Supplementary Information for

Liquid biopsy of cerebrospinal fluid enables selective genetic profiling of glioma molecular subtypes at first clinical presentation

Francesca Orzan[1§], Francesca De Bacco[1,2§], Elisabetta Lazzarini[3§], Giovanni Crisafulli[4], Alessandra Gasparini[3], Angelo Dipasquale[5,6], Ludovic Barault[7,2], Marco Macagno[7], Pasquale Persico[5,6], Federico Pessina[5,6], Beatrice Bono[5], Laura Giordano[5], Pietro Zeppa[8], Antonio Melcarne[9], Paola Cassoni[10], Diego Garbossa[8], Armando Santoro[5,6], Paolo M. Comoglio[4], Stefano Indraccolo[3,11], Matteo Simonelli[5,6,#] and Carla Boccaccio[1,2,#]*

[1] Laboratory of Cancer Stem Cell Research, Candiolo Cancer Institute, FPO-IRCCS, 10060 Candiolo, Turin, Italy

[2] Department of Oncology, University of Turin Medical School, 10060 Candiolo, Turin, Italy

[3] Basic and Translational Oncology Unit, Veneto Institute of Oncology IOV-IRCCS, 35128 Padua, Italy

[4] IFOM ETS – The AIRC Institute of Molecular Oncology, 20139, Milan, Italy

[5] IRCCS Humanitas Research Hospital, 20089 Rozzano, Milan, Italy

[6] Department of Biomedical Sciences, Humanitas University, 20090 Pieve Emanuele, Milan, Italy

[7] Laboratory of Cancer Epigenetics, Candiolo Cancer Institute, FPO-IRCCS, 10060 Candiolo, Turin, Italy

[8] Department of Neurosciences, University of Turin Medical School, 10126 Turin, Italy

[9] Città della Salute e della Scienza, Turin, Italy

[10] Department of Medical Sciences, University of Turin Medical School, 10126 Turin, Italy

[11] Department of Surgery, Oncology and Gastroenterology, University of Padova, 35123 Padua, Italy

