UNIVERSITÀ DI TORINO

AZIENDA OSPEDALIERO-UNIVERSITARIA
Città della Salute e della Scienza di Torino

Nuove frontiere applicative
-
Neoplasie gliali

L. Bertero

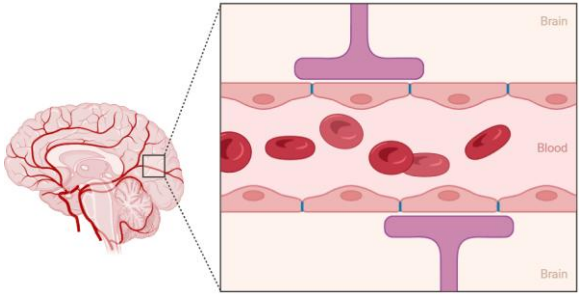
Pathology Unit

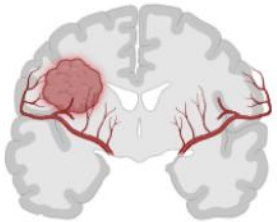
Dept. Medical Sciences, University of Turin, Italy

Dept. Laboratory Medicine, «Città della Salute e della Scienza» University Hospital, Turin, Italy

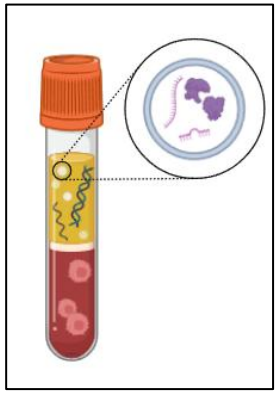
CNS tumors: the “snowflake” ones

Impact of BBB

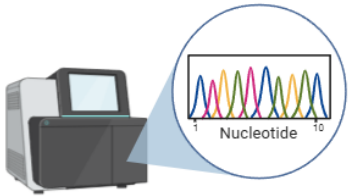


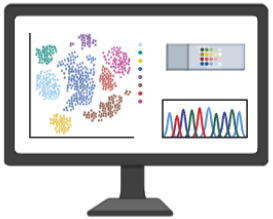


Clinical/diagnostic challenges (tumor location, heterogeneity…)



Role of molecular hallmarks





DNA methylation profiling

Role of BBB

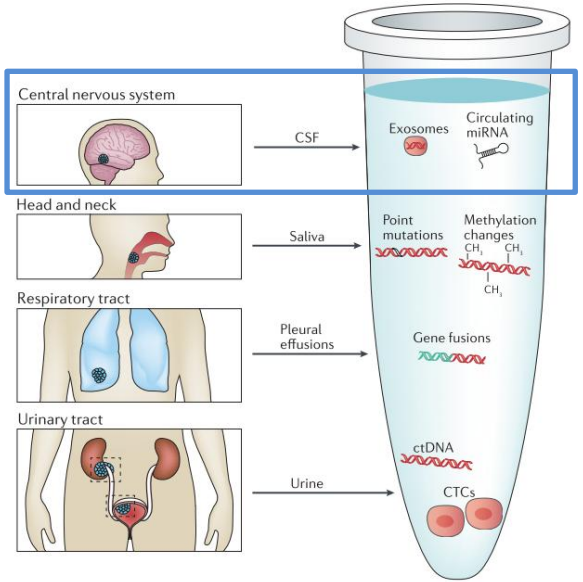


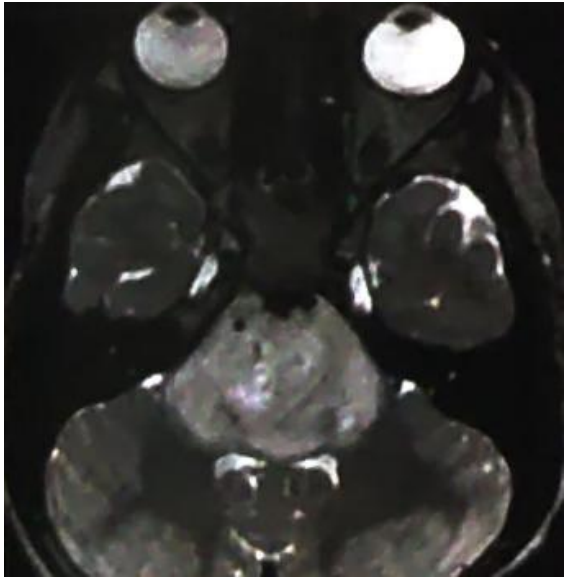
Table 1. Studies investigating circulating cfDNA in primary CNS tumours

	Tumour	*Positive CSF cytology*	*Positive CSF molecular profiling*	*Positive blood molecular profiling*
Rhodes *et al.* [1]	Glioblastoma	ND/NR	1/1 (100%)	ND/NR
Rhodes *et al.* [2]	Glioblastoma	ND/NR	1/1 (100%)	ND/NR
Boisselier *et al.* [7]	Glioma (low grade = 8, high grade = 17)	ND/NR	ND/NR	Low grade: 3/8 (37.5%) High grade: 12/17 (70.6%)
Salkeni *et al.* [30]	Glioblastoma	ND/NR	ND/NR	3/3 (100%)
Bettegowda *et al.* [19]	Glioma (n = 27) Medulloblastoma (n = 14)	ND/NR	ND/NR	Glioma: <10% Medulloblastoma: <50%
Pan *et al.* [36]	Meningioma (n = 1) Schwannoma (n = 1)	ND/NR	Meningioma: 1/1 (100%) Schwannoma: 0/1 (0%)	Meningioma: 0/1 (0%) Schwannoma: 0/1 (0%)
De Mattos-Arruda *et al.* [20]	Glioblastoma (n = 4) Medulloblastoma (n = 2)	ND/NR	Glioblastoma: 4/4 (100%) Medulloblastoma: 2/2 (100%)	Glioblastoma: 0/4 (0%) Medulloblastoma: 0/2 (0%)
Wang *et al.* [22]	Low-grade glioma (n = 8) High-grade glioma (n = 13) Ependymoma (n = 7) Medulloblastoma (n = 6) Other low-grade tumour (n = 1)	ND/NR	Low-grade glioma: 6/8 (75%) High-grade glioma: 13/13 (100%) Ependymoma: 5/7 (71%) Medulloblastoma: 5/6 (83%) Other low-grade tumour: 1/1 (100%)	ND/NR
Pentsova *et al.* [21]	Glioma (n = 8) Ependymoma (n = 1)	Glioma: 0/8 Ependymoma: 0/1	Glioma: 6/8 (75%) Ependymoma: 0/1 (0%)	ND/NR
Connolly *et al.* [23]	Ependymoma (n = 3)	ND/NR	0/3 (0%)	0/3 (0%)
Huang *et al.* [32]	Diffuse midline glioma (n = 5)	ND/NR	4/5 (80%)	ND/NR
Martìnez-Ricarte *et al.* [31]	High-grade glioma (n = 15) Low-grade glioma (n = 5)	ND/NR	High-grade glioma: 15/15 (100%) Low-grade glioma: 2/5 (40%)	ND/NR
Pan *et al.* [33]	Brainstem glioma	ND/NR	39/40 (98%)	3/8 (38%)
Panditharatna *et al.* [34]	Diffuse midline glioma	ND/NR	24/27 (89%)	34/40 (85%)
Hiemcke-Jiwa *et al.* [37]	Lymphoplasmacytic lymphoma (n = 6) PCNSL (n = 1)	Lymphoplasmacytic lymphoma: 2/6 (33%) PCNSL: 1/1 (100%)	Lymphoplasmacytic lymphoma: 5/6 (83%) PCNSL: 1/1 (100%)	ND/NR
Miller *et al.* [24]	Diffuse glioma (grade II-III-IV)	7/80 (9%) CSF cytology not available in five cases	42/85 (49%)	3/19 (16%)

Siravegna G et al. *Nat Rev Clin Oncol* 2017

Bertero L et al. *Neuropathol Appl Neurobiol 2019*

Primary brain tumors



Diffuse midline glioma, H3 K27-altered

Neuro-Oncology
25(10), 1731–1749, 2023 | https://doi.org/10.1093/neuonc/noad100 | Advance Access date 2 June 2023

Molecular diagnostic tools for the World Health Organization (WHO) 2021 classification of gliomas, glioneuronal and neuronal tumors; an EANO guideline

Felix Sahm, Sebastian Brandner, Luca Bertero, David Capper, Pim J. French, Dominique Figarella-Branger, Felice Giangaspero*, Christine Haberler, Monika E. Hegi, Bjarne W. Kristensen, Kathreena M. Kurian, Matthias Preusser, Bastiaan B. J. Tops, Martin van den Bent, Wolfgang Wick, Guido Reifenberger, and Pieter Wesseling

ADULT-TYPE DIFFUSE GLIOMAS	
Astrocytoma, IDH-mutant	***IDH1*** **p.R132**[1#] or ***IDH2*** **p.R172**[1#]**; no chr. 1p/19q**[5]***; ATRX***[2,3]***; CDKN2A/B***[4@]; *TP53*[2,3]; MP
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	***IDH1*** **p.R132**[1#] or ***IDH2*** **p.R172**[1#]**; chr. 1p/19q**[5]; *TERT* promoter[2]; MP; IHC: retained nuclear ATRX expression
Glioblastoma, IDH-wildtype	**IDH-wt and H3-wt**; ***TERT*** **promoter**[2]; ***EGFR***[6]**; chr. +7/−10**[7]; MP; predictive: *MGMT* promoter methylation[5]
PEDIATRIC-TYPE DIFFUSE LOW-GRADE GLIOMAS	
Diffuse astrocytoma, *MYB*- or *MYBL1*-altered	***MYB***[8]**,** ***MYBL1***[8]**; IDH1-wt and H3-wt; MP**
Angiocentric glioma	*MYB*[8]; MP
Polymorphous low-grade neuroepithelial tumor of the young (PLNTY)	**MAPK alteration such as** ***BRAF*** **p.V600**[1]**,** ***FGFR2***[8]**,** ***FGFR3***[8]**, or other; IDH-wt**; no 1p/19q[5]
Diffuse low-grade glioma, MAPK pathway-altered	**MAPK alteration; IDH-wt and H3-wt; no** ***CDKN2A/B***[4]; MP: absence of profile of other FGFR- or *BRAF*-altered tumor
PEDIATRIC-TYPE DIFFUSE HIGH-GRADE GLIOMAS	
Diffuse midline glioma, H3K27-altered	**IHC: loss of H3 p.K28me3 (K27me3) in tumor cell nuclei;** p.K28M (K27M) or pK28I (K27I) mutation in H3.3, H3.1, or H3.2 for H3 K27-mutant subtypes[1^]; ***EGFR***[2,6]**; EZHIP**[10]**; MP**
Diffuse hemispheric glioma, H3G34-mutant	**H3.3 p.G35 (G34)**[1^]; MP; loss of ATRX expression, diffuse p53 immunopositivity
Diffuse pediatric-type high-grade glioma, H3- and IDH-wildtype	***PDGFRA***[2,6]**,** ***EGFR***[2,6] **or** ***MYCN***[6]**; IDH-wt and H3-wt; MP**
Infant-type hemispheric glioma	**RTK alteration such as NTRK family gene**[8]**,** ***ROS1***[8]**,** ***MET***[8]**,** ***ALK***[8]**; MP**

