reverse transcription PCR. The relative low protein concentration of CSF (100–400 times lower as compared with serum) allows rapid screening, low sample consumption, and accurate identification or profiling by proteomic technologies, which have been facilitated by the recent publication of the normal human CSF proteome. Under pathological conditions, one may find altered levels of normal constitutive proteins or proteins that are usually absent from normal CSF. These proteins may have entered the CSF due to disruption of the blood–brain barrier or intrathecal secretion, or shedding by tumor cells of primary brain tumor or metastasis, and/or their microenvironment. By now, various candidate protein biomarkers for gliomas have been found in CSF.

In this review, different categories of tumor-associated circulating biomarkers, which have been identified in blood and CSF of glioma patients, are addressed including circulating tumor cells (CTCs), exosomes (microvesicles), circulating nucleic acids (DNA, RNA and miRNA), proteins, and metabolites.

Circulating Tumor Cells
CTCs are detected when diagnosing metastatic disease or tumor recurrence and may be used to monitor disease progression and therapeutic response. CTCs are found in the peripheral blood of patients with advanced stages of solid cancers with or without clinically detectable metastasis. It has been shown that the presence of CTCs is related to tumor response, progression-free survival, and overall survival in patients suffering from various tumor types and that the presence of CTCs may hint at the existence of a hitherto undiscovered primary tumor. Only one cell per 10^8 cells represents a CTC in the blood of patients with metastatic cancer, and the specificity and sensitivity of CTC detection is a technical challenge. Various detection technologies have been recently developed including microchips, filtration devices, quantitative reverse-transcription PCR assays, automated microscopy systems, and telomerase promoter-based assays. CTCs are particularly valuable for tumor characterization in situations in which tissue biopsies are unavailable or the collected tissue is of poor quality and/or insufficient quantity. To what extent CTCs represent the cell population in the solid tumor part remains questionable. Because CTCs reflect the molecular heterogeneity of the tumor, they are important for therapeutic strategies. CTCs are probably not only released from the primary tumor but also from metastatic sites. However, most cancer cells are rapidly destroyed in the circulation, and the metastatic potential of CTCs seems limited. Current clinical investigations of CTCs focus on their molecular characterization and the classification of heterogeneous subsets in relation to treatment resistance. CTCs are subjects of investigations in basal processes of epithelial-mesenchymal transition (EMT), collective cell migration and more, with the aim being to better understand the mechanisms of tumorigenesis, invasion, and metastasis.

Until recently, the spread of glial tumor cells outside the brain was considered to be a rare event. However, many isolated cases of metastasizing glial neoplasms have been reported. Several cases of transmission of metastatic GBM from donor to organ transplant recipients further supports the notion that appearance of glial cells in the circulation is not as rare as previously believed and that occurrence rates may match those of other solid tumors. Novel sensitive imaging techniques contribute to higher detection rates. So far, data on circulating CTCs associated with brain tumors are limited, and the use of CTCs as biomarkers in glioma patients is just beginning. The identification of CTCs was carried out by using markers for neural lineage in one study, and a telomerase promoter-based assay was used for the detection of these cells in another study (Table 1). There are limitations in the use of lineage markers for the identification of CTCs because of overlap in marker expression of tumor cells and normal cells. Further characterization of CTCs at the DNA, RNA, or protein level will improve the identification of true CTCs and their subfractions from other circulating cells.