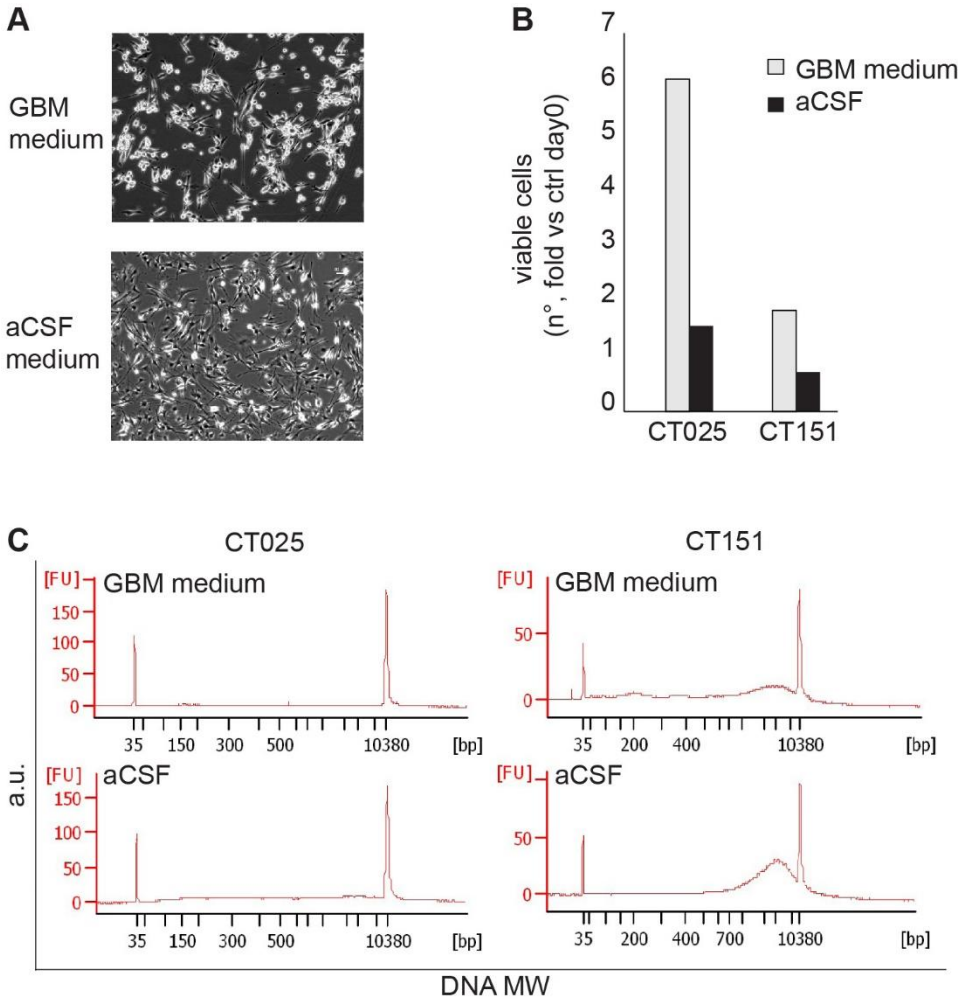
§Equal contribution

#Equal contribution

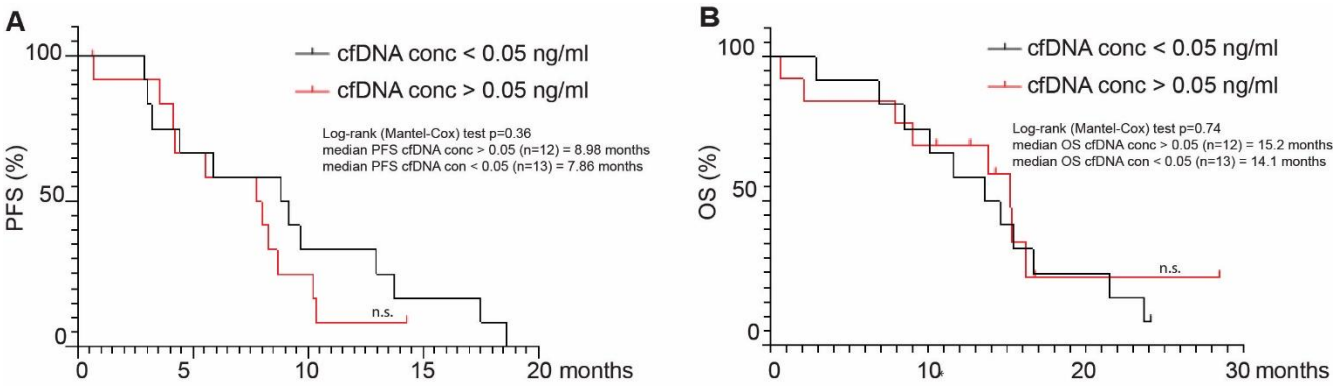
*Corresponding author: Carla Boccaccio, Candiolo Cancer Institute, FPO-IRCCS, Str. Prov. 142, Km. 3.95, 10060 Candiolo, Turin, Italy. Phone: +39-011-9933208. E-mail: carla.boccaccio@ircc.it.

Running title. Liquid biopsy of cerebrospinal fluid for glioma diagnosis

Inventory of Supplementary Information

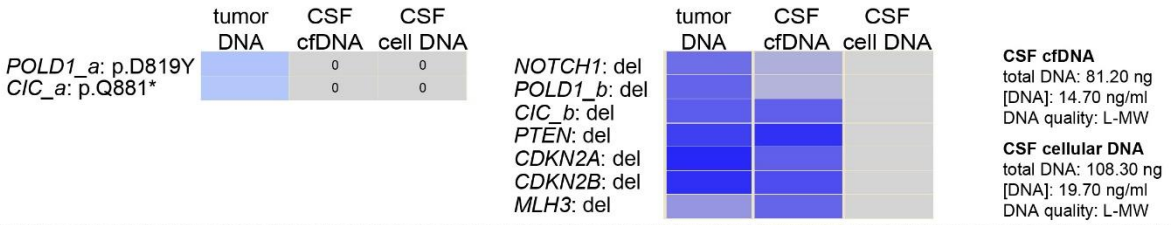


Supplementary Fig. S1 (related to Fig. 2D). Cell viability and DNA release in CSF. **A,** Representative images of CT025 GBM primary cells kept for 6 days in conventional culture medium (GBM medium, top) or in artificial cerebrospinal fluid (aCSF). **B,** Bar graph showing viability of CT025 and CT151 GBM primary cells after 6 days of culture in GBM medium or aCSF. Cells were counted and dead cells were excluded by trypan blue staining. Cell number relative increase vs. day 0 is shown. **C,** Molecular weight (MW) analysis of DNA released by CT025 or CT151 GBM primary cells in the supernatant after 6 days of culture in GBM medium or aCSF (Bioanalyzer output). In GBM medium, DNA release is minimal. In aCSF, high-MW DNA, typically associated with cell necrosis, is found, while low-MW DNA, resulting from apoptotic DNA nucleosomic fragmentation, is absent. bp = base pairs.

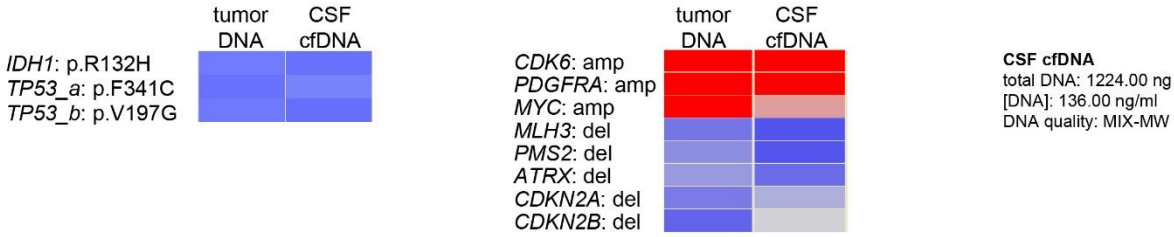


Supplementary Fig. S2 (related to Fig. 3E). LP-CSF DNA and prognostic correlations (Cohort 2). **A** and **B,** Correlation between cfDNA concentration in LP-CSF and progression free survival (PFS, **A**) and overall survival (OS, **B**), both not significant. 0.05 ng/ml: median cfDNA concentration in Cohort 2.