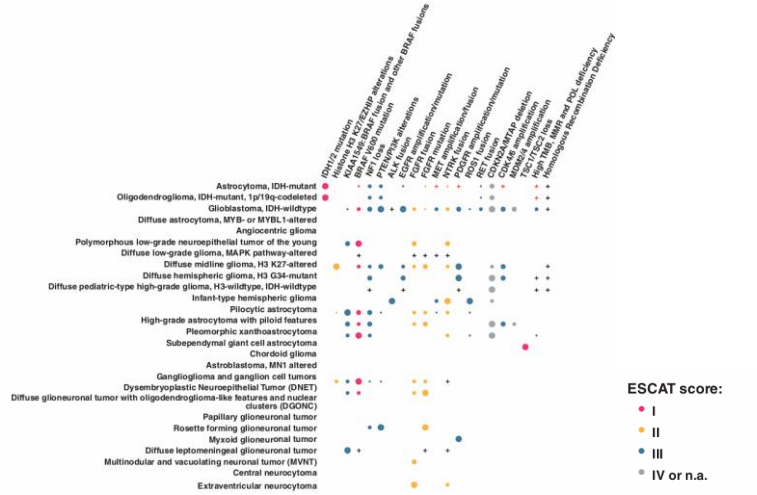
Primary brain tumors

Neuro-Oncology

27(2), 331–337, 2025 | https://doi.org/10.1093/neuonc/noae213 | Advance Access date 10 October 2024

Updated EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection—Update 1

Martin J. van den Bent, Enrico Franceschi, Mehdi Touat, Pim J. French, Ahmed Idbaih, Giuseppe Lombardi, Roberta Rudà, Leonille Schweizer, David Capper, Marc Sanson, Pieter Wesseling, Michael Weller, Marica Eoli, Elena Anghileri, Franck Bielle, Phillipp Euskirchen, Marjolein Geurts, Patrick Y. Wen, and Matthias Preusser

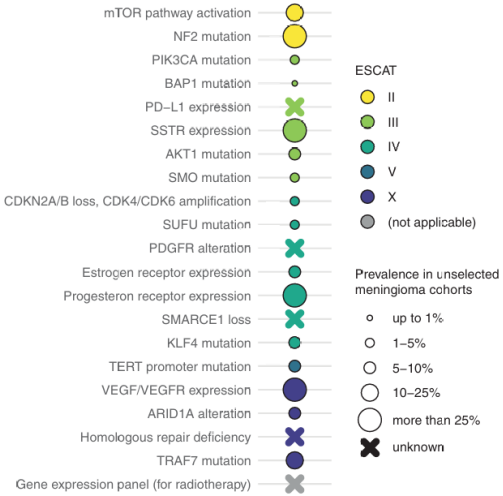


Neuro-Oncology

27(4), 869–883, 2025 | https://doi.org/10.1093/neuonc/noae253 | Advance Access date 23 November 2024

European Association of Neuro-Oncology guideline on molecular testing of meningiomas for targeted therapy selection

Felix Sahm, Luca Bertero, Sebastian Brandner, David Capper, Roland Goldbrunner, Michael D. Jenkinson, Michel Kalamarides, Katrin Lamszus, Nathalie L. Albert, Maximilian J. Mair, Anna S. Berghoff, Christian Mawrin, Hans-Georg Wirsching, Sybren L.N. Maas, David R. Raleigh, Guido Reifenberger, Leonille Schweizer, Abigail K. Suwala, Ghazaleh Tabatabai, Emeline Tabouret, Susan Short, Patrick Y. Wen, Michael Weller, Emilie Le Rhun, Pieter Wesseling, Martin van den Bent, and Matthias Preusser



However…

Acta Neuropathol Commun. 2022; 10: 137.
Published online 2022 Sep 14. doi: 10.1186/s40478-022-01442-4
PMCID: PMC9476256
PMID: 36104744

Posterior fossa ependymoma H3 K27-mutant: an integrated radiological and histomolecular tumor analysis

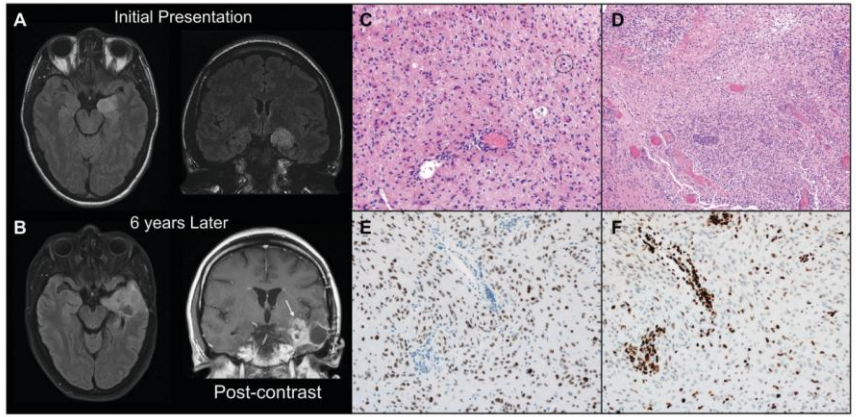
Cassandra Mariet,[1] David Castel,[2] Jacques Grill,[1,2] Raphaël Saffroy,[3] Volodia Dangouloff-Ros,[4,5] Nathalie Boddaert,[4,5] Francisco Llamas-Guttierrez,[6] Céline Chappé,[7] Stéphanie Puget,[8] Lauren Hasty,[9] Fabrice Chrétien,[9] Alice Métais,[9,10] Pascale Varlet,[9,10] and Arnault Tauziède-Espariat[9,10]

Journal of Neuropathology & Experimental Neurology, 2024, **83**, 357–364
https://doi.org/10.1093/jnen/nlae018
Advance Access Publication Date: 6 March 2024
Original Article

OXFORD

Diffuse hemispheric glioma with H3 p.K28M (K27M) mutation: Unusual non-midline presentation of diffuse midline glioma, H3 K27M-altered?

Kliment Donev, MD[1], Vanitha Sundararajan, MD[2,3], Derek Johnson, MD[4], Jagadheshwar Balan, MS[5], Meagan Chambers, MD[6], Vera A. Paulson, MD, PhD[7], Kathryn P. Scherpelz, MD, PhD[6], Zied Abdullaev, PhD[8], Martha Quezado, MD[8], Patrick J. Cimino, MD, PhD[8], Drew Pratt, MD[8], Ediel Valerio, MD[9], João Vıctor Alves de Castro, MD[9], Dirce Maria Carraro, PhD[10,11], Giovana Tardin Torrezan, PhD[10,11], Beatriz Martins Wolff, MS[12], Leslie Domenici Kulikowski, PhD[12], Felipe D'Almeida Costa, MD, PhD[8,13], Kenneth Aldape, MD[8], Cristiane M. Ida, MD[14,*]



Tumor DNA methylation profling

Neuro-Oncology Advances

7(1), vdae228, 2025 | https://doi.org/10.1093/noajnl/vdae228 | Advance Access date 3 January 2025

cIMPACT-NOW update 9: Recommendations on utilization of genome-wide DNA methylation profiling for central nervous system tumor diagnostics

Kenneth Aldape, David Capper, Andreas von Deimling, Caterina Giannini, Mark R. Gilbert, Cynthia Hawkins, Jürgen Hench, Thomas S. Jacques, David Jones, David N. Louis, Sabine Mueller, Brent A. Orr, MacLean Nasrallah, Stefan M. Pfister, Felix Sahm, Matija Snuderl, David Solomon, Pascale Varlet and , Pieter Wesseling

Input requirement:
up to 500 ng DNA

Key Points

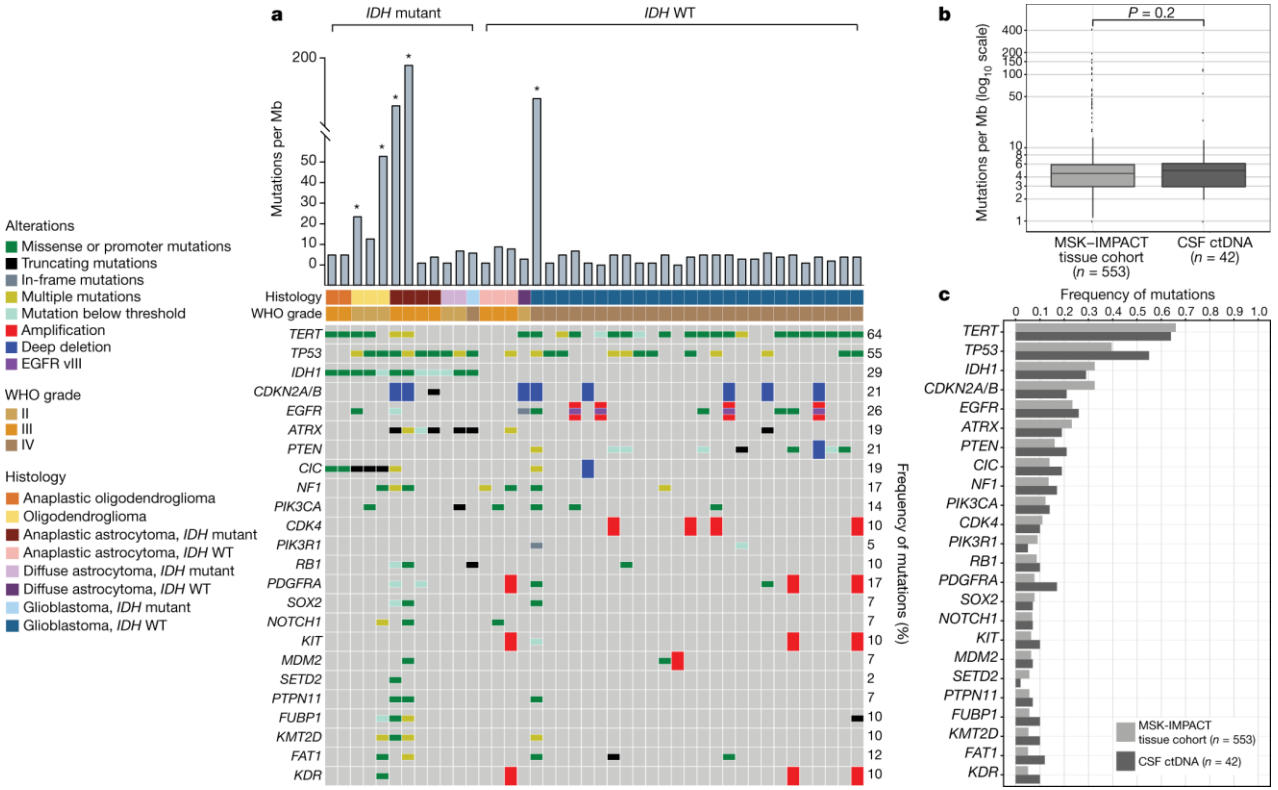
- DNA methylation profiling is a valuable tool for the diagnosis of central nervous system tumors.
- Results should be interpreted within the entire context of the case.
- Inclusion of methylation results into a layered diagnostic format is recommended.