Circulating Tumor-derived Exosomes
Exosomes (microvesicles or extracellular vesicles) are 30–100 nm in diameter and are released into the microenvironment of cells or into surrounding body fluids by both normal and cancer cells, where they perform a variety of functions. Exosomes can be taken up by particular host cells and thus provide signaling between various cell types including cancer cells. Circulating tumor-derived exosomes contain a variable spectrum of molecules representative of the parental cells including proteins, nucleic acids, lipids, metabolites, and other molecules. Cancer cell exosomes carry molecular signatures and effectors of diseases such as mutant oncoproteins, oncogenic transcripts, microRNA, and DNA sequences. Their contents can help identify the cells of origin for the exosomes, thereby offering the opportunity to identify biomarkers or therapeutic targets in body fluids. Circulating exosomes in the body fluids of brain tumor patients may be used to decipher molecular features of the neoplasms or measure their responses to therapy. So far, various tumor-related molecules with altered expression patterns have been found in circulating exosomes of glioma patients including EGFRVIII, EGFR, podoplanin (PDPN), phosphatase and tensin homolog (PTEN), miR-21, and mutant IDH1 mRNA (Table 1). Exosomes may carry substantial amounts of bound antibody-recognizing tumor antigens (autoantibodies), which can be used to reveal the presence of tumor antigens; exosome-based immunotherapy is under development. Although exosomes are promising targets of biomarker research, their tracing and quantification in clinical samples remain challenging. New technologies, such as ExoScreen, are being developed for eventual clinical use.

Circulating Tumor-associated Nucleic Acids
Circulating nucleic acids (CNAs) have been identified in blood and other bodily fluids of patients with various diseases. CNAs are promising targets for development as tumor biomarkers (circulating tumor-associated nucleic acids [ctNAs]) because of the possibility to profile tumors at the genomic and transcriptomic levels. Nucleic acids appear in body fluids...
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Abbreviations: AII, astrocytoma WHO grade 2; AIII, astrocytoma WHO grade III; AOA, anaplastic oligoastrocytoma; BEAMing, beads, emulsion, amplification, magnets; CSF, cerebrospinal fluid; CTC, circulating tumor cell; DAPK, death-associated protein kinase 1; ddPCR, droplet digital PCR; EGFvIII, endothelial growth factor variant III; GBM, glioblastoma (or astrocytoma WHO grade IV); Glioma NOS, glioma not otherwise specified; HGG, high-grade glioma; IDH1, isocitrate dehydrogenase 1; MeDIP, methylated DNA immunoprecipitation; Methyl, methylation; MGMT, O6-alkylguanine DNA alkyltransferase; miR-, microRNA; NA, not available; OII, oligodendroglioma WHO grade II; OIII, oligodendroglioma WHO grade III; PTEN, phosphatase and tensin homolog; qRT-PCR, real-time reverse-transcription PCR; RARbeta, retinoic acid receptor beta; RASSF1A, Ras association domain-containing protein 1A; THBS1, thrombospondin1; TIMP-3, metalloproteinase inhibitor 3; TPBA, telomerase promoter-Based Assay; intraop, intraoperative.

All studies listed in Table 1 were observational studies.
as a sequel of apoptotic tumor cells or tumor necrosis, but they may also be actively secreted into the circulation. Levels of CNAs are influenced by many factors: the turnover of (tumor) cell populations, cell degradation rates, filtering processes present in the blood or lymphatic circulation, clearance by liver and kidney, infection, age, sex, treatment, stress on epigenetic mechanisms, diet, lifestyle, and more. Although nucleic acids are valuable as biomarkers because they can be measured in sensitive high-throughput PCR detection assays, the identification, quantitation, and validity of ctRNAs remain challenging. In order to link the presence of ctDNA, circulating tumor-associated RNA (ctRNA), or circulating tumor-associated microRNA (ctmiRNA) in body fluids of cancer patients to tumor-specific molecular events, the preanalytic conditions must be well defined and standardized.

Circulating Tumor-associated DNA

cDNA may harbor the genetic and epigenetic aberrations present in tumors and their metastases including point mutations, rearrangements, amplifications, and aneuploidy. The aberrations may be highly specific for an individual tumor and may also represent its molecular heterogeneity. ctDNAs have been detected in patients with tumors of breast, bladder, colon, liver, lung, ovaries, pancreas, and prostate as well as non-Hodgkin's lymphoma and melanoma. ctDNAs have also been detected in glioma patients, and the aberrations found included IDH1 mutation, loss of heterozygosity for 1p, 10q, 19q12, EGFRvIII mutation, as well as abnormal methylation of the promoters of MGMT, DAPK, RASSF1A, p73, RARβ, PTEN, p15, p16INK4B, and p14ARF. At this point, the clinical utility has not been validated for any of the candidate ctDNAs as biomarkers for glioma patients. Prospective settings are needed for clinically applicable tests.