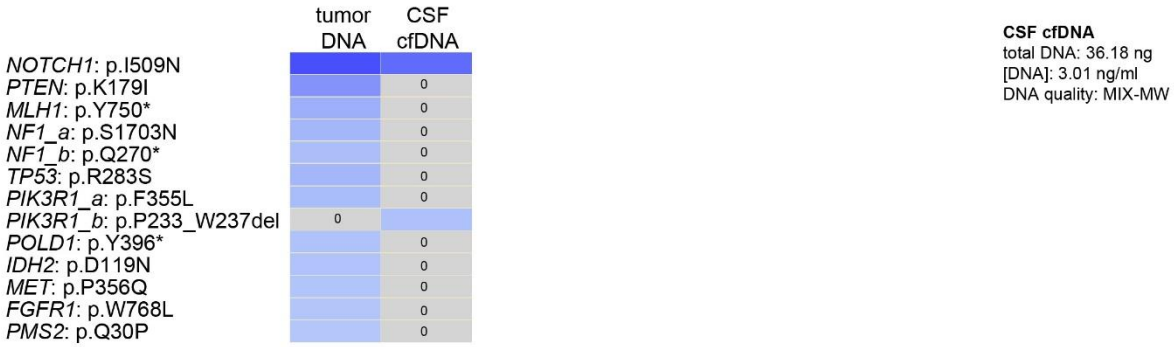
A MG2063 (CSF cfDNA + CSF cellular DNA)



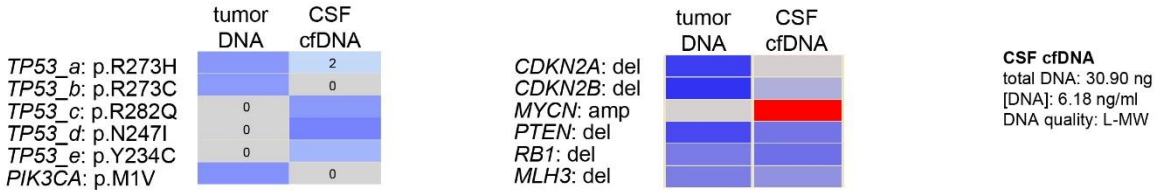
B MG1927 (CSF cfDNA)



C MG1942 (CSF cfDNA)

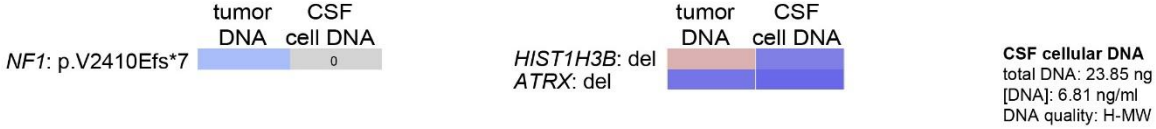


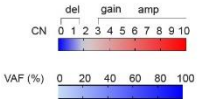
D MG2170 (CSF cfDNA)