CSF cfDNA – Primary brain tumors in adults

- Analysis of a series of 85 adult gliomas:

46/85 (54%) WHO grade 4 gliomas
26/85 (31%) WHO grade 3 gliomas
13/85 (15%) WHO grade 2 gliomas

- Detection of one tumour-derived genetic alteration in 42/85 (49.4%)



Miller A et al. Nature 2019

CSF cfDNA – Primary brain tumors

Table 2
Selection of published studies investigating clinical applications of CSF ctDNA.

Disease	Study	Patient population	Method	Key findings
Glioma	Martinez-Ricarte (2018)	20 diffuse gliomas	ddPCR, NGS (Sn=85 %)	• CSF ctDNA allowed for glioma subtyping in 17/20 (85 %) pts
	Pan (2019)	57 brainstem gliomas	NGS (Sn=83 %)	• CSF ctDNA allowed for glioma subtyping in 47/57 (83 %) pts
	Fujioka (2021)	34 diffuse gliomas	ddPCR (Sn=59 %)	• CSF ctDNA allowed for glioma subtyping in 20/34 (59 %) pts
	Miller (2019)	85 diffuse gliomas	NGS (Sn=50 %)	• CSF ctDNA detection associated with tumor burden, radiographic progression, and survival
	Cantor (2022)	24 DMG (prospective CT)	ddPCR (Sn=97 %)	• Decrease in CSF ctDNA associated with longer PFS (13 pts with nonrecurrent tumor)
Other primary brain tumors	Bobillo (2021)	7 CNS lymphoma (1 primary, 6 secondary)	NGS, WES (Sn=86 %, Sp=100 %)	• VAF increases with progression and decreases with treatment response (4 pts) • CSF ctDNA detected in one pt with systemic lymphoma but negative CSF flow cytometry, who later developed CNS lymphoma involvement
	Liu (2021)	123 medulloblastomas (prospective CT)	sWGS (Sn=64 %, Sp=100 %)	• Detectable CSF ctDNA (MDR) during/after treatment associated with subsequent progression

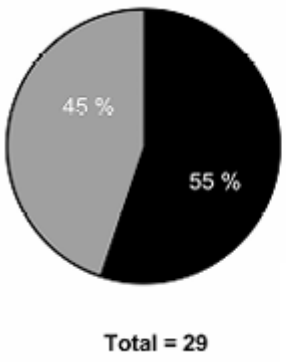
Diaz M et al. Translational Oncology 2024

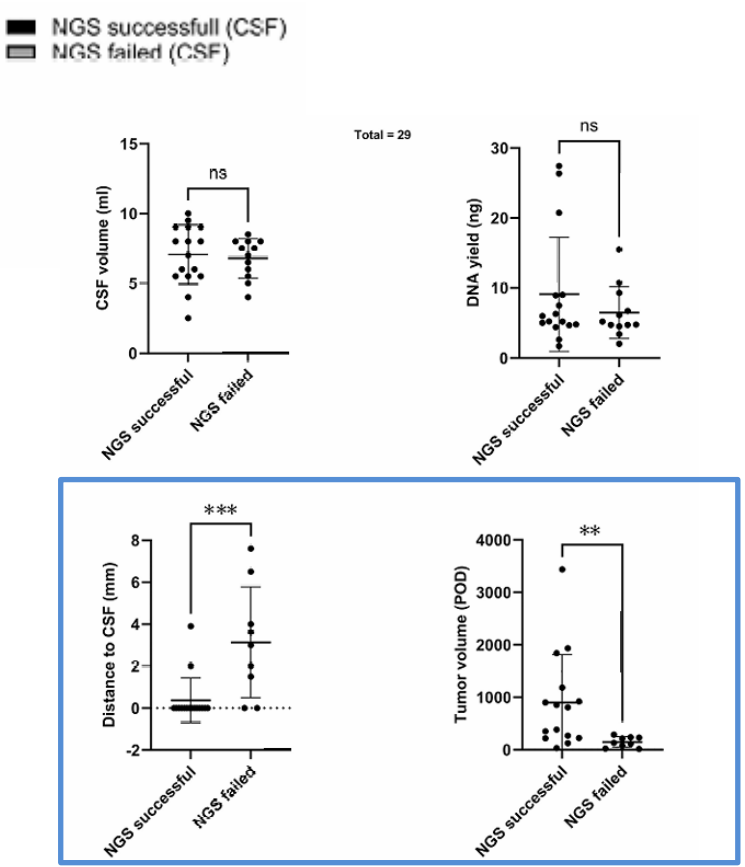
CSF cfDNA – Primary brain tumors

Table 1. Clinical characteristics of included patients

	Overall (*N*=29)
Age (years)	Mean: 55.4
Sex	
Male	15 (51.7%)
Female	14 (48.3%)
Disease entity	
AEG	1 (3.4%)
Astrocytoma, high grade	1 (3.4%)
Breast cancer	5 (17.2%)
Germinoma	2 (6.9%)
Glioblastoma	5 (17.2%)
LPD	1 (3.4%)
Lung cancer	3 (10.3%)
Lymphoma	1 (3.4%)
Melanoma	4 (13.8%)
non-neoplastic	3 (10.3%)
Unknown	3 (10.3%)
Blood analysis	
Failed	0 (0%)
Successful	29 (100%)
CSF analysis	
Failed	13 (44.8%)
Successful	16 (55.2%)

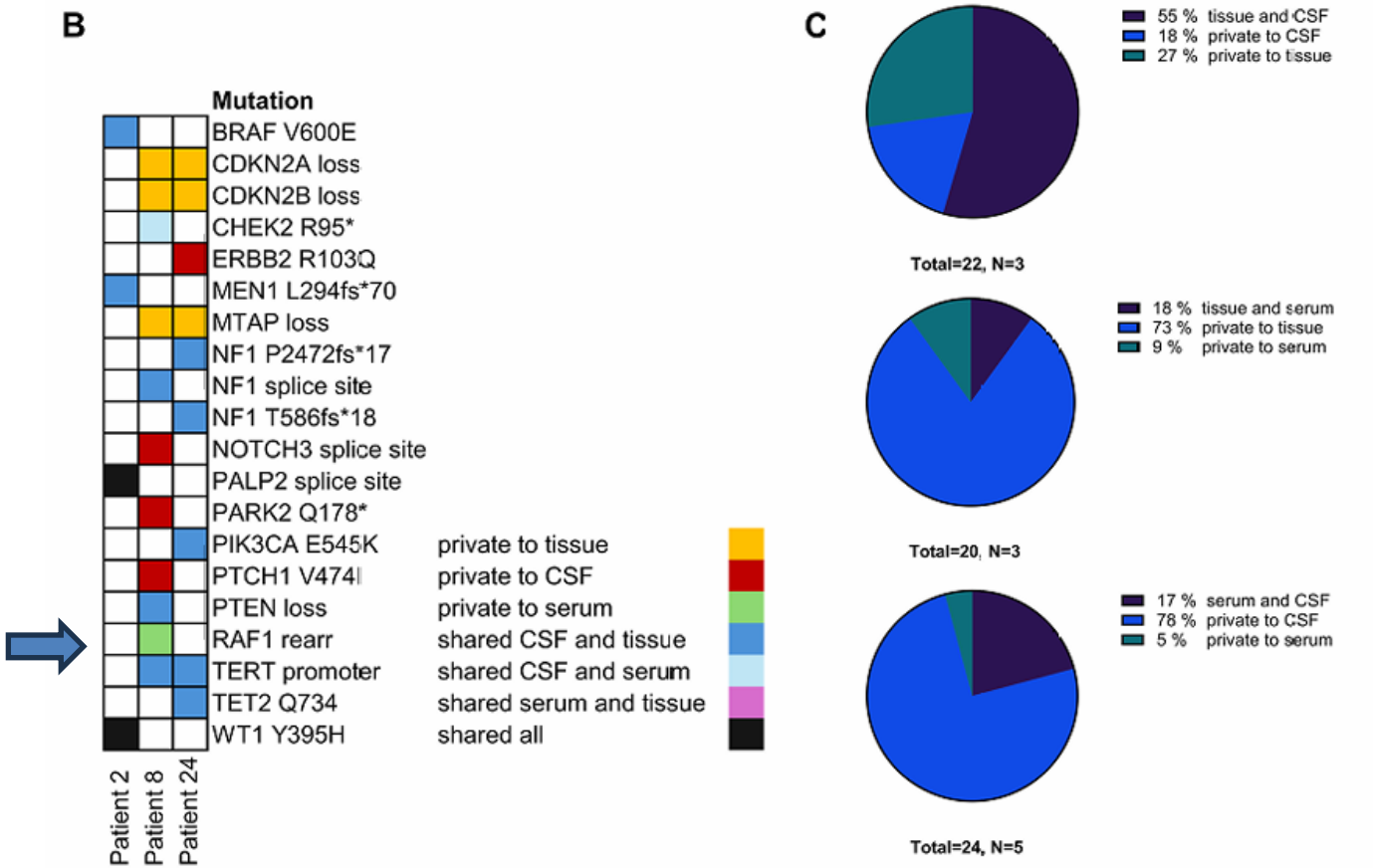
Abbreviations: AEG, esophagogastric junctional adenocarcinoma; CSF, cerebrospinal fluid; LPD, lymphoproliferative disorders; unknown, no final diagnosis available.





