Cancer Letters 480 (2020) 24–28

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Liquid biopsies for diagnosing and monitoring primary tumors of the central nervous system

Emilie Le Rhun[a,b,c,d,e], Joan Seoane[f], Michel Salzet[g], Riccardo Soffietti[h], Michael Weller[a,*]

[a] Department of Neurology & Clinical Neuroscience Center, University Hospital and University of Zurich, Zurich, Switzerland
[b] University of Lille, U-1192, F-59000 Lille, France
[c] Inserm, U-1192, F-59000 Lille, France
[d] CHU Lille, Neuro-Oncology, General and Stereotaxic Neurosurgery Service, F-59000 Lille, France
[e] Oscar Lambret Center, Neurology, F-59000 Lille, France
[f] Vall D'Hebron Institute of Oncology (VHIO), Vall D'Hebron University Hospital (HUVH), Universitat Autònoma de Barcelona. Institució Catalana de Recerca I Estudis Avançats (ICREA), CIBERONC, Barcelona, Spain
[g] University of Lille, Inserm, U-1192, Laboratoire Protéomique, Réponse Inflammatoire et Spectrométrie de Masse-PRISM, Lille, F-59000, France
[h] Department of Neuro-Oncology, University and City of Health and Science Hospital, Turin, Italy

ARTICLE INFO

Keywords:
Biopsy
Brain
CSF
Glioma
Liquid
Plasma
Tumor

ABSTRACT

Obtaining diagnostic specimens, notably to monitor disease course in cancer patients undergoing therapy, is an emerging area of research, however, with few clinical implications so far in the field of Neuro-oncology. Specifically for patients with primary brain tumors where repeat biosampling from the tumor and clinical decision making based on neuroimaging alone remain challenging, this area may assume a central role. In principle, sampling could focus on blood, cerebrospinal fluid or urine with differential sensitivities and specificities of findings that differ between specific parameters and target molecules. These include protein, mRNA, miRNA, cell-free DNA, either freely circulating or as cargo of extracellular vesicles, as well circulating tumor cells. The most solid biomarkers are those directly reflecting neoplastic disease, e.g., in the case of primary brain tumors isocitrate dehydrogenase mutation or epidermal growth factor receptor variant III. Importantly, the main goals of liquid biopsy marker development are to better understand response to therapy, natural evolution and emergence of resistant clones, rather than obviating the need for surgical interventions which remain to be a mainstay of therapy for the vast majority of primary brain tumors.

1. Introduction

Liquid biopsies refer to the analysis of body fluids to inform on the presence or on the molecular evolution of neoplastic disease. The body fluids studied include blood, cerebrospinal fluid (CSF), and urine, but also any other conceivable compartmentalized fluid collection like cysts or reservoirs. While the use of liquid biopsies in systemic cancer has long been considered an interesting tool, much less is known about the potential clinical value of liquid biopsies in locally circumscribed diseases like primary brain tumors, notably given the common absence of extra-central nervous system spread in these instances. Yet, specifically with primary brain tumors where repeat tissue sampling may be challenging, easily accessible sources of molecular data to monitor the disease course such as urine or plasma and even CSF are highly attractive.

How and why the liquid biomarkers reach these compartments and determinants of their half-life therein remains incompletely understood, but these topics are areas of ongoing research efforts. The blood brain barrier serves the purpose of excluding molecules and particles from the brain parenchyma, but whether it plays any role in keeping these within the brain and restricting their exiting from the tumor tissue into blood, lymphatic system or CSF remains largely unclear.

Soluble biologically active molecules may not only be freely circulating, but may also constitute part of cargo circulating within subcellular structures referred to as extracellular (micro)vesicles. Since cancer cells may release more such material than normal cells, cancer cell-derived vesicles may contribute to overall pathogenesis of cancer [1]. Characterization of these structures and of their content requires specific techniques of centrifugation and sample processing [2–4]. These extracellular vesicles may shape not only the microenvironment,

* Corresponding author. Laboratory of Molecular Neuro-Oncology, Department of Neurology, University Hospital and University of Zurich, Frauenklinikstrasse 26, CH-8091, Zurich, Switzerland.
E-mail address: michael.weller@usz.ch (M. Weller).

https://doi.org/10.1016/j.canlet.2020.03.021
Received 21 February 2020; Received in revised form 16 March 2020; Accepted 18 March 2020

but also modulate systemic processes in cancer patients including glioblastoma [5]. Here we review recent developments of liquid biopsies for primary brain tumors, excluding primary central nervous system lymphoma.

2. Serum

2.1. Proteins

The detection of proteins as tumor markers in serum or plasma remains challenging because of the high protein content of serum and because proteins only prove the presence of neoplastic disease if they themselves are affected by mutation in the disease context, e.g., the detection of epidermal growth factor receptor (EGFR) variant III (EGFRvIII) protein in serum would prove the existence of an EGFRvIII-mutant tumor somewhere. Among primary brain tumors, only subgroups of germ cell tumors can be diagnosed based on the synthesis and release of high amounts of α1-fetoprotein or β-human choriogonadotrophin. Other protein biomarkers play no role in clinical routine so far.