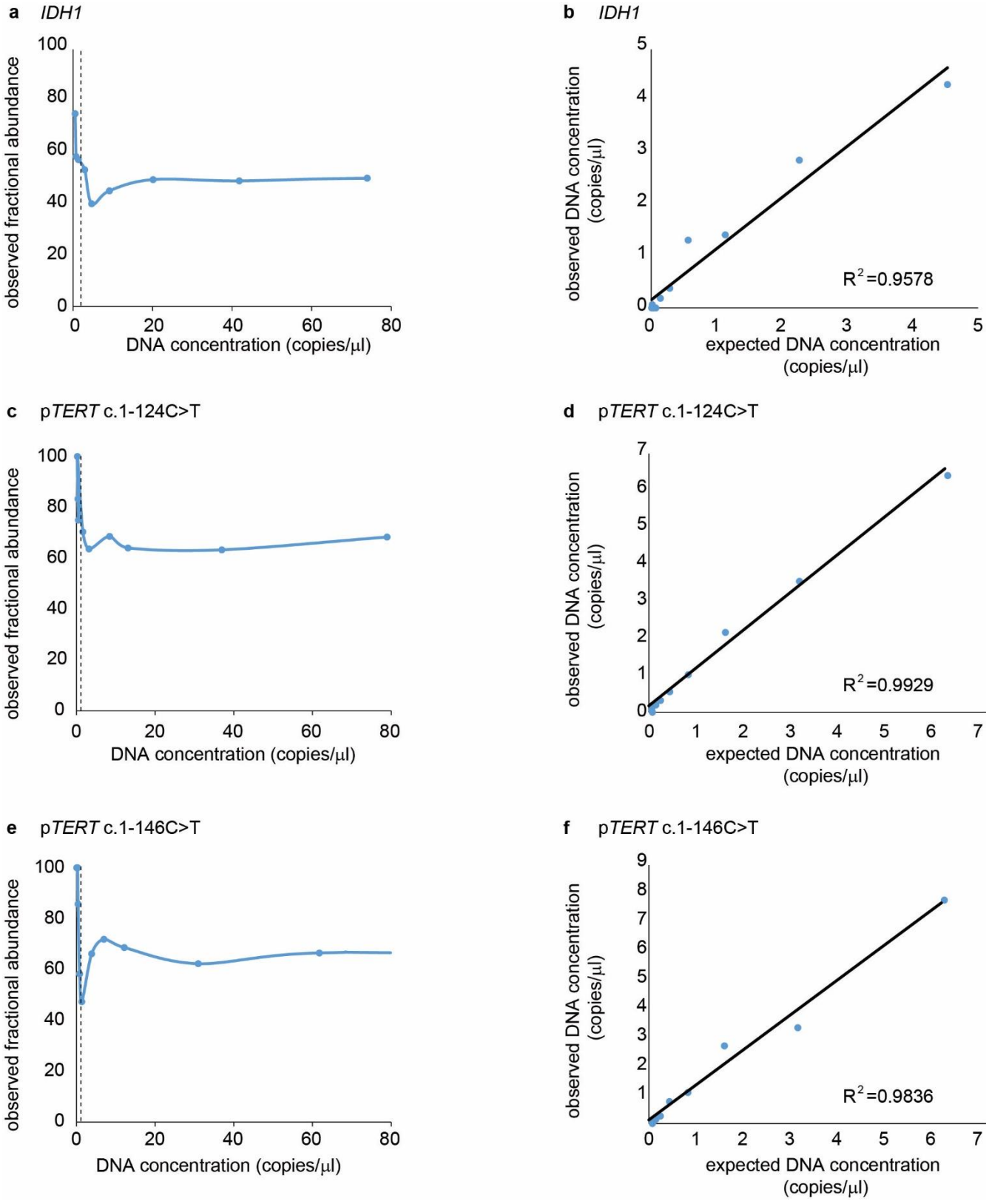
E MG2166 (CSF cellular DNA)

F MG2062 (CSF cellular DNA)





Supplementary Fig. S3 (related to Fig. 3G). Cohort 2, overall NGS analysis of tumor tissues and CSFs. **A-F,** Comparative analysis of DNA extracted from the indicated cases, each including tumor tissue and CSF (cfDNA from supernatant and/or cellular DNA from pellet). CSF DNA total quantity, concentration (ng/ml) and quality (L-MW: low-MW; H-MW: high-MW; MIX-MW: mixed high and low molecular weight) were indicated. Heatmaps represent gene copy number (CN) and variant allele frequency (VAF). VAF < 10% were reported.


a IDH1
observed fractional abundance
DNA concentration (copies/µl)
b IDH1
observed DNA concentration (copies/µl)
expected DNA concentration (copies/µl)
R2=0.9578
c pTERT c.1-124C>T
observed fractional abundance
DNA concentration (copies/µl)
d pTERT c.1-124C>T
observed DNA concentration (copies/µl)
expected DNA concentration (copies/µl)
R2=0.9929
e pTERT c.1-146C>T
observed fractional abundance
DNA concentration (copies/µl)
f pTERT c.1-146C>T
observed DNA concentration (copies/µl)
expected DNA concentration (copies/µl)
R2=0.9836

Supplementary Fig. S4 (related to Fig. 4C). Linearity of DNA quantification across a low concentration dynamic range. **A,** A range of input cell line DNA concentrations (0.1 to 80 copies/ul of reaction, sum of WT and mutant) was analyzed using the *IDH1* p.R132H in CT075 tumor. **B,** The average absolute output for the DNA concentrations (expected versus observed) is shown for the *IDH1* p.R132H. **C,** A range of input cell line DNA concentrations (0.1 to 80 copies/ul of reaction, sum of WT and mutant) was analyzed using the *TERT* c.1-124C>T in BT328 neurosphere line. **D,** The average absolute output for the DNA concentrations (expected versus observed) is shown for *TERT* c.1-124C>T. **E,** A range of input cell line DNA concentrations (0.1 to 80 copies/ul of reaction, sum of WT and mutant) was analyzed using the *TERT* c.1-146C>T in BT205 neurosphere line. **F,** The average absolute output for the DNA concentrations (expected versus observed) is shown for *TERT* c.1-146C>T.

Supplementary Table S1 (related to Fig. 1A and 2C). Cohort 1, clinical characteristics and tumor imaging features.