Circulating tumor-associated RNA and microRNA

The characterization of ctRNAs has not been explored to the extent of ctDNA, and the tumor specificity of these CNAs has been challenged more vigorously. One reason is that cell-free RNA is prone to degradation by the ubiquitous presence of RNA-degrading enzymes, which are generally elevated in the serum of cancer patients. Extracellular RNA is usually present in the exosomes. Aberrant RNA expression has been associated with stage, progression, and spread of various cancer types. In patients with gliomas, exosomes and platelets have been used as sources for detecting tumor-associated RNA profiles, among which are mutant EGFRvIII and mutant IDH1. As with the ctDNAs, the ctRNAs have also not been validated as biomarkers for introduction into clinical practice.

miRNAs (miRNAs) are noncoding, single-stranded RNAs of ~22 nucleotides that constitute a novel class of gene regulators and function as tumor suppressors and oncogenes. Because miRNAs, unlike RNA, are relatively stable and are present in blood and other bodily fluids, they are potential tumor biomarkers and may be more sensitive and specific for detecting tumors than currently available methods for early diagnosis of cancer. The peripheral blood contains large amounts of stable miRNAs derived from various tissues, and alterations in these miRNAs have been reported for many tumors including gliomas. Deviant miRNA expression patterns in the blood of glioma patients include miR-10b, miR-15b, miR-17–5p, miR-20a, miR-21, miR-23a, miR-31, miR-106, miR-128, miR-133a, miR-146b, miR-148a, miR-150, miR-193a, miR-197, miR-200b, miR-221, miR-222, miR-342–3p, miR-497, and miR-548b-5p. Some significant technological pitfalls and limitations need to be addressed before the miRNAs can be introduced as clinically applicable glioma biomarkers.

Circulating Tumor-associated Protein Biomarkers

Efforts have been made over the last decades to identify candidate protein biomarkers for gliomas that could be measured in body fluids (eg, urine, serum/plasma, or CSF) for making a diagnosis, detecting recurrence, or monitoring tumor activity following therapy (Table 2). Recent advances in proteomics have led to an explosion of activity in the field of biomarker research, particularly that related to cancers. The identification of biomarkers in body fluids such as serum is difficult due to the large dilution factor and the abundance of other constitutive serum proteins. Sample enrichment is necessary to enhance the sensitivity, and extensive validation of the methodology is necessary to ensure the specificity of candidate biomarkers. Several reports on the analysis of the glioma proteome, in which tumor tissue, serum, plasma, CSF or cyst fluids have been implicated, have also been made to identify biomarkers by using xenografts in animal models.