Köpp A et al. Neurooncol Adv 2026

CSF cfDNA – Primary brain tumors



Köpp A et al. Neurooncol Adv 2026

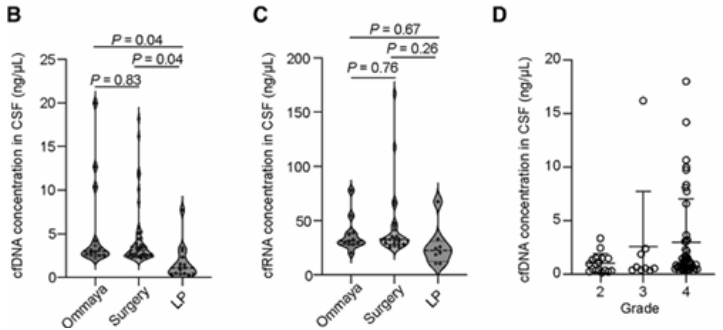
CSF cfDNA/cfRNA

- GlioKit: a cfDNA/cfRNA qPCR-based assay targeting 8 clinically relevant glioma genes (IDH1, IDH2, H3F3A, HIST1H3B, TERT, BRAF, EGFR, MET)
- 71 glioma CSF samples:
  - cfDNA mutations detected in 55 (77%) with a tumor-concordance rate of 89%
  - cfRNA alterations detected in 15/44 (34%) evaluable samples with tumor-concordance rate of 73%
- Comparison with NGS:
  - GlioKit: 33/35 (94.3%) sensitivity
  - NGS: 30/35 (85.7%) sensitivity

Table 2. Characteristics of cfDNA/cfRNA in the CSF of glioma patients

Variable		CSF samples
DNA sample size		71
DNA-positive		55/71
DNA-negative		16/71
Concordance of pathology for DNA		49/55
RNA sample size		44
RNA-positive		15/44
RNA-negative		29/44
Concordance of pathology for RNA		11/15
Frequency of pathogenic DNA mutated genes	IDH1_R132H	31/55
	IDH2_R172K	0/55
	H3C2-K28M	0/55
	H3-3A_K28M/ H3-3A_G35R/ H3-3A_G35V	4/55
	TERT-228CT/ TERT-250CT	25/55
	BRAF_V600E	2/55
Frequency of pathogenic RNA genes	EGFR Fusion	11/15
	MET Fusion	4/15

Abbreviation: CSF, cerebrospinal fluid.



Sun M et al. Neurooncol Adv 2026

CSF cfDNA – Primary brain tumors in children and AYA

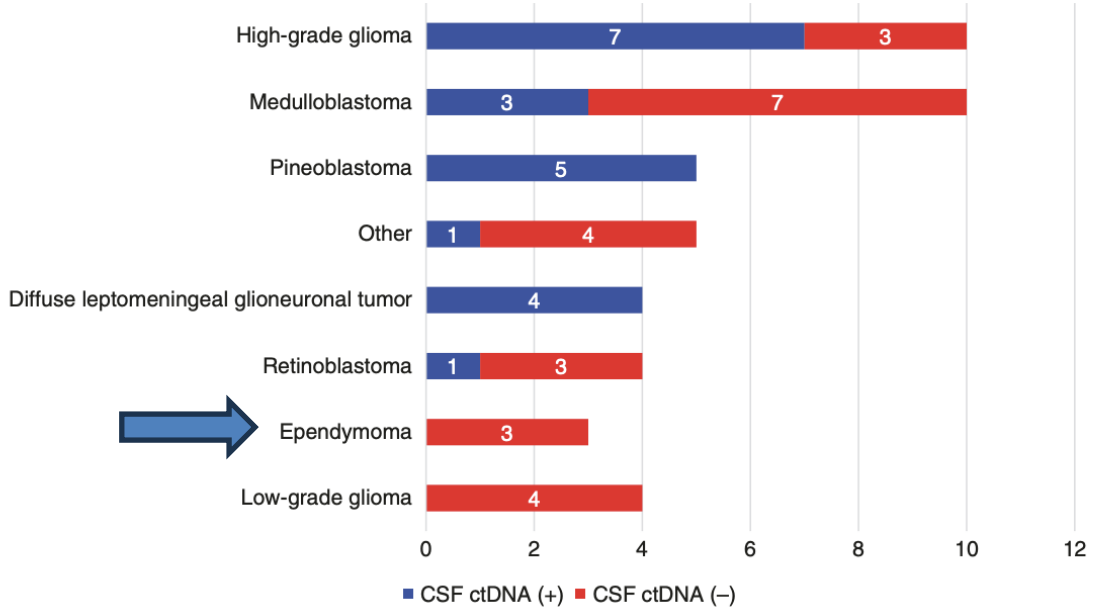
Table 1. Clinical Correlates of CSF cfDNA

Patient Characteristics (n = 45)	
Demographics	
Median age [years] (range)	14.4 (8 months–40 years)
Pediatric [*n*] (%)	25 (55.5)
Adolescent/Young Adult (AYA)	20 (44.4)
Male	30 (66.7)
Female	15 (33.3)
Pathological diagnosis	
High-grade glioma	10 (22.2)
Medulloblastoma	10 (22.2)
Pineoblastoma	5 (11.1)
Low-grade Glioma	4 (8.8)
Diffuse leptomeningeal glioneuronal tumor	4 (8.8)
Retinoblastoma	4 (8.8)
Ependymoma	3 (6.6)
Other	5 (11.1)
Number of samples per patient	
One sample	35 (77.8)
Two samples	6 (13.3)
Three or more samples	4 (8.9)
Sample Characteristics (n = 64)	
Disease stage at the time of CSF collection	
Newly diagnosed	16 (25.0)
Obtained at recurrence	21 (32.8)
Obtained during treatment	17 (26.6)
Obtained during surveillance	10 (15.6)
Disease status at the time of CSF collection	
Disseminated disease	27 (42.1)
Localized disease	24 (37.5)
No evidence of disease	13 (20.3)
Prior therapy	
Prior surgery	58 (90.6)
Prior treatment	46 (71.9)
Disseminated disease at the time of CSF collection	
Leptomeningeal disease (LMD) on imaging	26 (40.6)
Positive CSF cytology	13 (20.3)
LMD on imaging or positive CSF cytology	27 (42.2)

Miller A et al. Neuro-Oncology 2022

CSF cfDNA – Primary brain tumors in children and AYA

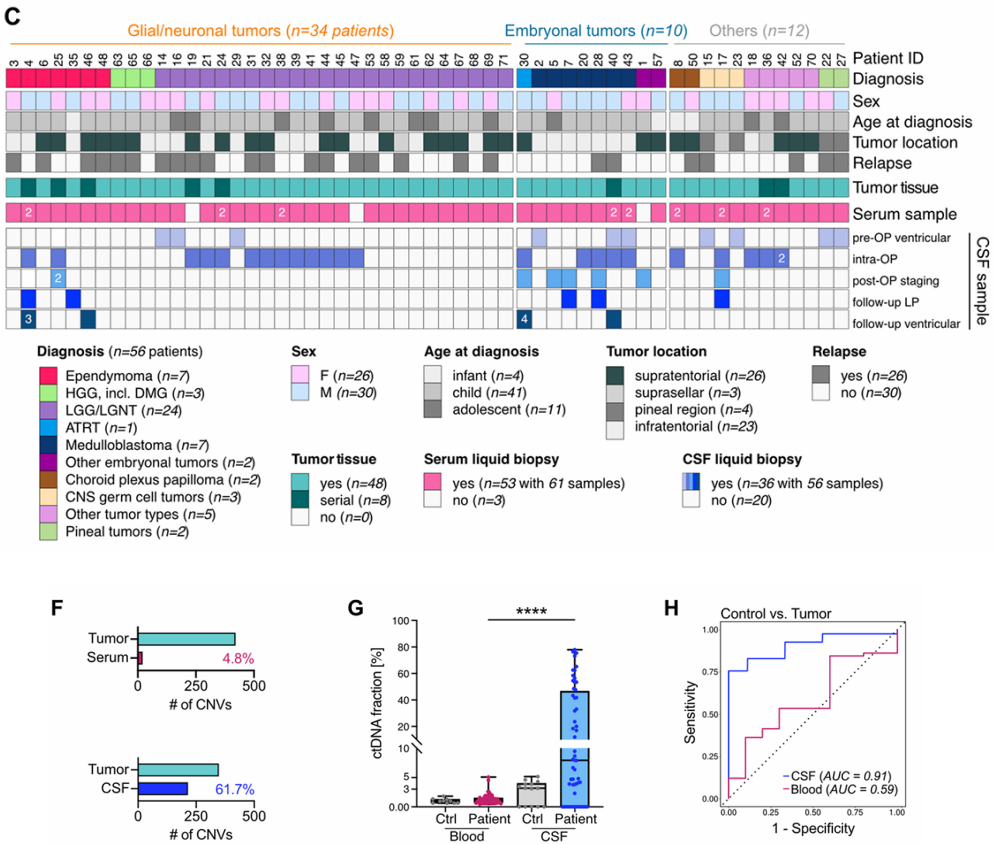
- Tumor derived mutations were detected in about 50% of samples
- CSF cfDNA was associated with the presence of disseminated disease



Miller A et al. Neuro-Oncology 2022

CSF cfDNA – Primary brain tumors in children and AYA

- Low-coverage WGS (lcWGS) assay from picogram-level cfDNA
- Analysis of 61 serum and 56 CSF samples
- Based on CNV, ctDNA was detected in 2/61 (3%) serum and 25/56 (45%) CSF samples
- Potential for disease monitoring



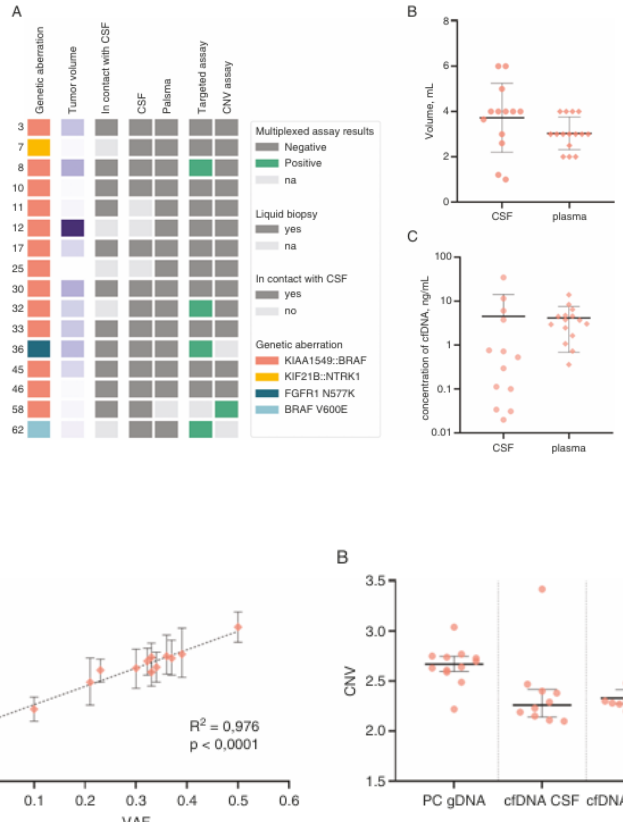
Fischer TT et al. Acta Neuropathol Commun 2025

CSF cfDNA – Evaluation of KIAA1549-BRAF fusion

- 5/13 positive liquid biopsy
  - 3/5 detection of KIAA1549-BRAF fusion (1 by CNV analysis and 2 by fusion-specific probe)
  - 2/5 SNV (1 BRAF V600E and 1 FGFR1 N577K)

Table 1. Clinical data and information on WGS results and CSF samples when available. The larger CNVs were detected on WGS data from tumor DNA.