Sample ID	Age	Gender	Tumor location	Tumor area D (mm) x d (mm)	Contrast enhancement	Tumor abutting CSF space	CSF space	Diagnosis (WHO 2021)
CT025	81	F	T	28x16	yes	no	no	GBM IDH-wt
CT027	68	F	FT	63x57	yes	yes	ventricles	GBM IDH-wt
CT046	55	M	F	52x41	yes	yes	ventricles	GBM IDH-wt
CT047	64	F	TO	31x28	yes	no	no	GBM IDH-wt
CT048	57	F	FT	42x35	yes	no	no	GBM IDH-wt
CT052	66	F	insular (T)	27x20	yes	yes	ventricles	GBM IDH-wt
CT053	62	M	TP	40x36	yes	yes	ventricles	GBM IDH-wt
CT054	63	F	F	3x2	yes	no	no	GBM IDH-wt
CT056	37	F	PO	47x32	yes	no	no	GBM IDH-wt
CT058	72	M	T	29x20	yes	yes	cisterns	GBM IDH-wt
CT059	61	M	P	30x26	yes	no	no	GBM IDH-wt
CT062	75	M	T	34x28	yes	yes	ventricles	GBM IDH-wt
CT063	56	M	T	52x54	yes	yes	cisterns	GBM IDH-wt
CT064	40	M	F	33x24	yes	no	no	GBM IDH-wt
CT070	66	M	F	27x12	yes	no	no	GBM IDH-wt
CT073	73	F	F	17x15	yes	no	no	GBM IDH-wt
CT074	73	M	F	46x43	yes	yes	ventricles	GBM IDH-wt
CT075	40	F	F	14x13	yes	no	no	grade 3 IDH-mutant oligodendroglioma
CT076	68	F	T	8x7	yes	no	no	GBM IDH-wt
CT077	74	M	F	32x30	yes	no	no	GBM IDH-wt
CT078	63	M	insular (T)	49x30	yes	no	no	GBM IDH-wt
CT079	66	M	PO	40x36	yes	yes	ventricles	GBM IDH-wt
CT080	66	F	T	50x48	yes	yes	ventricles	GBM IDH-wt
CT081	47	F	T	34x23	yes	yes	ventricles	GBM IDH-wt
CT082	75	M	F	45x43	yes	no	no	GBM IDH-wt
CT084	69	M	F	18x15	yes	no	no	GBM IDH-wt
CT085	74	F	F	60x32	yes	yes	ventricles	GBM IDH-wt
CT086	66	F	multicentric	34x34	yes	yes	ventricles	GBM IDH-wt
CT087	76	F	multicentric	53x27	yes	yes	ventricles	GBM IDH-wt
CT088	68	F	T	41x23	yes	yes	ventricles	GBM IDH-wt
CT089	60	F	P	50x24	yes	yes	ventricles	GBM IDH-wt
CT092	42	M	F	24x18	yes	no	no	GBM IDH-wt
CT098	80	F	FT	44x43	yes	no	no	GBM IDH-wt
CT104	50	M	FT	5x6	yes	no	no	GBM IDH-wt
CT115_rec	44	M	FP	32x21	yes	no	no	recurrent GBM IDH-wt
CT116	76	M	F	25x15	yes	no	no	GBM IDH-wt
CT122	68	M	insular (F)	38x38	yes	no	no	GBM IDH-wt
CT124	62	M	multicentric	11x8	yes	yes	ventricles	GBM IDH-wt
CT132	44	F	FP	25x27	yes	yes	ventricles	grade 1 ganglioglioma
CT133	78	M	F	31x23	yes	no	no	GBM IDH-wt
CT137	63	M	T	34x20	yes	yes	ventricles	GBM IDH-wt
CT146	53	M	T	40x24	yes	no	no	GBM IDH-wt
CT149	58	F	insular (F)	41x36	yes	no	no	GBM IDH-wt
CT151	67	M	TPO	44x38	yes	yes	ventricles	GBM IDH-wt
CTR003_rec	54	F	TP	20x15	yes	no	no	recurrent grade 4 IDH-mutant astrocytoma

Tumor location: T, temporal; F, frontal; P, parietal; O, occipital.

Supplementary Table S2 (related to Fig. 1B). Cohort 1, genetic alterations in tumors (targeted analysis: Sanger sequencing and qPCR).