2.2. mRNA

Freely circulating mRNA may be rapidly degraded, but extracellular vesicles may contain mRNA that disclose the presence of cancer including glioblastoma, e.g., EGFRvIII mRNA was detected in serum-derived extracellular vesicles from 7 of 25 glioblastoma patients, although two of these patients reportedly had EGFRvIII-non-mutant tumors [5]. In a study of WHO grade III and IV gliomas, EGFRvIII mRNA was detected in 39.5% of tumor samples, but in 44.7% of paired serum exosome samples, resulting in a sensitivity of 81% and a specificity of 79%, setting tissue detection as the reference. Very surprisingly, given the need for EGFR amplification to acquire the EGFRvIII mutation, wild-type EGFR mRNA was exclusively found in tumor tissue in this study, but never in the exosomes [6].

2.3. miRNA

In an almost historical study, it was explored whether specific miRNA fingerprints can be detected in the peripheral blood of glioblastoma patients. Of 1158 miRNA examined, 52 were significantly deregulated, and two miRNA, miR-128 which was increased and miR-342–3p which was decreased in tumor patients relative to healthy controls, remained significant after correction for multiple testing. Furthermore, a machine learning algorithm was able to distinguish between glioblastoma samples and control samples with specificity of 79% and sensitivity of 83% [7]. More recently, miR-100 has been proposed to be decreased in the serum of glioblastoma patients, and its levels normalized after treatment [8]. Like other soluble markers, miRNA species may also be circulating with microvesicles [9], and elevated levels of the glioblastoma-associated miR-21 were also detected in extracellular vesicles in serum from glioblastoma patients [5].

2.4. Cell-free tumor DNA

Compared with metastatic cancers, detection of ctDNA in the serum of patients with primary brain tumors turned out to be more challenging and may require more sophisticated technology [10].

O^6-methyl guanine DNA methyltransferase (MGMT) promoter methylation is the single most important predictive biomarker in glioblastoma although establishing a widely accepted methodology for its assessment has remained challenging. Since MGMT promoter methylation rarely changes in the course of disease [11], its monitoring in the periphery is of limited clinical relevance. Yet, the technical feasibility of detecting promoter-methylated MGMT gene sequences has in principle been demonstrated [12]. In a more extensive study, paired serum and tumor samples were assessed for MGMT promoter methylation status by methylation-specific PCR or by pyrosequencing. Methylation was detected in serum by both methods, but concordance of serum data with tissue results was low. Overall sensitivities were 31% and 38% whereas specificity was good; it was higher for methylation-specific PCR (96%) than for pyrosequencing (76%) [13]. Thus this technology may not yet be used for clinical decision making.

In general, detection of ctDNA requires the presence of typical mutations that can be readily detected by simple sequencing techniques and which prove the presence of tumor. Typical examples in gliomas include EGFRvIII or isocitrate dehydrogenase (IDH) 1 or 2 mutations. Furthermore, promoter-mutated telomerase reverse transcriptase (TERTp) is a tumor-defining molecular lesion e.g. detected in the peripheral blood of a patient with metastatic spinal myxopapillary ependymoma [14]. Various clinical trial activities, notably the development of a specific vaccine, rindopepimut, for patients with glioblastomas carrying the EGFRvIII mutation triggered efforts at determining EGFRvIII status by peripheral liquid biopsies, at least as a means to monitor immune-mediated elimination of the target antigen. Moreover, EGFRvIII has the advantage of being without exception a tumor-defining molecular marker. Yet, the negative outcome of the ACT IV trial decreased interest in EGFRvIII as a target in glioblastoma which also was observed not to be stably expressed over time [15], although agents targeting EGFRvIII, including bispecific antibodies, continue to be developed. Recent large screening efforts indicate that half of the patients with primary brain tumors may reveal relevant mutations when plasma cell-free DNA is subjected to next generation panel sequencing [16].

2.5. Circulating tumor cells (CTC)

In contrast to the detection of the biomarkers discussed above, the detection of tumor cells outside the central nervous system provides direct evidence of systemic spread of primary brain tumors and nourishes the unresolved question of why there are so few clinically diagnosed metastases from primary brain tumors outside the central nervous system. Several methods have been employed to detect CTC in the peripheral blood of primary brain tumor patients (Table 1). The detection rates varied from 20% to 70%. Where studied, detection of CTC has been associated with inferior outcome. The relevance of CTC clusters that have so far only been reported once in a glioblastoma patient [17] remains obscure.

3. CSF

3.1. Proteins

As indicated above for plasma, α1-fetoprotein or β-human choriogonadotrophin can also be assessed in the CSF as a means to aid in the differential diagnosis of suspected intracranial germ cell tumors. Other CSF protein biomarkers play no role in the clinic.