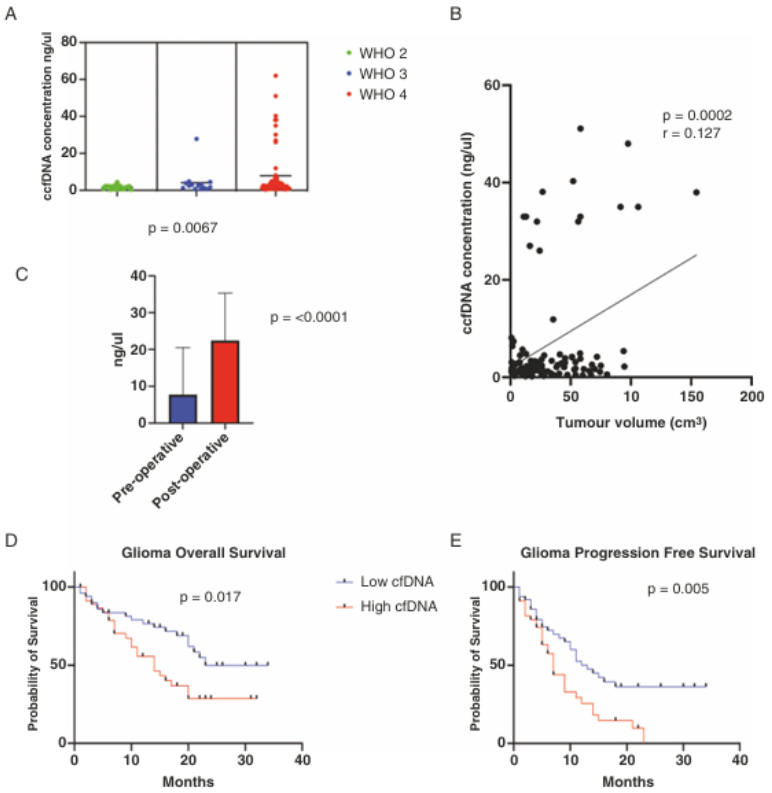
UPN	Age	Tumor size (cm^3)	Anatomical location	In contact with CSF	Method of CSF collection	Genetic aberrations in tumor	Larger CNVs	Relapse (Y/N)
11	9 years	3.5 × 2.5 × 4	4th ventricle	y	–	*KIAA1549::BRAF* fusion	chr 5,6 gain	Y
30	12 years	5.5 × 5 × 5	Vermis and cerebellum	y	Surgery	*KIAA1549::BRAF* fusion	–	N
32	3 years	5.5 × 4 × 4.5	Right cerebellar hemisphere	n	Surgery	*KIAA1549::BRAF* fusion	–	N
33	6 years	5.5 × 5 × 4	Vermis, 4th ventricle	y	Surgery	*KIAA1549::BRAF* fusion	–	N
10	8 years	6 × 1.5 × 2	Medulla oblongata, C4	y	Biopsy	*KIAA1549::BRAF* fusion	–	N
45	14 years	4 × 4.5 × 5	4th ventricle	y	Surgery	*KIAA1549::BRAF* fusion	–	N
46	7 years	3 × 3 × 2	4th ventricle	y	Surgery	*KIAA1549::BRAF* fusion	chr 7 gain	N
12	7 years	7 × 7 × 6	Supratentorial	n	–	*KIAA1549::BRAF* fusion	–	N
36	10 years	6.5 × 6.5 × 3	Frontal skull base	y	Biopsy	*FGFR1* N577K, *PTPN11* E76K)	chr 12 gain	N
3	13 years	4.6 × 4.7 × 5.4	Cerebellum	y	Surgery	*KIAA1549::BRAF* fusion	chr 5,6,7,11,12 gain	N
7	12 years	3 × 3 × 2.5	Right globus pallidus	n	Surgery	*KIF21B::NTRK1*, *NOS1AP::KIF21B* fusions	–	N
8	10 years	7 × 5 × 4	4th ventricle	y	Surgery	*KIAA1549::BRAF* fusion	–	Y
17	5 years	4 × 4.5 × 5	Cerebellum	y	Surgery	*KIAA1549::BRAF* fusion	–	Y
25	2 years	1 × 1 × 1	Cerebellum	n	–	*KIAA1549::BRAF* fusion	–	N
58	11 months	3,5 × 2,5 × 4	Spinal, leptomeningeal spreading mesencephalon, cerebellum, hippocampus	y	Lumbar puncture	N/A	N/A	Y
62	5 months	4.5 × 3.5 × 3	Supratentorial/suprasellar	n	VP-Shunt	*BRAF*V600E	N/A	N



Krynina O et al. Neurooncol Adv 2024

Plasma-based liquid biopsy

- 110 glioma patients
- 359 plasma samples (median 4 samples/patient)
- Method: ddPCR
- Total cfDNA associated with tumor grade, tumor volume, PFS and OS for all gliomas and glioblastoma subgroup, but not with postoperative tumor volume/progression
- IDH1 mutation overall sensitivity: 84% (77% in preoperative samples), but no association with pre-/postoperative progression or survival
- TERTp mutations sensitivity:
  - C228T: 88%
  - C250T: 49%
- EGFRvIII sensitivity: 71% (5/7)



Jones JJ et al. Neuroonc Adv 2024

Plasma-based liquid biopsy

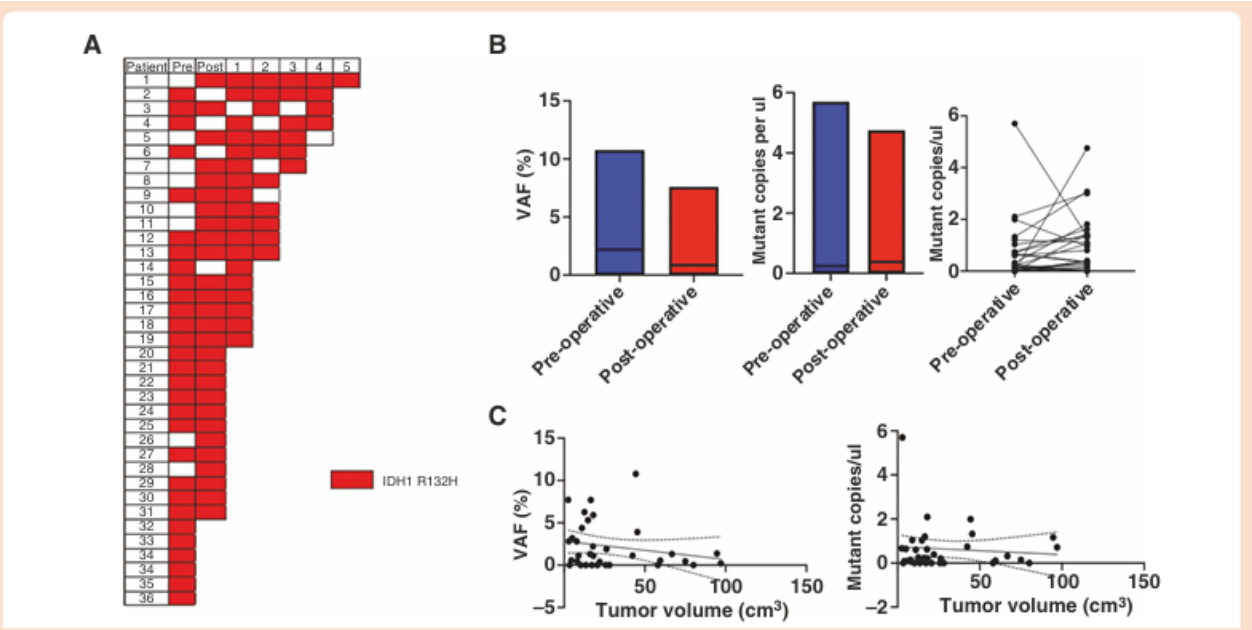
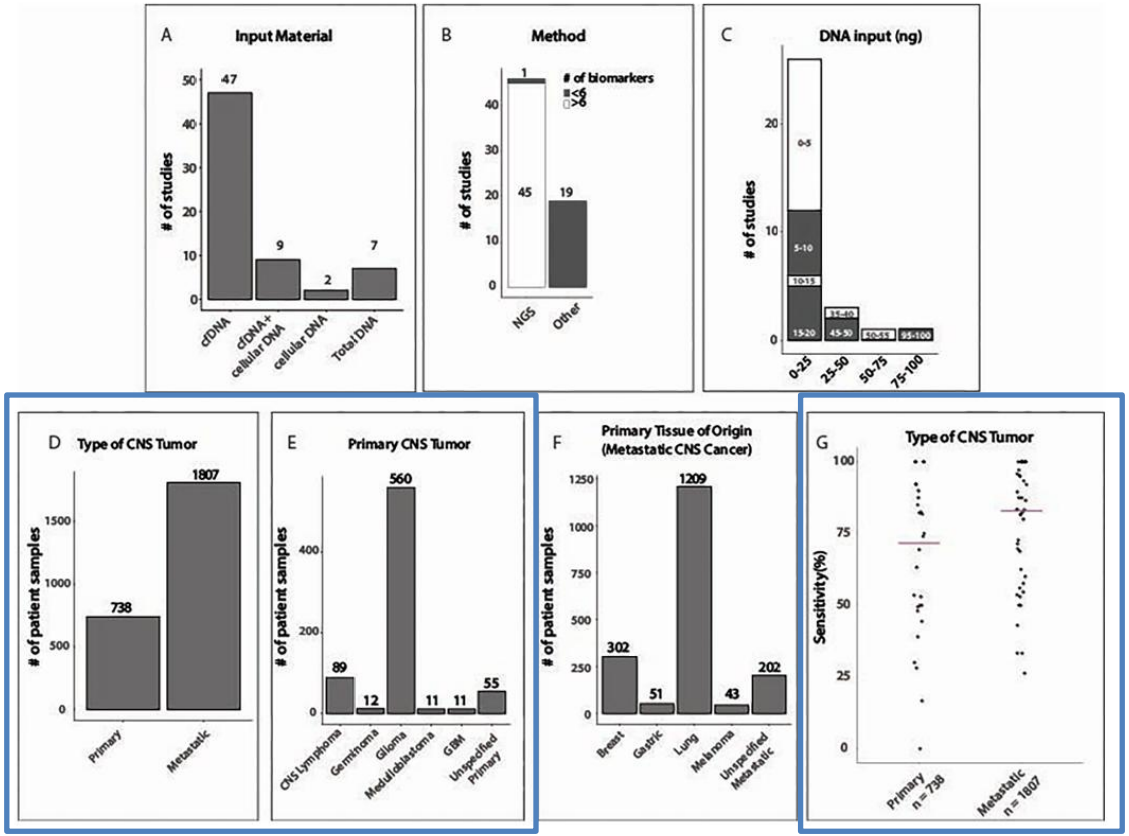


Figure 2. (A) Schematic showing results of IDH1m ddPCR in 36 patients, ranked from greatest to least by number of plasma samples. Red solid square = IDH1m detected, blank square = not detected. (B) Bar graphs showing mean levels of IDH1m preoperatively (blue) and postoperatively (red) with median value shown as black line. VAF% (left panel), mutant copies per milliliter (center panel), and paired difference (right panel). (C) Scatterplot showing correlation between MRI-based tumor volume and IDH1m VAF% (left panel) and mutant copies/mL (right panel). Trend lines with confidence intervals are indicated.