Sample ID	Mutation					CNV			
	IDH1	*IDH2*	*TERT promoter*	*PTEN*	*TP53*	*EGFR*	*CDKN2A*	*PDGFRA*	*CDK4*
CT025	wt	wt	c.1-124C>T	wt	wt	wt	del	amp	wt
CT027	wt	wt	c.1-124C>T	del	p.R306*	wt	wt	wt	amp
CT046	wt	wt	c.1-124C>T	wt	wt	wt	wt	wt	amp
CT047	wt	wt	c.1-124C>T	p.335*	wt	wt	del	wt	wt
CT048	wt	wt	wt	wt	wt	wt	wt	wt	gain
CT052	wt	wt	c.1-124C>T	p.T319fs*4	wt	wt	del	wt	wt
CT053	wt	wt	c.1-124C>T	p.P95delP	p.R196P	gain	wt	gain	amp
CT054	wt	wt	c.1-124C>T	wt	wt	amp	del	wt	wt
CT056	wt	wt	c.1-124C>T	del	N/A	amp	del	gain	wt
CT058	wt	wt	c.1-124C>T	wt	wt	wt	wt	wt	wt
CT059	wt	wt	c.1-124C>T	p.I32T	wt	amp	del	wt	wt
CT062	wt	wt	c.1-146C>T	wt	wt	amp	wt	wt	wt
CT063	wt	wt	c.1-124C>T	wt	c.673-21_677TGGGCCTGTGTTATCTCCTAGGTTGG>AGGGCCTGTGTTAT	gain	wt	wt	wt
CT064	wt	wt	c.1-124C>T	p.N329fs*14 + p.K330N + p.K332R	wt	amp	wt	wt	amp
CT070	wt	wt	c.1-146C>T	p. K183fs*15	p.H214R	wt	del	amp	wt
CT073	wt	wt	c.1-124C>T	wt	wt	gain	wt	gain	gain
CT074	wt	wt	c.1-124C>T	wt	wt	gain	wt	wt	wt
CT075	p.R132H	wt	c.1-124C>T	wt	p.R213R + p.R273C	wt	wt	gain	amp
CT076	wt	wt	c.1-124C>T	wt	wt	gain	wt	gain	gain
CT077	wt	wt	c.1-124C>T	del	p.G245C	gain	wt	wt	amp
CT078	wt	wt	c.1-146C>T	wt	wt	amp	wt	wt	amp
CT079	wt	wt	c.1-124C>T	p.R130*	p.M246R	wt	del	amp	amp
CT080	wt	wt	c.1-124C>T	wt	p.R248Q	wt	wt	wt	amp
CT081	wt	wt	c.1-124C>T	wt	wt	gain	wt	wt	wt
CT082	wt	wt	c.1-124C>T	wt	wt	amp	del	amp	amp
CT084	wt	wt	c.1-124C>T	del	wt	amp	del	wt	wt
CT085	wt	wt	wt	p.E43V + p.G208D	p.L308Afs*32	amp	del	wt	gain
CT086	wt	wt	c.1-124C>T	del	wt	amp	del	wt	wt
CT087	wt	wt	c.1-124C>T	p.S229*	wt	amp	del	wt	wt
CT088	wt	wt	c.1-146C>T	del	wt	wt	del	wt	wt

continued

Sample ID	Mutation					CNV			
	IDH1	***IDH2***	***TERT promoter***	***PTEN***	***TP53***	***EGFR***	***CDKN2A***	***PDGFRA***	***CDK4***
CT089	wt	wt	c.1-124C>T	wt	p. C275Y	wt	wt	wt	wt
CT092	wt	wt	c.1-124C>T[a]	wt	wt	wt	wt	wt	wt
CT098	wt	wt	c.1-124C>T + c.1-146C>T	wt	wt	wt	wt	wt	wt
CT104	wt	wt	wt	wt	wt	wt	wt	wt	wt
CT115_rec	wt	wt	c.1-124C>T	wt	wt	wt	wt	wt	wt
CT116	wt	wt	c.1-124C>T	wt	wt	wt	wt	wt	wt
CT122	wt	wt	c.1-146C>T	p.D331fs*13	wt	wt	wt	gain	wt
CT124	wt	wt	wt	wt	wt	wt	wt	wt	gain
CT132	wt	wt	wt	wt	wt	wt	wt	wt	wt
CT133	wt	wt	c.1-124C>T	del	wt	amp	del	wt	wt
CT137	wt	wt	c.1-124C>T	wt	wt	wt	wt	wt	wt
CT146	wt	wt	c.1-146C>T	wt	wt	amp	del	wt	wt
CT149	wt	wt	wt	wt	p.R282W	wt	del	wt	wt
CT151	wt	wt	c.1-124C>T	wt	wt	wt	del	wt	gain
CTR003_rec	p.R132H	wt	wt	wt	p.R273C	wt	wt	amp	wt

wt: wild-type; gain: 3<CNV<5; amp: CNV>5; del: CNV<1.5.

[a]: VAF = 0.2%, considered not informative for further analysis in CSF DNA.

Supplementary Table S3 (related to Fig. 2A-D). Cohort 1, features of peritumoral CSF and its DNA content.