Growth Factors and Angiogenesis-related Biomarkers

Vascular Endothelial Growth Factor

Gliomas are highly vascularized tumors, and the process of angiogenesis is progressive throughout tumor development. Obviously, the newly formed vessels are attractive targets for antiangiogenic therapy. Vascular endothelial growth factor (VEGF) is a key molecule for triggering the process of angiogenesis in pathological conditions including neoplasms. Tumor hypoxia due to increased cell density triggers the angiogenic switch by upregulating VEGF. Given the importance of VEGF in tumor angiogenesis, several drugs to suppress VEGF signaling have been developed. Bevacizumab is the most well-characterized anti-angiogenic drug currently being used for the treatment of human GBM. Bevacizumab is a humanized monoclonal antibody that binds to circulating VEGF and prevents its interaction with the VEGF receptor suppressing VEGF signaling. Antiangiogenic strategies are targeted to endothelial cells, although the main sources of VEGF are the glial tumor cells. Patients usually become resistant to anti-VEGF therapy after an initial response due to various compensation mechanisms. VEGF has been considered to be a potential protein biomarker in CSF and serum/plasma of glioma patients, and its elevated levels have appeared to correlate with the microvascular density of the tumors. Because VEGF levels of serum are also
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<td>Obser</td>
<td>Serum</td>
<td>Pretreatment</td>
<td>ELISA</td>
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<td>AGG</td>
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<td>Obser</td>
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<td>ELISA</td>
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<td>VEGF</td>
<td>Obser</td>
<td>CSF</td>
<td>Pre/Intraoperative</td>
<td>ELISA</td>
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<td>AGG</td>
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<td>NA</td>
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<td>ELISA</td>
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<td>NA</td>
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Abbreviations: AGG, all grade of gliomas; AII, astrocytoma WHO grade 2; AIII, astrocytoma WHO grade III; AACT, alpha-1-antichymotrypsin; ABL, N-terminal residue of albumin; AHSG, 2-Heremans-Schmid glycoprotein; Ang2, angiopoietin2; APRIL, a proliferation-inducing ligand; CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; FGF-ß, basic fibroblast growth factor; GBM, glioblastoma; GFAP, glial fibrillary acidic protein; HGG, high-grade glioma; IGFBP-2, insulin-like growth factor-binding protein 2; IGFBP-5, insulin-like growth factor-binding protein 5; intraop, intraoperative; L-CaD, low molecular; LGG, low-grade glioma; MIF, macrophage migration inhibitory factor; MMP2/9/10, matrix metalloproteinase-2/9/10; NCAM, neural cell adhesion molecule; NOS, not otherwise specified; Obser, observational studies; OPN, osteopontin; PAI-1, plasminogen activator inhibitor 1; PBEF1, pre-B-cell colony enhancing factor 1; PDGF, platelet-derived growth factor; PlGF, placental growth factor; SDF-1α, stromal cell-derived factor 1; Tie2, angiopoietin receptors; TTHY, transthyretin; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; Wb, Western blot; YKL-40, (tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight).
increased in other systemic cancers, including breast cancers,\textsuperscript{183,185} lung cancer,\textsuperscript{166,167} and colon cancer,\textsuperscript{188,189} they are not specific to glial tumors. In several clinical studies, it was demonstrated that the serum VEGF levels of cancer patients, including gliomas, remained significantly high following surgery, radiotherapy, or chemotherapy.\textsuperscript{179,191} Moreover, particular inhibitors of the VEGF receptor tyrosine kinases induce increased levels of serum VEGF.\textsuperscript{192} Taken together, the value of VEGF as a biomarker has not been established in glioma.

**Other Growth Factors and Angiogenesis-associated Molecules**

Aside from VEGF, various growth factors and other angiogenesis-associated molecules have been used to monitor the effects of antiangiogenic therapy in gliomas.\textsuperscript{38,138,155,168,193,194} Growth factors are potential targets for therapeutic strategies because they are essential for tumor progression. Changes in plasma placental growth factor, basic fibroblast growth factor (FGF-B), soluble VEGF receptor 1, soluble VEGF receptor 2, stromal cell-derived factor-1alpha (SDF-1alpha), and soluble Tek/Tie2 receptor were all used to monitor the effects of cediranib (a pan-VEGF receptor tyrosine kinase inhibitor) in several clinical studies.\textsuperscript{168,193,194} All of these molecules reportedly correlated with radiological response and overall survival.\textsuperscript{194} FGF-B levels related to tumor progression and overall survival were also evaluated apart from the cediranib trial.\textsuperscript{38,138} The factors or molecules evaluated in more than one study include platelet-derived growth factor (PDGF)\textsuperscript{41,150,182} insulin-like growth factor binding protein 2 (IGFBP-2),\textsuperscript{149,195} and angiopoietin2 (Ang2)\textsuperscript{182,194} (Table 1). Endothelial nitric oxide synthase (eNOS), a specific isofrom of the nitric oxide-producing enzyme of endothelial cells (ECs), is a well-characterized marker of ECs.\textsuperscript{196} This molecule is activated in the process of angiogenesis and vasculogenesis and plays an intimate role in VEGF signaling.\textsuperscript{197} The blood level of eNOS largely reflects the activity of cells with endothelial lineage.\textsuperscript{196,197} The expression of low molecular isoform of caldesmon (l-Cad), a cytoskeleton-associated protein, was also increased in CSF\textsuperscript{20} and serum\textsuperscript{198} in glioma patients. The expression of l-Cad in blood vessels was further confirmed in tissue sections of glioma patients.\textsuperscript{199,200} A zebrafish l-Cad knockdown model further confirmed that this molecule plays a crucial role in vasculogenesis and angiogenesis in vivo.\textsuperscript{201} So far, the performance of l-Cad and eNOS as biomarkers for glioma has not been tested in additional studies.