3.2. mRNA

Detection of mutant EGFRvIII became a prime target also in the CSF because of its tumor specificity and the interest in monitoring response to EGFRvIII-directed treatment, notably vaccination [15]. EGFRvIII mRNA was detected in CSF-derived extracellular vesicles of 14 of 23 patients with EGFRvIII-mutant tumors whereas only one of 48 patients with EGFRvIII-non-mutant tumors showed EGFRvIII mRNA in the CSF, for a sensitivity of 61% and a specificity of 98% for analysis of CSF extracellular vesicles to detect positive EGFRvIII status [18]. These authors reported similar sensitivity and specificity when comparing lumbar versus cisternal CSF.

3.3. miRNA

miRNA profiles in the CSF of glioblastoma patients have been

Table 1

Reference	Tumors	Parameter	Technology	Findings
Serum				
Skog et al., 2008	Glioblastoma	mRNA in extracellular vesicles	RT-PCR	EGFRvIII mRNA can be detected
Manda et al., 2018	« high-grade » gliomas	mRNA in exosomes	RT-PCR	EGFRvIII mRNA more often detected in exosomes than in tumors
Roth et al., 2011	Glioblastoma	miRNA	miRNA chip hybridization	Specific miRNA profiles can be detected in glioblastoma
Zhang et al., 2019	Glioblastoma	miR-100	RT-PCR	miR-100 decreased in glioblastoma, normalization after treatment
Santangelo et al., 2018	Gliomas	miR-21, miR-222, miR-124–3p	RT-PCR	expression of miR-21, miR-222 and miR-124–3p was measured in exosomes isolated from serum
Estival et al., 2019	Glioblastoma	ctDNA	Methylation-specific PCR or pyrosequencing	MGMT promoter methylation status can be assessed in plasma, but sensitivity remains low
Piccioni et al., 2019	Primary brain tumors	ctDNA	sequencing	50% detection rate of relevant mutations
Macarthur et al., 2014		CTC	Telomerase activity-based assays	72% detection rate
Sullivan et al., 2014	Glioblastoma	CTC	Antibody cocktail (SOX2, tubulin, EGFR, A2B5, c-MET)	39% detection rate
Müller et al., 2014	Glioblastoma	CTC	Immune staining for glial fibrillary acidic protein	21% detection rate
Gao et al., 2016	Gliomas	CTC	CEP8-FISH (centromeric probe for chromosome 8 fluorescent in situ hybridization	77% detection rate of CTC
Krol et al., 2018	Glioblastoma	CTC	Antigen-independent enrichment using Parsortix® technology	First report of CTC clusters in glioblastoma
CSF				
Figueroa et al., 2017	Glioblastoma	mRNA in extracellular vesicles	RT-PCR of vesicles isolated by ultracentrifugation	EGFRvIII status scan be detected with low sensitivity, but high specificity
Akers et al., 2017	Glioblastoma	miRNA	RT-PCR	Glioblastoma CSF shows a characteristic miRNA signature
Wang et al., 2015	Primary brain tumors	ctDNA	Sequencing	The majority of tumor-specific mutations can be detected in the CSF
Pentsova et al., 2016	Primary brain tumors and brain metastases	ctDNA	Sequencing	ctDNA assessment can be used for monitoring
Connolly et al., 2017	Intramedullary spinal ependymoma	ctDNA	digital droplet PCR	No reliable detection of ctDNA
Huang et al., 2017	Brain stem glioma	ctDNA	Sequencing or mutation-specific PCR	Histone H3 mutations can be detected in the CSF
Martinez Ricarte et al., 2018	Adult diffuse gliomas	ctDNA	targeted exome sequencing and droplet digital PCR	Sequencing of IDH2, TP53, TERT, ATRX, H3F3A, and HIST1H3B allows to diagnose the majority of diffuse gliomas
Panditharatna et al., 2018	Diffuse midline glioma	H3K27 M	digital droplet PCR	ctDNA can be used to detect H3K27 M mutations and to document response to radiotherapy
Miller et al., 2019	Gliomas	ctDNA	Sequencing	ctDNA assessment can be used to monitor tumor evolution
Pan et al., 2019	Brain stem gliomas	ctDNA	Gene panel sequencing	Very high sensitivity of ctDNA in the CSF
Gershanov et al., 2017	Medulloblastoma	CTC	Fluorescence lifetime imaging microscopy (FLIM)	Novel technique to assess tumor cell spread in the CSF

reported to correlate with those found in the tumor tissue. A signature of nine miRNA to distinguishing glioblastoma patients from patients with other conditions was identified. In a prospective validation study, sensitivity and specificity of this signature were 67% and 80% for cisternal CSF, and 28% and 95% for lumbar CSF. The authors furthermore reported no difference when analyzing extracellular vesicles from CSF versus crude CSF [19].