Sample ID	Site of sampling	CSF volume (ml)	Blood traces	Total cfDNA amount (ng)	cfDNA concentration (ng/ml)	cfDNA molecular weight (MW)
CT025	subarachn. sp.	5.00	no	296.80	59.36	H-MW
CT027	subarachn. sp.	0.35	no	72.80	208.00	H-MW
CT046	subarachn. sp.	0.14	yes	49.56	354.00	L-MW
CT047	subarachn. sp.	0.27	no	70.00	259.26	L-MW
CT048	subarachn. sp.	0.46	no	17.80	38.70	n.d.
CT052	subarachn. sp.	1.97	yes	57.96	29.42	n.d.
CT053	subarachn. sp.	0.85	yes	50.12	58.96	n.d.
CT054	subarachn. sp.	0.62	no	55.44	89.42	L-MW
CT056	subarachn. sp.	0.07	no	14.20	202.86	H-MW
CT058	subarachn. sp.	1.31	no	194.48	148.46	MIX-MW
CT059	subarachn. sp.	0.45	no	47.32	105.16	n.d.
CT062	subarachn. sp.	0.59	no	59.64	101.08	H-MW
CT063	subarachn. sp.	0.10	no	0.00	0.00	n.d.
CT064	subarachn. sp.	0.41	no	13.40	32.68	n.d.
CT070	subarachn. sp.	0.20	no	61.32	306.60	n.d.
CT073	subarachn. sp.	0.75	no	0.00	0.00	n.d.
CT074	subarachn. sp.	0.97	yes	79.52	81.98	n.d.
CT075	subarachn. sp.	0.50	no	38.60	77.20	H-MW
CT076	subarachn. sp.	0.31	yes	135.10	435.81	n.d.
CT077	subarachn. sp.	0.08	no	34.60	411.91	MIX-MW
CT078	subarachn. sp.	0.85	yes	1740.00	2047.06	H-MW
CT079	subarachn. sp.	0.27	no	133.00	492.59	MIX-MW
CT080	subarachn. sp.	0.81	no	12.60	15.56	H-MW
CT081	subarachn. sp.	1.05	no	48.72	46.40	L-MW
CT082	subarachn. sp.	0.45	no	153.00	340.00	MIX-MW
CT084	subarachn. sp.	0.19	no	0.00	0.00	n.d.
CT085	subarachn. sp.	0.53	yes	45.40	85.66	H-MW
CT086	subarachn. sp.	0.75	yes	21.20	28.27	H-MW
CT087	subarachn. sp.	0.25	yes	145.00	580.00	MIX-MW
CT088	ventricle	0.96	yes	5180.00	5395.83	H-MW
CT089	ventricle	1.50	no	19320.00	12880.00	H-MW
CT092	subarachn. sp.	1.10	no	36.68	33.35	n.d.
CT098	subarachn. sp.	0.22	no	13.60	61.82	H-MW
CT104	subarachn. sp.	5.24	no	24.00	4.58	H-MW
CT115_rec	subarachn. sp.	1.40	no	0.00	0.00	L-MW
CT116	subarachn. sp.	1.68	no	856.00	509.52	H-MW
CT122	subarachn. sp.	2.13	no	21.60	10.14	H-MW
CT124	subarachn. sp.	1.40	no	19.80	14.14	H-MW
CT132	subarachn. sp.	0.80	no	0.00	0.00	n.d.

continued

Sample ID	Site of sampling	CSF volume (ml)	Blood traces	Total cfDNA amount (ng)	cfDNA concentration (ng/ml)	cfDNA molecular weight (MW)
CT133	subarachn. sp.	0.25	no	0.00	0.00	n.d.
CT137	subarachn. sp.	1.40	yes	13640.00	9742.86	MIX-MW
CT146	subarachn. sp.	0.30	no	0.00	0.00	n.d.
CT149	subarachn. sp.	1.50	no	73.40	48.93	MIX-MW
CT151	subarachn. sp.	4.40	no	104.47	23.74	MIX-MW
CTR003_rec	subarachn. sp.	4.90	no	480.00	97.96	L-MW

Peritumoral CSFs assessed in targeted NGS are highlighted in blue.
subarachn. sp.: subarachnoid space.
DNA molecular weight: H-MW, high molecular weight; L-MW, low molecular weight; MIX-MW, mixed high and low molecular weight.
n.d.: not detectable for DNA concentration under sensitivity threshold or for poor quality of Bioanalyzer profile.

Supplementary Table S4 (related to Fig. 2E and F). Cohort 1, genetic alterations in CSF and tumors (targeted analysis, ddPCR).