**Matrix Metalloproteinase**

Matrix metalloproteinases (MMPs) represent a family of degrading enzymes involved in the breakdown of extracellular matrices necessary for invasion of tumor cells.\textsuperscript{211,212} The zinc- and calcium-dependent MMP family also plays a role in various physiological processes such as embryonic development, angiogenesis, wound healing, and more.\textsuperscript{212,213} MMPs comprise a relatively large and ever-growing family, and more than 20 enzymes are now known.\textsuperscript{214} MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are the most abundant MMPs in malignant gliomas.\textsuperscript{214–216} In glial tumors, MMP-9 in particular enables tumor cells to migrate or infiltrate, and its level is upregulated by tumor cells to the surrounding brain tissue. Among the many proteins that serve in this context are thrombospondin-1 and-2 (TSP1, TSP2), tenascin-C (TNC), secreted protein acidic and rich in cysteine (SPARC), osteopontin (OPN), angiopoietin-like protein 4 (ANGPTL4), CCN family members cysteine-rich angiogenic inducer 61 (Cyr61/CCN1) and CCN6, peristin, and more.\textsuperscript{204} Some of these proteins have been scrutinized for their value as glioma biomarkers in CSF and serum (eg, OPN\textsuperscript{30,32,207} and tenascin).\textsuperscript{43} CSF levels of tenascin were reportedly higher in anaplastic gliomas as compared with astrocytomas of lower malignancy.\textsuperscript{43} Increased expression of OPN has been associated with the presence of a variety of cancers including breast cancer, ovarian cancer, melanoma, and glioblastoma.\textsuperscript{208} The presence of metastases was also found to be associated with high OPN levels.\textsuperscript{209,210} CSF and serum levels of OPN appeared to be higher in patients with gliomas as compared with patients with other primary brain tumors or systemic cancers, and the levels were associated with worse outcomes.\textsuperscript{30,33,207} However, no correlation between the radiographic properties of the tumor and the OPN level was found. Interestingly, significant differences in OPN levels between patients with gliomas of WHO grades II, III, and IV were found, and the survival times of patients with high serum OPN levels (>20 ng/mL) appeared to be significantly shorter than those of patients with low OPN levels. Postoperative levels of OPN were, however, not measured in these studies.\textsuperscript{30,33,207} So far, the value of OPN for monitoring treatment response is unclear. Since OPN levels were reportedly higher in CSF of patients with atypical teratoid/rhabdoid tumors\textsuperscript{211} and other tumors,\textsuperscript{208} OPN is not specific for glioma and cannot be used as a diagnostic biomarker.