3.4. Cell-free tumor DNA

Many academic institutions have introduced gene panel sequencing into their standard work-up of primary brain tumors. In principle, such gene panels can also easily be applied to CSF samples [20]. Such approaches may be particularly useful in situations where tissue availability is limited or where biopsies may be refused by patients and caregivers or not feasible because of lack of local surgical expertise or where extent of resection is no prognostic factor, e.g., in patients with brain stem gliomas [21–23]. Using mutations detected in the tumor as a reference, the rate of positive CSF findings has been estimated at 74%, and lack of lesion proximity to the CSF space correlated with negative findings [24]. Assessing the mutational status of IDH2, TP53, TERT, ATRX (α-thalassemia/mental retardation syndrome, nondeletion type, X-linked), histone H3F3A, and HIST1H3B, which allows to classify the majority of diffuse gliomas of adulthood, in the CSF resulted in the correct diagnosis in 17 of 20 cases [25]. CSF ctDNA profiling can also be used to detect newly acquired mutations in the course of disease or in response to treatment [26,27]. However, sensitivity overall remains to vary by tumor burden, proximity to CSF space, and grade of malignancy, and, e.g., no ctDNA was detected in three patients with intramedullary spinal ependymoma [28].

3.5. Metabolomics

Metabolomic studies to characterize the CSF of primary brain tumor patients are limited, but have potential for specific treatment monitoring situations. Pathway analyses revealed different alterations in various metabolic pathways when comparing CSF from patients with IDH-mutant and IDH-wildtype gliomas, and CSF from patients with IDH-mutant gliomas showed increased levels of D-2-hydroxyglutarate in the CSF [29].

4. Urine

Urine has been less well studied as a source of biochemical information in brain tumor patients. Elevated levels of tissue inhibitor of metalloproteinases 3 and basic fibroblast growth factor have been advanced as specific for juvenile pilocytic astrocytoma [30].

Urine may be more suitable for metabolic studies than for the assessment of other biomarkers covered herein. While two studies failed to demonstrate altered levels of the putative oncometabolite, 2-hydroxyglutarate, in the urine of patients with IDH-mutant gliomas [31], the latter study somewhat surprisingly reported that its urine levels are decreased in these patients, accordingly, the ratio of plasma/urine is increased [32]. Neither the biological basis nor clinical implications are clear at this point. Furthermore, a subsequent study indeed, while confirming no change in serum levels, reported rather increased levels of 2-hydroxyglutarate in patients with IDH-mutant gliomas [33].

5. Outlook

Potential clinical applications of liquid biopsies for patients with primary brain tumors include the initial diagnostic work-up, potentially scenarios where knowledge of molecular status would impact surgical strategies, monitoring of response to therapy, and the differentiation of tumor progression from pseudoprogression which refers to scenarios where imaging suggests progression while there is none. Furthermore, metabolic profiling has been advanced as a potential tool to identify individuals at risk of developing a glioma [34].

Liquid biopsies from various body fluids hold a lot of promise to complement the current diagnostic repertoire for patients with primary brain tumors. Furthermore, the power of genome wide characterization of *tumor-educated platelets* remains to be applied to patients with primary brain tumors [35]. To implement results from liquid biopsies into clinical practice will require standardization of sampling, storage and analysis in prospective studies on defined tumor entities and disease stages to identify the clinical scenarios in which liquid biopsies may be of most value.

Moreover, liquid biopsies are a valuable research tool that should be incorporated increasingly into prospective clinical trials in Neuro-Oncology. In particular, liquid biopsies may turn out to be useful as a complementary diagnostic approach to clinical assessment and neuroimaging in clinical situations which have remained challenging to date, e.g., differentiation of progression from pseudoprogression, assessment of tumor evolution associated with emerging druggable mutations, or assessment of disease extension in brain tumors associated with leptomeningeal spread. In contrast, liquid biopsies should not be primarily conceptualized as an opportunity to obviate the need for surgical interventions or disease monitoring. This is because surgery has commonly a therapeutic, not only a diagnostic intent, and neuroimaging provides essentially different information than liquid biopsies.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

ELR has received research grants from Mundipharma and Amgen, honoraria for lectures or advisory board from Tocagen, Abbvie, Daiichy Sankyo, Mundipharma and Novartis.

JS is a co-founder of Mosaic Biomedicals and Board member of Northern Biologics. He received grant/research support from Mosaic Biomedicals, Northern Biologics, Roche/Glycart, Isarna and Ridgeline.

MS declares no conflicts of interest.

RS has received honoraria for lectures or advisory board from Boards from MSD, Roche, Merck Serono, Celldex Therapeutics, Novartis, Mundipharma and Puma Technologies.

MW has received research grants from Abbvie, Adastra, Bristol Meyer Squibb (BMS), Dracen, Merck, Sharp & Dohme (MSD), Merck (EMD), Novocure, Piqur and Roche, and honoraria for lectures or advisory board participation or consulting from Abbvie, Basilea, Bristol Meyer Squibb (BMS), Celgene, Merck, Sharp & Dohme (MSD), Merck (EMD), Novocure, Orbus, Roche and Tocagen.