- 12 patients with 4 or more follow-up plasma samples available: IDH1m concentration and VAF were not relibly associated
- In 4/12 patients only mutant copy concentration and/or VAF reflected changes in tumor volume

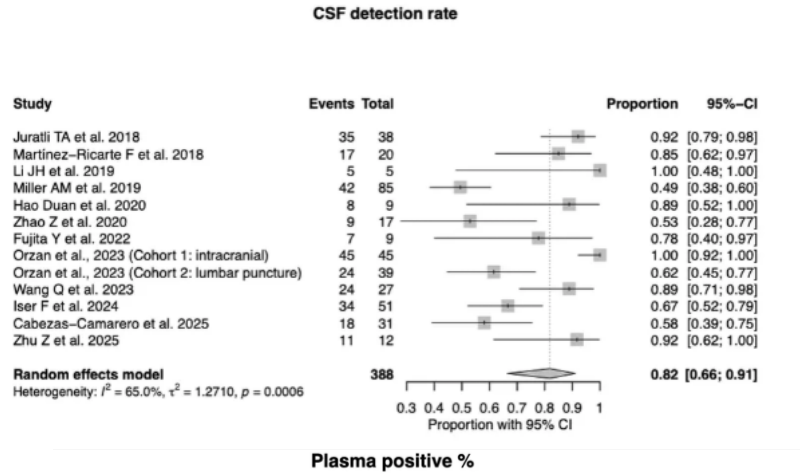
Jones JJ et al. Neuroonc Adv 2024

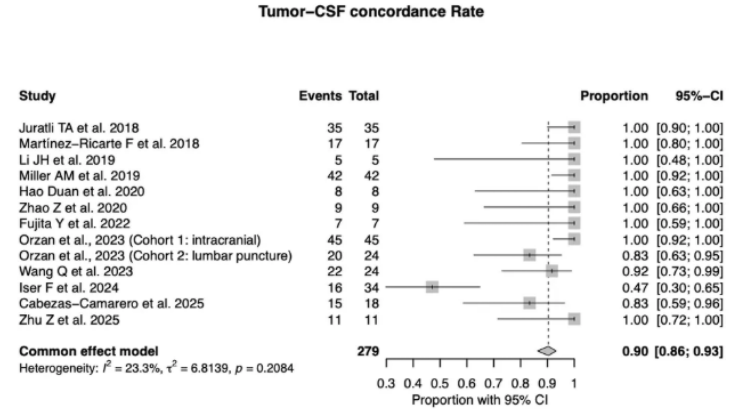
CSF-based liquid biopsy – Overall efficacy

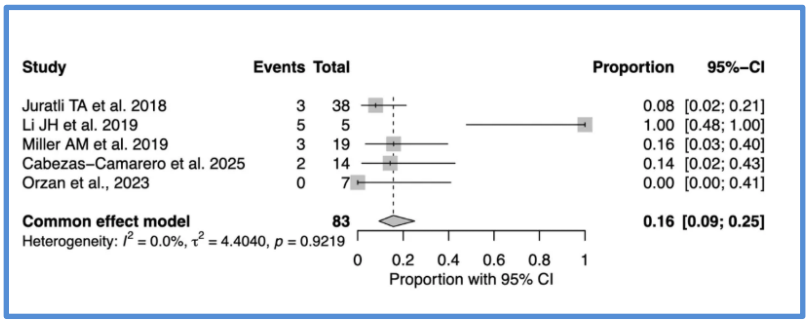


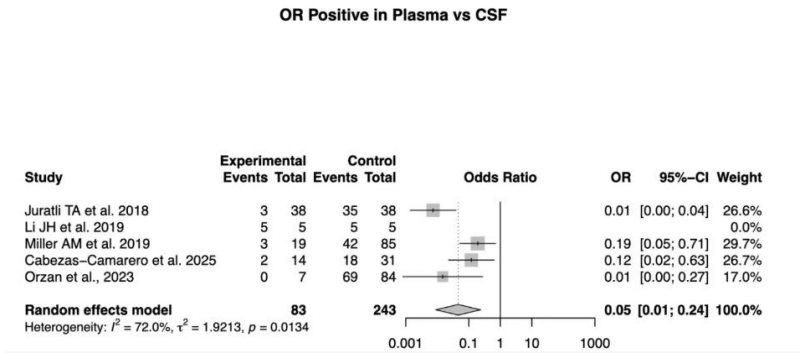
Khurana S et al. J Liq Biopsy 2025

Liquid biopsy for CNS tumors – Overall efficacy









Perez-Alfayate R et al. Front Oncol 2025

Disease monitoring

- PXA, BRAF V600E-mutant with extra-CNS metastases
- Monitoring by plasma LB using ddPCR for BRAF V600E during treatment with dabrafenib/trametinib

NIH NATIONAL CANCER INSTITUTE
Center for Cancer Research

METHYLATION PROFILING REPORT

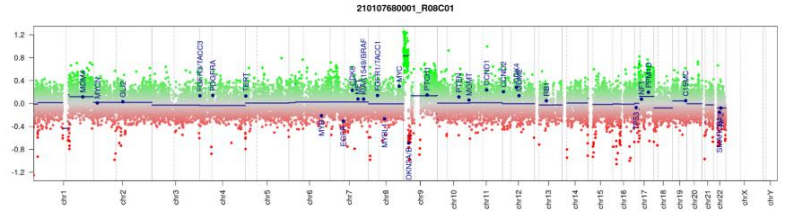
Top hit classifiers results

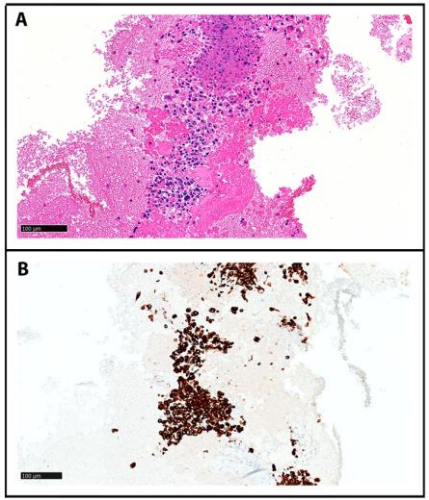
	Best.match	Scores	Comment
Family	Intermediate_grade_IDH_wildtype_gliomas	0.99	Matched
Class	PXA	0.986	Matched

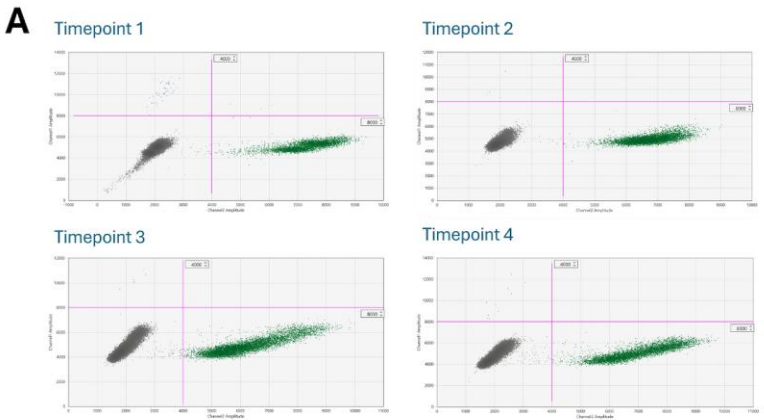
MGMT Status

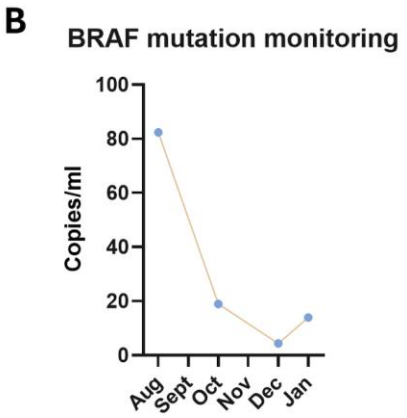
Promoter	Status	pred
MGMT	Methylated	0.6606759272972
		source: mgmstp27 R package

Copy Number Variation Profile



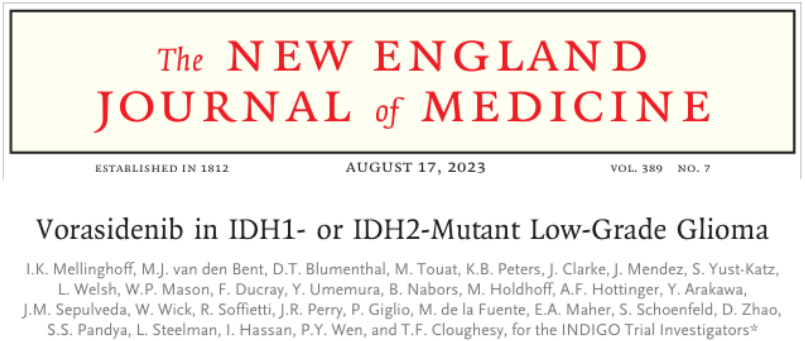






Raschio B et al. Neurooncol Adv (Submitted)

IDH-mutant gliomas: monitoring during vorasidenib treatment



Clinical/Neuro-oncological monitoring (Prof. Rudà)

- Tumor status/treatment
- Neurological status (including seizures)

Imaging

- Volumetric MRI (Prof. Morana)
- PET-based monitoring (Prof. Morbelli)