**Matricellular Proteins**

The group of matricellular proteins consists of structurally diverse glycoproteins that are secreted by tumor cells and neighboring stromal cells.\textsuperscript{204–206} These proteins are secreted into the extracellular environment, and they interact with cell-surface receptors, proteases, hormones, and structural matrix proteins such as collagens.\textsuperscript{205} Matricellular proteins are also involved in various aspects of tumor biology such as EMT, angiogenesis, cell proliferation and survival, motility, and ECM degradation.\textsuperscript{204–206} Glial tumor cells need to break down the environmental substances in order to infiltrate diffusely into the surrounding brain tissue. Among the many proteins that serve in this context are thrombospondin-1 and-2 (TSP1, TSP2), tenascin-C (TNC), secreted protein acidic and rich in cysteine (SPARC), osteopontin (OPN), angiopoietin-like protein 4 (ANGPTL4), CCN family members cysteine-rich angiogenic inducer 61 (Cyr61/CCN1) and CCN6, peristin, and more.\textsuperscript{204} Some of these proteins have been scrutinized for their value as glioma biomarkers in CSF and serum (eg, OPN\textsuperscript{30,32,207} and tenascin).\textsuperscript{43} CSF levels of tenascin were reportedly higher in anaplastic gliomas as compared with astrocytomas of lower malignancy.\textsuperscript{43} Increased expression of OPN has been associated with the presence of a variety of cancers including breast cancer, ovarian cancer, melanoma, and glioblastoma.\textsuperscript{208} The presence of metastases was also found to be associated with high OPN levels.\textsuperscript{209,210} CSF and serum levels of OPN appeared to be higher in patients with gliomas as compared with patients with other primary brain tumors or systemic cancers, and the levels were associated with worse outcomes.\textsuperscript{30,33,207} However, no correlation between the radiographic properties of the tumor and the OPN level was found. Interestingly, significant differences in OPN levels between patients with gliomas of WHO grades II, III, and IV were found, and the survival times of patients with high serum OPN levels (>20 ng/mL) appeared to be significantly shorter than those of patients with low OPN levels. Postoperative levels of OPN were, however, not measured in these studies.\textsuperscript{30,33,207} So far, the value of OPN for monitoring treatment response is unclear. Since OPN levels were reportedly higher in CSF of patients with atypical teratoid/rhabdoid tumors\textsuperscript{211} and other tumors,\textsuperscript{208} OPN is not specific for glioma and cannot be used as a diagnostic biomarker.
that leukocytes secrete MMP-9, and increased numbers of leukocytes in CSF or serum can cause increased levels of MMP-9. Serum or CSF levels of MMP-9 in glioma patients may therefore be influenced by concomitant inflammation. Because MMPs are crucial for angiogenesis, tumor invasion, tumor growth, and metastatic potential, MMPs are promising targets for potential therapies.

**Proteins Associated With Cell Lineage**

**Glial Fibrillary Acidic Protein**

Glial fibrillary acidic protein (GFAP) is an intermediate filament-associated protein, and its immunohistochemistry is used for revealing astrocytic lineage of glial cells and glial tumor cells. Serum levels of GFAP have been analyzed in several clinical studies and were significantly elevated in high-grade gliomas, as compared with those of nonglial tumors, with 100% specificity for the diagnosis of gliomas. Jung et al prospectively examined 50 patients with GBM, 18 with anaplastic gliomas, 13 with low-grade gliomas, 17 with a single cerebral metastasis, and 50 healthy controls. Serum GFAP levels were measured by ELISA and were detectable in 40 of the 50 GBM patients (median, 0.18 µg/L; range, 0–5.6 µg/L). Only 2 patients with gliomas of low malignancy grade had detectable serum GFAP levels. Serum GFAP levels in patients with metastases and healthy people were below the detection limit (<0.012 µg/L). The GFAP serum levels correlated with both tumor volumes and estimated volumes of tumor necrosis in the GBM patients. Brommeland et al found GFAP serum levels with a broad range from 30–1210 ng/L (mean, 239 ng/L) and demonstrated a significant association between preoperative serum GFAP levels and tumor volume in 31 high-grade glioma patients by using ELISA. An ELISA has been recently developed for an autoantibody to GFAP, to be used for detection of glioma. The GFAP serum level may well become a useful protein biomarker. At this point, GFAP should be validated in appropriate clinical studies.