References

[1] R. Xu, A. Rai, M. Chen, W. Suwakulsiri, D.W. Greening, R.J. Simpson, Extracellular vesicles in cancer - implications for future improvements in cancer care, Nat. Rev. Clin. Oncol. 15 (2018) 617–638, https://doi.org/10.1038/s41571-018-0036-9.

[2] A.-N. Murgoci, D. Cizkova, P. Majerova, E. Petrovova, L. Medvecky, I. Fournier, M. Salzet, Brain-cortex microglia-derived exosomes: nanoparticles for glioma therapy, ChemPhysChem 19 (2018) 1205–1214, https://doi.org/10.1002/cphc.201701198.

[3] Q. Lemaire, A. Raffo-Romero, T. Arab, C. Van Camp, F. Drago, S. Forte, J.-P. Gimeno, S. Begard, M. Colin, J. Vizioli, P.-E. Sautière, M. Salzet, C. Lefebvre, Isolation of microglia-derived extracellular vesicles: towards miRNA signatures and neuroprotection, J. Nanobiotechnol. 17 (2019) 119, https://doi.org/10.1186/s12951-019-0551-6.

[4] M. Duhamel, M. Rose, F. Rodet, A.N. Murgoci, L. Zografidou, A. Régnier-Vigouroux, F.V. Abeele, F. Kobeissy, S. Nataf, L. Pays, M. Wisztorski, D. Cizkova, I. Fournier, M. Salzet, Paclitaxel treatment and proprotein convertase 1/3 (PC1/3) knockdown in macrophages is a promising antiglioma strategy as revealed by proteomics and cytotoxicity studies, Mol. Cell. Proteomics 17 (2018) 1126–1143, https://doi.org/10.1074/mcp.RA117.000443.

[5] J. Skog, T. Würdinger, S. van Rijn, D.H. Meijer, L. Gainche, M. Sena-Esteves, W.T. Curry, B.S. Carter, A.M. Krichevsky, X.O. Breakefield, Glioblastoma

microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat. Cell Biol. 10 (2008) 1470–1476, https://doi.org/10.1038/ncb1800.
[6] S.V. Manda, Y. Kataria, B.R. Tatireddy, B. Ramakrishnan, B.G. Ratnam, R. Lath, A. Ranjan, A. Ray, Exosomes as a biomarker platform for detecting epidermal growth factor receptor-positive high-grade gliomas, J. Neurosurg. 128 (2018) 1091–1101, https://doi.org/10.3171/2016.11.JNS161187.
[7] P. Roth, J. Wischhusen, C. Happold, P.A. Chandran, S. Hofer, G. Eisele, M. Weller, A. Keller, A specific miRNA signature in the peripheral blood of glioblastoma patients, J. Neurochem. 118 (2011) 449–457, https://doi.org/10.1111/j.1471-4159.2011.07307.x.
[8] H. Zhang, J. Wang, Z. Wang, C. Ruan, L. Wang, H. Guo, Serum miR-100 is a potential biomarker for detection and outcome prediction of glioblastoma patients, Canc. Biomarkers 24 (2019) 43–49, https://doi.org/10.3233/CBM-181416.
[9] A. Santangelo, P. Imbrucè, B. Gardenghi, L. Belli, R. Agushi, A. Tamanini, S. Munari, A.M. Bossi, I. Scambi, D. Benati, R. Mariotti, G. Di Gennaro, A. Sbarbati, A. Eccher, G.K. Ricciardi, E.M. Ciceri, F. Sala, G. Pinna, G. Lippi, G. Cabrini, M.C. Dechecchi, A microRNA signature from serum exosomes of patients with glioma as complementary diagnostic biomarker, J. Neuro Oncol. 136 (2018) 51–62, https://doi.org/10.1007/s11060-017-2639-x.
[10] C. Bettegowda, M. Sausen, R.J. Leary, I. Kinde, Y. Wang, N. Agrawal, B.R. Bartlett, H. Wang, B. Luber, R.M. Alani, E.S. Antonarakis, N.S. Azad, A. Bardelli, H. Brem, J.L. Cameron, C.C. Lee, L.A. Fecher, G.L. Gallia, P. Gibbs, D. Le, R.L. Giuntoli, M. Goggins, M.D. Hogarty, M. Holdhoff, S.-M. Hong, Y. Jiao, H.H. Juhl, J.J. Kim, G. Siravegna, D.A. Laheru, C. Lauricella, M. Lim, E.J. Lipson, S.K.N. Marie, G.J. Netto, K.S. Oliner, A. Olivi, L. Olsson, G.J. Riggins, A. Sartore-Bianchi, K. Schmidt, le-M. Shih, S.M. Oba-Shinjo, S. Siena, D. Theodorescu, J. Tie, T.T. Harkins, S. Veronese, T.-L. Wang, J.D. Weingart, C.L. Wolfgang, L.D. Wood, D. Xing, R.H. Hruban, J. Wu, P.J. Allen, C.M. Schmidt, M.A. Choti, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, N. Papadopoulos, L.A. Diaz, Detection of circulating tumor DNA in early- and late-stage human malignancies, Sci. Transl. Med. 6 (2014) 224ra24, , https://doi.org/10.1126/scitranslmed.3007094.
[11] J. Felsberg, N. Thon, S. Eigenbrod, B. Hentschel, M.C. Sabel, M. Westphal, G. Schackert, F.W. Kreth, T. Pietsch, M. Löffler, M. Weller, G. Reifenberger, J.C. Tonn, German Glioma Network, Promoter methylation and expression of MGMT and the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in paired primary and recurrent glioblastomas, Int. J. Canc. 129 (2011) 659–670, https://doi.org/10.1002/ijc.26083.
[12] C. Balaña, J.L. Ramirez, M. Taron, Y. Roussos, A. Ariza, R. Ballester, C. Sarries, P. Mendez, J.J. Sanchez, R. Rosell, 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. Canc. Res. 9 (2003) 1461–1468.
[13] A. Estival, C. Sanz, J.-L. Ramirez, J.M. Velarde, M. Domenech, C. Carrato, R. de Las Peñas, M. Gil-Gil, J. Sepúlveda, R. Armengol, I. Cardiel, A. Berrocal, R. Luque, A. Herrero, C. Balana, Pyrosequencing versus methylation-specific PCR for assessment of MGMT methylation in tumor and blood samples of glioblastoma patients, Sci. Rep. 9 (2019) 11125, https://doi.org/10.1038/s41598-019-47642-2.
[14] A. Deniel, F. Marguet, L. Beaussire, A.-C. Tobenas-Dujardin, C. Peillon, M.-A. Gambirasio, O. Veresezan, N. Magne, F. Di Fiore, A. Laquerrière, N. Sarafan-Vasseur, M. Fontanilles, TERTp mutation detection in plasma by droplet-digital polymerase chain reaction in spinal myxopapillary ependymoma with lung metastases, World Neurosurg 130 (2019) 405–409, https://doi.org/10.1016/j.wneu.2019.07.111.
[15] M. Weller, N. Butowski, D.D. Tran, L.D. Recht, M. Lim, H. Hirte, L. Ashby, L. Mechtler, S.A. Goldlust, F. Iwamoto, J. Drappatz, D.M. O'Rourke, M. Wong, M.G. Hamilton, G. Finocchiaro, J. Perry, W. Wick, J. Green, Y. He, C.D. Turner, M.J. Yellin, T. Keler, T.A. Davis, R. Stupp, J.H. Sampson, ACT IV trial investigators, Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial, Lancet Oncol. 18 (2017) 1373–1385, https://doi.org/10.1016/S1470-2045(17)30517-X.