Liquid biopsy

- IDH mutation tracking (Prof. Bertero)
- 2-HG oncometabolite (Prof. Mengozzi)

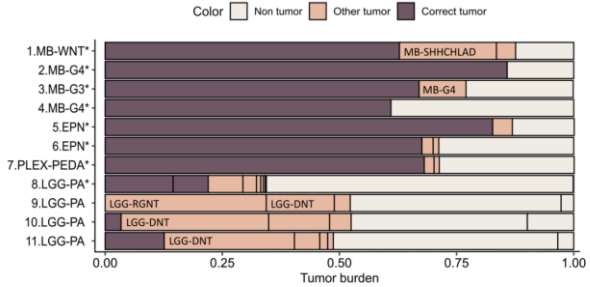
Fondazione VERONESI
Grant to AA Ricci

Ministero della Salute

INNOVA Advanced Diagnostic Platform

Progetto – PNC- E3-2022-23683266 – DIAGNOSTICA AVANZATA (HLS-DA) DAL TITOLO “INNOVA” – Piano Nazionale Complementare Ecosistema Innovativo della Salute

CSF cfDNA – DNA methylation profiling



Clinical Chemistry 70:1
250–260 (2024)

Cancer Diagnostics

Classification of Brain Tumors by Nanopore Sequencing of Cell-Free DNA from Cerebrospinal Fluid

Ann-Kristin Afflerbach,[a,b] Christian Rohrandt,[c] Björn Brändl,[d] Marthe Sönksen,[a] Jürgen Hench,[e] Stephan Frank,[e] Daniela Börnigen,[f] Malik Alawi,[f] Martin Mynarek,[a] Beate Winkler,[a] Franz Ricklefs,[g] Michael Synowitz,[h] Lasse Dührsen,[g] Stefan Rutkowski,[a] Annika K. Wefers,[i,j] Franz-Josef Müller,[d,k] Melanie Schoof,[a,b,†] and Ulrich Schüller[a,b,i,*,†]

Home › Acta Neuropathologica Communications › Article

DNA methylation profiling from cerebrospinal fluid as a diagnostic tool for pineoblastoma

Case Report | Open access | Published: 08 March 2025
Volume 13, article number 52 (2025) Cite this article

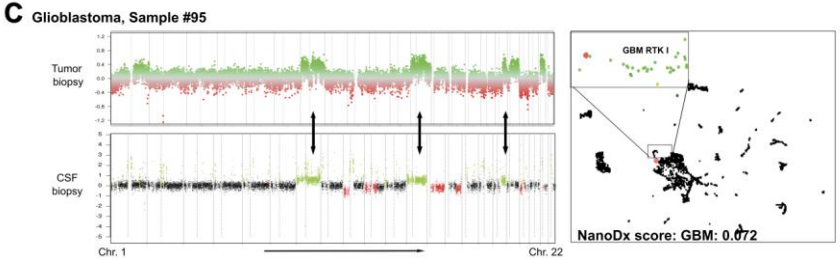
Cornelli *et al. Clinical Epigenetics* (2024) 16:87
https://doi.org/10.1186/s13148-024-01696-w

Clinical Epigenetics

RESEARCH Open Access

Diagnosis of pediatric central nervous system tumors using methylation profiling of cfDNA from cerebrospinal fluid

Lotte Cornelli[1,2,3], Ruben Van Paemel[1,3,4], Maísa R. Ferro dos Santos[1,2,3], Sofie Roelandt[1,2,3], Leen Willems[4,5], Jelle Vandersteene[6], Edward Baert[6], Liselot M. Mus[1,3,4], Nadine Van Roy[1,3], Bram De Wilde[1,3,4,5†] and Katleen De Preter[1,2,3*†]



EPIGENOMICS
2026, VOL. 18, NO. 3, 269–272
https://doi.org/10.1080/17501911.2026.2641518

Taylor & Francis
Taylor & Francis Group

EDITORIAL

Unlocking diagnostic potential: DNA methylation profiling in cerebrospinal fluid for central nervous system tumors

Celeste Antonacci, Luana Abballe, Sara Patrizi and Evelina Miele

Onco-Hematology, Cell Therapy, Gene Therapies and Hemopoietic Transplant, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

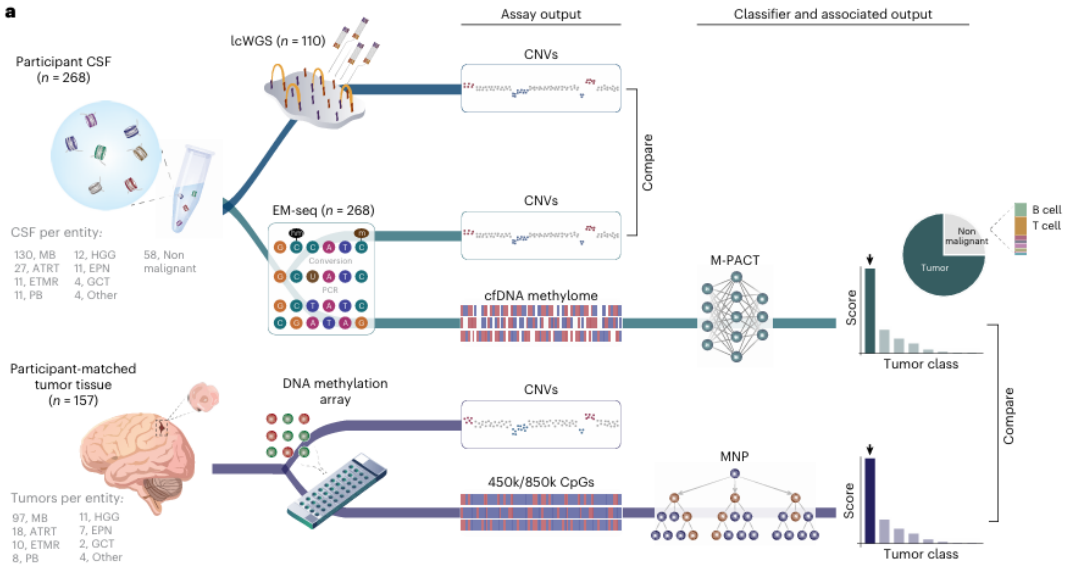
ARTICLE HISTORY Received 9 January 2026; Accepted 3 March 2026

KEYWORDS Central nervous system tumors; DNA methylation profiling; epigenetics; cerebrospinal fluid; liquid biopsy; molecular diagnostics; prognosis and disease monitoring

CSF cfDNA – DNA methylation profiling

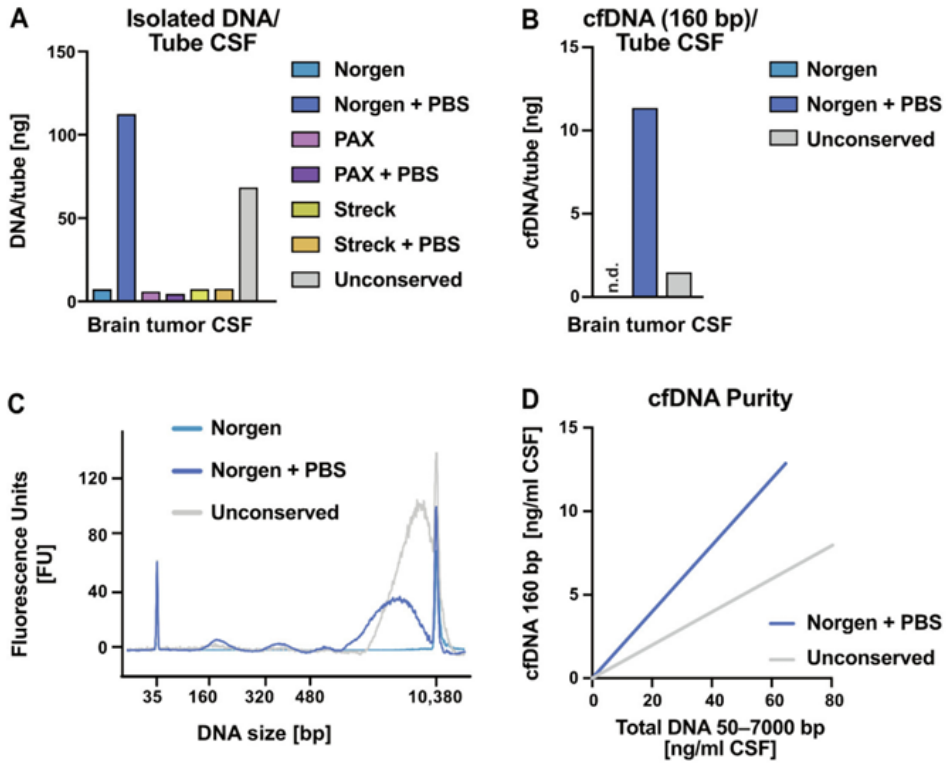
- M-PACT (methylation-based predictive algorithm for CNS tumors): a deep neural network to classify pediatric brain tumors from subnanogram-input cfDNA methylomes

- Evaluation in a benchmarking cohort of 79 embryonal CNS tumors and 58 validation cohort resulting 92% and 88% accuracy, respectively



Smith KS et al. Nat Cancer 2026

Pre-analytical workflow – CSF



Maass KK et al. Cancers 2021

«If the Mountain won't go…»