**Embryonic Antigens**

Carcinoembryonal antigen (CEA), human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) are useful markers for the differential diagnosis between primary brain tumors and metastases or germ cell tumors (GCTs). The cell adhesion molecule CEA is an embryonic antigen and is produced in gastrointestinal tissues during fetal development. CEA is an embryonic antigen and is produced in gastrointestinal tissues during fetal development. Levels of CEA were monitored in the serum, CSF, plasma, and tumor cyst fluid of patients with primary brain tumors and cerebral metastases. CEA levels in patients with cerebral metastases and leptomeningeal dissemination were consistently higher than those in patients with primary brain tumors. In a study with postoperative follow-up, it was found that patients with metastatic brain tumors and leptomeningeal tumor spread showed high levels of CEA in CSF preoperatively (which normalized following surgery). These data support the use of CEA levels in CSF for the differential diagnosis of primary and metastatic brain tumors. The detection of hCG in serum or CSF supports the diagnosis of intracranial GCTs and proves the presence of trophoblastic cells.

**Miscellaneous Proteins and Circulating Oncometabolites**

**2-hydroxyglutamate**

Cancer-associated IDH mutations produce the metabolite 2-hydroxyglutarate (2HG). Circulating levels of 2HG are significantly elevated in patients with cholangiocarcinoma and acute myeloid leukemia. In a recent study, it was reported that the concentration of the metabolite 2HG in serum from glioma patients did not correlate with the IDH1 mutational status or the size of the tumor. More clinical studies are required to evaluate the clinical utility of 2HG.

**YKL-40**

YKL-40 (tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight) is also known as chitinase-3-like-1 or human cartilage glycoprotein-39. The value of YKL-40 as a serum marker was evaluated during a follow-up period of 27 months after surgery for high-grade glioma. Levels of YKL-40 were significantly correlated with radiographic evidence of disease and survival times in GBM (n = 76) and anaplastic glioma (n = 66). In a prospective study in which 1740 MRI matched serum samples of 343 anaplastic glioma patients were implicated, the YKL-40 levels appeared to be significantly lower in patients with no radiographic tumor progression as compared with patients with progressive disease. Increases in YKL-40 levels were also associated with worse survival. In various other clinical studies, serum YKL-40 levels of glioma patients were also elevated and correlated with radiographic evidence of disease and worse overall survival. Since serum levels of YKL-40 are also correlated with poor outcome in various cancers, additional validation studies need to be done, focusing on the specificity of YKL-40 and testing of its value as a glioma biomarker in prospective, controlled settings.
Other proteins
Various other proteins, which are not discussed here, are listed in Table 1 and include galectin-1, nerve growth factor (NGF), macrophage migration inhibitory factor (MIF), alpha-1-antichymotrypsin (AAT), transhydrogen (THY), gelsolin, 2-Heremans-Schmid glycoprotein (AHSG), Pre-B-cell colony enhancing factor 1 (PBEF1), plasminogen activator inhibitor 1 (PAI-1), neural cell adhesion molecule (NCAM), EGFR, attractin, a proliferation-inducing ligand (APRIL), Cathepsin D, recoverin, CD95, G-22, and somatomedins.

Concluding Remarks
Current strategies in the therapy for patients suffering from primary brain tumors necessitate the development of practical and standardized assays for monitoring disease activity and therapy effects. Intracranial tumors are not accessible for frequent sampling, and therefore body fluids such as blood and CSF are preferable sources for biomarkers. A large number of candidate biomarkers have been discovered, but neither circulating tumor cells, nor their exosomes, DNA, RNA, and particular proteins have passed the requirements of the Tumor Marker Utility Grading System Levels of Evidence/NCCN for clinical application or for serving as monitors in trials. The road from the discovery of new candidate biomarkers to their clinical validation is long. Many issues need to be addressed including biological relevance, sensitivity, specificity, and reproducibility of the measurements. Technical standardization is crucial to achieve clinical utility for candidate biomarkers. Collaborating consortia are needed for standardization and validation of sample collection and isolation, and large prospective multicenter studies are needed to reach the level of evidence required for introducing new biomarkers into clinical practice.

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