[16] D.E. Piccioni, A.S. Achrol, L.A. Kiedrowski, K.C. Banks, N. Boucher, G. Barkhoudarian, D.F. Kelly, T. Juarez, R.B. Lanman, V.M. Raymond, M. Nguyen, J.D. Truong, A. Heng, J. Gill, M. Saria, S.C. Pingle, S. Kesari, Analysis of cell-free circulating tumor DNA in 419 patients with glioblastoma and other primary brain tumors, CNS Oncol 8 (2019) CNS34, https://doi.org/10.2217/cns-2018-0015.
[17] I. Krol, F. Castro-Giner, M. Maurer, S. Gkountela, B.M. Szczerba, R. Scherrer, N. Coleman, S. Carreira, F. Bachmann, S. Anderson, M. Engelhardt, H. Lane, T.R.J. Evans, R. Plummer, R. Kristeleit, J. Lopez, N. Aceto, Detection of circulating tumour cell clusters in human glioblastoma, Br. J. Canc. 119 (2018) 487–491, https://doi.org/10.1038/s41416-018-0186-7.
[18] J.M. Figueroa, J. Skog, J. Akers, H. Li, R. Komotar, R. Jensen, F. Ringel, I. Yang, S. Kalkanis, R. Thompson, L. LoGuidice, E. Berghoff, A. Parsa, L. Liau, W. Curry, D. Cahill, C. Bettegowda, F.F. Lang, E.A. Chiocca, J. Henson, R. Kim, X. Breakefield, C. Chen, K. Messer, F. Hochberg, B.S. Carter, Detection of wild-type EGFR amplification and EGFRvIII mutation in CSF-derived extracellular vesicles of glioblastoma patients, Neuro Oncol. 19 (2017) 1494–1502, https://doi.org/10.1093/neuonc/nox085.
[19] J.C. Akers, W. Hua, H. Li, V. Ramakrishnan, Z. Yang, K. Quan, W. Zhu, J. Li, J. Figueroa, B.R. Hirshman, B. Miller, D. Piccioni, F. Ringel, R. Komotar, K. Messer, D.R. Galasko, F. Hochberg, Y. Mao, B.S. Carter, C.C. Chen, A cerebrospinal fluid microRNA signature as biomarker for glioblastoma, Oncotarget 8 (2017) 68769–68779, https://doi.org/10.18632/oncotarget.18332.
[20] J. Seoane, L. De Mattos-Arruda, E. Le Rhun, A. Bardelli, M. Weller, Cerebrospinal fluid cell-free tumour DNA as a liquid biopsy for primary brain tumours and central nervous system metastases, Ann. Oncol. (2018), https://doi.org/10.1093/annonc/mdy544.
[21] T.Y. Huang, A. Piunti, R.R. Lulla, J. Qi, C.M. Horbinski, T. Tomita, C.D. James, A. Shilatifard, A.M. Saratsis, Detection of Histone H3 mutations in cerebrospinal fluid-derived tumor DNA from children with diffuse midline glioma, Acta Neuropathol Commun 5 (2017) 28, https://doi.org/10.1186/s40478-017-0436-6.
[22] C. Pan, B.H. Diplas, X. Chen, Y. Wu, X. Xiao, L. Jiang, Y. Geng, C. Xu, Y. Sun, P. Zhang, W. Wu, Y. Wang, Z. Wu, J. Zhang, Y. Jiao, H. Yan, L. Zhang, Molecular profiling of tumors of the brainstem by sequencing of CSF-derived circulating tumor DNA, Acta Neuropathol. 137 (2019) 297–306, https://doi.org/10.1007/s00401-018-1936-6.
[23] L. De Mattos-Arruda, R. Mayor, C.K.Y. Ng, B. Weigelt, F. Martínez-Ricarte, D. Torrejon, M. Oliveira, A. Arias, C. Raventos, J. Tang, E. Guerini-Rocco, E. Martínez-Sáez, S. Lois, O. Marín, X. de la Cruz, S. Piscuoglio, R. Towers, A. Vivancos, V. Peg, S. Ramon y Cajal, J. Carles, J. Rodon, M. González-Cao, J. Tabernero, E. Felip, J. Sahuquillo, M.F. Berger, J. Cortes, J.S. Reis-Filho, J. Seoane, Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma, Nat. Commun. 6 (2015) 8839, https://doi.org/10.1038/ncomms9839.
[24] Y. Wang, S. Springer, M. Zhang, K.W. McMahon, I. Kinde, L. Dobbyn, J. Ptak, H. Brem, K. Chaichana, G.L. Gallia, Z.L. Gokaslan, M.L. Groves, G.I. Jallo, M. Lim, A. Olivi, A. Quinones-Hinojosa, D. Rigamonti, G.J. Riggins, D.M. Sciubba, J.D. Weingart, J.-P. Wolinsky, X. Ye, S.M. Oba-Shinjo, S.K.N. Marie, M. Holdhoff, N. Agrawal, L.A. Diaz, N. Papadopoulos, K.W. Kinzler, B. Vogelstein, C. Bettegowda, Detection of tumor-derived DNA in cerebrospinal fluid of patients with primary tumors of the brain and spinal cord, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 9704–9709, https://doi.org/10.1073/pnas.1511694112.
[25] F. Martínez-Ricarte, R. Mayor, E. Martínez-Sáez, C. Rubio-Pérez, E. Pineda, E. Cordero, M. Cicuéndez, M.A. Poca, N. López-Bigas, S. Ramon Y Cajal, M. Vieito, J. Carles, J. Tabernero, A. Vivancos, S. Gallego, F. Graus, J. Sahuquillo, J. Seoane, Molecular diagnosis of diffuse gliomas through sequencing of cell-free circulating tumor DNA from cerebrospinal fluid, Clin. Canc. Res. 24 (2018) 2812–2819, https://doi.org/10.1158/1078-0432.CCR-17-3800.