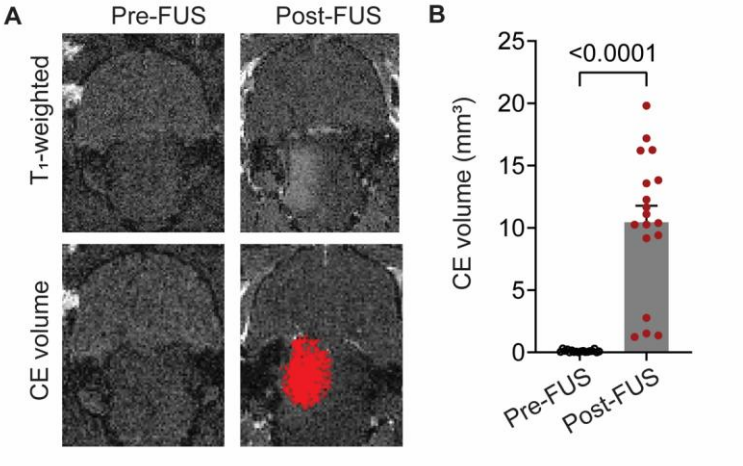
JOURNAL ARTICLE ACCEPTED MANUSCRIPT

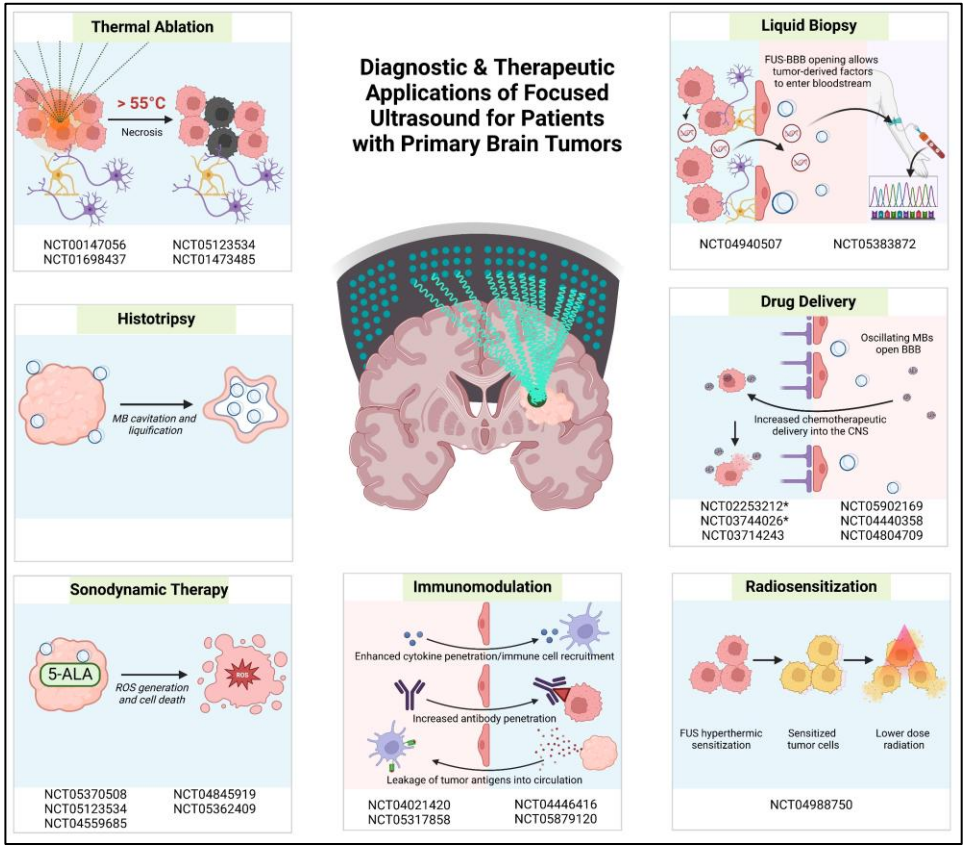
Noninvasive enrichment of circulating tumor biomarkers in a mouse model of diffuse midline glioma using focused ultrasound

Dingyue Zhang, Yimei Yue, Yan Gong, Leqi Yang, Kevin Xu, Jinyun Yuan ✉, Hong Chen ✉

Neuro-Oncology Advances, vdaf126, https://doi-org.bibliopass.unito.it/10.1093/noajnl/vdaf126

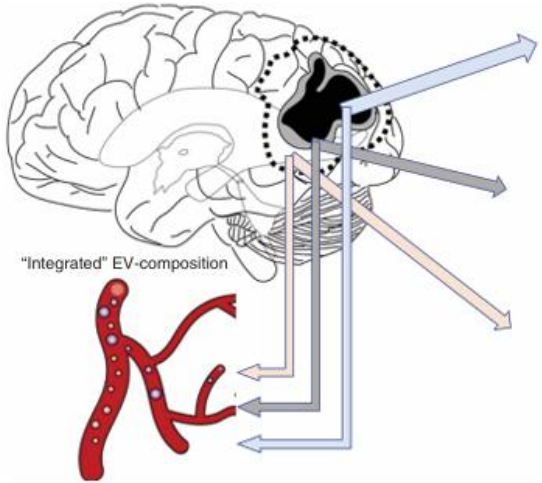
Published: 19 March 2026 **Article history** ▾





Ozair A et al. Neuroonc Adv 2026

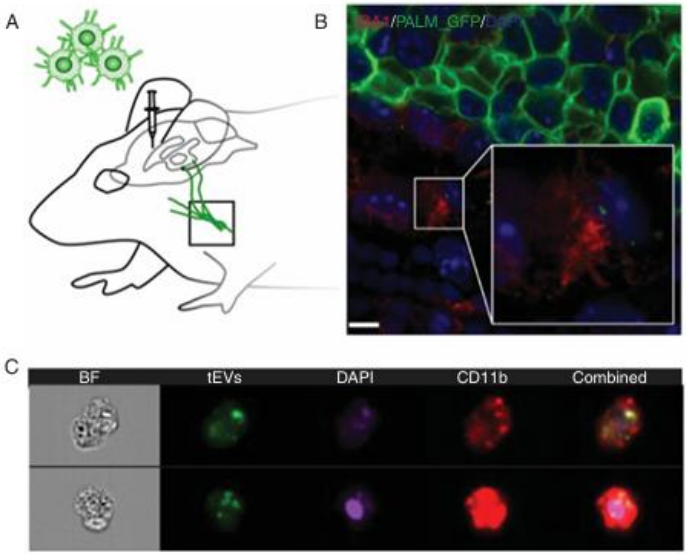
CTC and Extracellular Vesicles



Zone 1 : Necrosis
- Hypoxic endothelium
- Apoptotic cells (apoptotic bodies)
- Hypoxia adapted tumor cells
-

Zone 2 contrast enhancing tumor
- Classical/Proneural/Mesenchymal tumor cells
- Prenecrotic pseudopalisades
- Macrophages/Lympocytes/(Microglia)
- Endothelial cells

Zone 3 gradient of infiltrative cells
- Single glioma cells in "migration program"
- Reactive astrocytes, microglia, (macrophages)
- "Leaky" blood vessels



Westphal M et al. Neuroonc Adv 2022

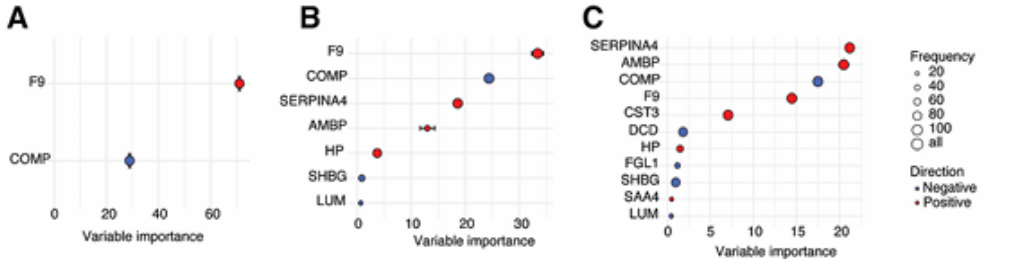
Proteomics

Neuro-Oncology Advances

8(1), vdag015, 2026 | https://doi.org/10.1093/noajnl/vdag015 | Advance Access publication February 5, 2026

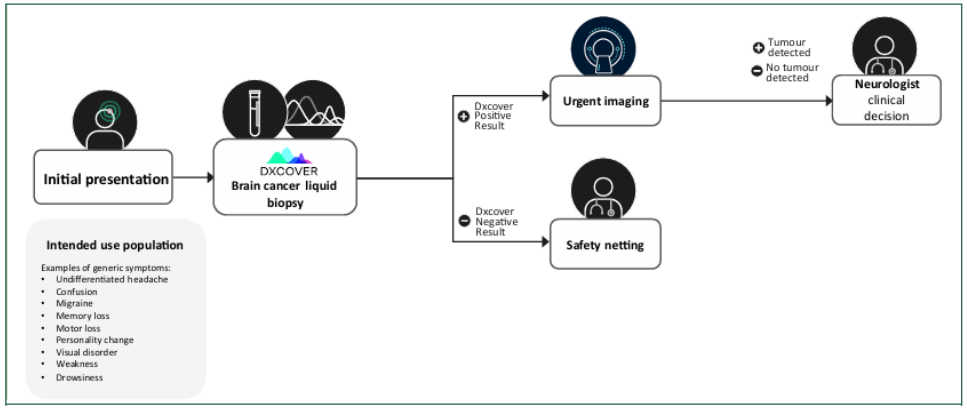
Noninvasive detection and monitoring of glioblastoma subtypes via dual-marker plasma proteomics

Patricia Rojas-Sanchez, Kirstine Juul-Elbaek, Henriette Pedersen, Dorte Schou Nørøxe, Aleena Azam, Hui Guo, Cong Zhou, Jiri Bartek, Jane Skjøth-Rasmussen, Ulrik Lassen, Erwin Schoff, and Petra Hamerlik



CNS tumors screening

- EMBRACE study aimed at earlier detection of brain tumors
- Spectroscopic-based liquid biopsy
- 2324 eligible patients enrolled at 7 centers
- Overall sensitivity: 77%
- Negative predictive value: 99%



		Count	Detected	Detection Rate %
Tumour Grade	1	127	89	70
	2	68	43	63
	3	20	16	80
	4	353	304	86
	Unknown	129	81	63
Tumour Group	Glioma	352	277	79
	Meningioma	152	101	66
	Brain Metastases	80	72	90
	Rare Brain Tumour	63	43	68
	Pituitary Tumours	30	21	70
	CNS Lymphoma	16	15	94
	Unclear	4	4	100

Brennan PM et al. ESMO Open 2026

Take home messages

- Liquid biopsy yields a great clinical potential in primary CNS tumors due to the clinical settings and the critical role played by molecular profiling in their diagnostic workup and monitoring

- This specific tumor location poses additional challenges which have to be addressed using highly-sensitivity methods and/or innovative approaches

- Novel strategies are enabling comprehensive molecular profiling including current diagnostic assays like tumor DNA methylation profiling

- Although clinical utility has been demonstrated in multiple settings, a role for the systematic implementation of liquid biopsy in the management of primary CNS tumors has to be defined yet

UNIVERSITÀ DI TORINO

AZIENDA OSPEDALIERO - UNIVERSITARIA
Città della Salute e della Scienza di Torino

Pathology Division,
Dept. of Medical Sciences

Luca Bertero
Alessia Andrea Ricci
Luca Mangherini
Paola Francia Di Celle
Paola Cassoni

Division of Radiotherapy, Dept. of Oncology

Umberto Ricardi
Mario Levis
Alessio Gastino
Erica Cuffini

Division of Neuroradiology

Giovanni Morana
Federica Ferrio
Annalisa Romano

Division of Neuro-Oncology,
Dept. of Neurosciences

Roberta Rudà
Alessia Pellerino
Marzia Borgognone
Francesco Bruno
Edoardo Pronello

Division of Neurosurgery, Dept. of Neurosciences

Diego Garbossa
Fabio Cofano
Flavio Panico
Francesca Rizzo