[26] E.I. Pentsova, R.H. Shah, J. Tang, A. Boire, D. You, S. Briggs, A. Omuro, X. Lin, M. Fleisher, C. Grommes, K.S. Panageas, F. Meng, S.D. Selcuklu, S. Ogilvie, N. Distefano, L. Shagabayeva, M. Rosenblum, L.M. DeAngelis, A. Viale, I.K. Mellinghoff, M.F. Berger, Evaluating cancer of the central nervous system through next-generation sequencing of cerebrospinal fluid, J. Clin. Oncol. 34 (2016) 2404–2415, https://doi.org/10.1200/JCO.2016.66.6487.
[27] A.M. Miller, R.H. Shah, E.I. Pentsova, M. Pourmaleki, S. Briggs, N. Distefano, Y. Zheng, A. Skakodub, S.A. Mehta, C. Campos, W.-Y. Hsieh, S.D. Selcuklu, L. Ling, F. Meng, X. Jing, A. Samoila, T.A. Bale, D.W.Y. Tsui, C. Grommes, A. Viale, M.M. Souweidane, V. Tabar, C.W. Brennan, A.S. Reiner, M. Rosenblum, K.S. Panageas, L.M. DeAngelis, R.J. Young, M.F. Berger, I.K. Mellinghoff, Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid, Nature 565 (2019) 654–658, https://doi.org/10.1038/s41586-019-0882-3.
[28] I.D. Connolly, Y. Li, W. Pan, E. Johnson, L. You, H. Vogel, J. Ratliff, M. Hayden Gephart, A pilot study on the use of cerebrospinal fluid cell-free DNA in intramedullary spinal ependymoma, J. Neuro Oncol. 135 (2017) 29–36, https://doi.org/10.1007/s11060-017-2557-y.
[29] L.Y. Ballester, G. Lu, S. Zorofchian, V. Vantaku, V. Putluri, Y. Yan, O. Arevalo, P. Zhu, R.F. Riascos, A. Sreekumar, Y. Esquenazi, N. Putluri, J.-J. Zhu, Analysis of cerebrospinal fluid metabolites in patients with primary or metastatic central nervous system tumors, Acta Neuropathol Commun 6 (2018) 85, https://doi.org/10.1186/s40478-018-0588-z.
[30] K. Pricola Fehnel, M. Duggins-Warf, D. Zurakowski, M. McKee-Proctor, R. Majumder, M. Raber, X. Han, E.R. Smith, Using urinary bFGF and TIMP3 levels to predict the presence of juvenile pilocytic astrocytoma and establish a distinct biomarker signature, J. Neurosurg. Pediatr. 18 (2016) 396–407, https://doi.org/10.3171/2015.12.PEDS15448.
[31] D. Capper, M. Simon, C.-D. Langhans, J.G. Okun, J.C. Tonn, M. Weller, A. von Deimling, C. Hartmann, German Glioma Network, 2-Hydroxyglutarate concentration in serum from patients with gliomas does not correlate with IDH1/2 mutation status or tumor size, Int. J. Canc. 131 (2012) 766–768, https://doi.org/10.1002/ijc.26425.
[32] G. Lombardi, G. Corona, L. Bellu, A. Della Puppa, A. Pambuku, P. Fiduccia, R. Bertorelle, M.P. Gardiman, D. D'Avella, G. Toffoli, V. Zagonel, Diagnostic value of plasma and urinary 2-hydroxyglutarate to identify patients with isocitrate dehydrogenase-mutated glioma, Oncol. 20 (2015) 562–567, https://doi.org/10.1634/theoncologist.2014-0266.
[33] A.T. Fathi, B.V. Nahed, S.A. Wander, A.J. Iafrate, D.R. Borger, R. Hu, A. Thabet, D.P. Cahill, A.M. Perry, C.P. Joseph, A. Muzikansky, A.S. Chi, Elevation of urinary 2-hydroxyglutarate in IDH-mutant glioma, Oncol. 21 (2016) 214–219, https://doi.org/10.1634/theoncologist.2015-0342.
[34] J. Huang, S.J. Weinstein, C.M. Kitahara, E.D. Karoly, J.N. Sampson, D. Albanes, A prospective study of serum metabolites and glioma risk, Oncotarget 8 (2017) 70366–70377, https://doi.org/10.18632/oncotarget.19705.
[35] M.G. Best, N. Sol, S.G.J.G. In 't Veld, A. Vancura, M. Muller, A.-L.N. Niemeijer, A.V. Fejes, L.-A. Tjon Kon Fat, A.E. Huis, In 't Veld, C. Leurs, T.Y. Le Large, L.L. Meijer, I.E. Kooi, F. Rustenburg, P. Schellen, H. Verschueren, E. Post, L.E. Wedekind, J. Bracht, M. Esenkbrink, L. Wils, F. Favaro, J.D. Schoonhoven, J. Tannous, H. Meijers-Heijboer, G. Kazemier, E. Giovannetti, J.C. Reijneveld, S. Idema, J. Killestein, M. Heger, S.C. de Jager, R.T. Urbanus, I.E. Hoefer, G. Pasterkamp, C. Mannhalter, J. Gomez-Arroyo, H.-J. Bogaard, D.P. Noske, W.P. Vandertop, D. van den Broek, B. Ylstra, R.J.A. Nilsson, P. Wesseling, N. Karachaliou, R. Rosell, E. Lee-Lewandrowski, K.B. Lewandrowski, B.A. Tannous, A.J. de Langen, E.F. Smit, M.M. van den Heuvel, T. Wurdinger, Swarm intelligence-enhanced detection of non-small-cell lung cancer using tumor-educated platelets, Canc. Cell 32 (2017) 238–252, https://doi.org/10.1016/j.ccell.2017.07.004 e9.