Sample ID	Gene	cDNA/amino acid variant	Tumor DNA		CSF cfDNA		Plasma cfDNA	
			CNV	VAF (%)	CNV	VAF (%)	CNV	VAF (%)
CT025	*CDKN2A*		0.76		0.90			
CT027	*TP53*	p.R306*		17.00		31.10		
	CDK4		32.60		24.40			
CT046	*CDK4*		18.70		10.00		2.70	
CT047	*PTEN*	p.R335*		46.45		41.60		0.00
CT052	*PTEN*	p.T319fs*1		41.85		0.00		0.00
CT053	*CDK4*		40.95			4.60		2.90
CT054	*pTERT*	c.1-124C>T		23.20		0.00		
	CDKN2A		0.93		no amp		no amp	
	EGFR		11.91		2.00		2.00	
CT056	*EGFR*		6.05		3.90			
	pTERT	c.1-124C>T		38.41		5.16		
CT058	*pTERT*	c.1-124C>T		11.08		no amp		
CT059	*CDKN2A*		0.44		no amp		no amp	
	EGFR		44.75		no amp		1.70	
CT062	*EGFR*		6.41		5.70		2.20	
CT063	*pTERT*	c.1-124C>T		29.50		0.00		0.00
CT064	*CDK4*		10.39		8.00			
CT070	*PDGFRA*		6.71		no amp			
CT073	*CDKN2A*		1.55		1.20			
CT074	*pTERT*	c.1-124 C>T		43.80		0.00		
CT075	*CDK4*		27.65		1.95			
	IDH1	p.R132H		46.20		0.00		
CT076	*PDGFRA*		2.12		5.10			
CT077	*pTERT*	c.1-124C>T		37.86		27.54		
CT078	*CDK4*		33.50		11.50			
CT079	*PDGFRA*		8.90		19.90			
CT080	*CDK4*		15.20		9.10			
CT081	*pTERT*	c.1-124C>T		1.50		0.00		
CT082	*EGFR*		25.40		25.80			
CT084	*EGFR*		71.30		2.45			
	pTERT	c.1-124C>T		31.00		no amp		
CT085	*EGFR*		33.50		26.70			
CT086	*EGFR*		51.30		2.35			
	pTERT	c.1-124C>T		38.01		0.00		
CT087	*EGFR*		45.30		43.00			

continued

Sample ID	Gene	cDNA/amino acid variant	Tumor DNA		CSF cfDNA		Plasma cfDNA	
			CNV	VAF (%)	CNV	VAF (%)	CNV	VAF (%)
CT088	*CDKN2A*		1.60		2.04			
	pTERT	c.1-146C>T		10.35		7.36		
CT089	*TP53*	p.C275Y		39.50		83.80		
CT098	*pTERT*	c.1-124C>T		1.50		0.00		
CT115_rec	*pTERT*	c.1-124C>T		0.27		8.50		
CT116	*pTERT*	c.1-124C>T		14.90		12.10		
CT122	*pTERT*	c.1-146C>T		15.00		0.00		
CT133	*EGFR*		27.50		no amp			
	pTERT	c.1-124C>T		12.48		no amp		
CT137	*pTERT*	c.1-124C>T		22.57		12.09		
CT146	*EGFR*		32.60		15.80			
	pTERT	c.1-146C>T		30.48		4.40		
CT149	*CDKN2A*		1.35		2.05			
	TP53	p.R282W		24.00		0.00		
CT151	*pTERT*	c.1-124C>T		2.50		6.60		
CTR003_rec	*IDH1*	p.R132H		15.10		41.10		

no amp: ddPCR failure.

Supplementary Table S5 (related to Fig. 2I). Cohort 1, NGS analysis of tumors and CSFs.

Sample ID		SNV[a]				CNV[b]		
		Gene ID	cDNA Variant	Amino Acid Variant	VAF (%)	Gene ID	Amp/Del	CN
CT087	tumor DNA	*PTEN*	c.686C>A	p.S229*	25.00	*EGFR*	amp	41.00
		ASCL1	c.114_128del	p.A43_A47del	18.99			
	peritumoral CSF cfDNA	*PTEN*	c.686C>A	p.S229*	32.00	*EGFR*	amp	32.50
		ASCL1	c.114_128del	p.A43_A47del	17.86			
CT116	tumor DNA	*RB1*	c.1703dup	p.L569Ffs*3	9.23			
		TP53	c.814G>A	p.V272M	5.90			
		TP53	c.796G>A	p.G266R	10.01			
		PIK3R1	c.1392_1403dup	p.D464_Y467dup	0.00			
	peritumoral CSF cfDNA	*RB1*	c.1703dup	p.L569Ffs*3	19.67			
		TP53	c.814G>A	p.V272M	12.15			
		TP53	c.796G>A	p.G266R	21.65			
		PIK3R1	c.1392_1403dup	p.D464_Y467dup	11.07			
CTR003_rec	tumor DNA	*PTEN*	c.388C>G	p.R130G	3.97	*AKT1*	gain/amp	5.00
		IDH1	c.395G>A	p.R132H	14.70	*PDGFRA*	amp	14.80
		ATRX	c.865C>T	p.Q289*	12.90			
		TP53	c.817C>T	p.R273C	20.70			
		MSH6	c.1372C>T	p.H458Y	5.14			
	peritumoral CSF cfDNA	*PTEN*	c.388C>G	p.R130G	52.90	*AKT1*	amp	22.20
		IDH1	c.395G>A	p.R132H	38.77	*PDGFRA*	amp	42.20
		ATRX	c.865C>T	p.Q289*	39.03			
		TP53	c.817C>T	p.R273C	72.80			
		MSH6	c.1372C>T	p.H458Y	48.60			

SNV: single nucleotide variant; CNV: copy number variation; VAF: variant allele frequency; CN: copy number.
[a]: variants predicted to be pathogenic based on MutationTaster (https://www.genecascade.org/MutationTaster2021/), COSMIC (https://cancer.sanger.ac.uk/cosmic) and IARC (https://tp53.isb-cgc.org/) databases.
[b]: CNV predicted to be pathogenic based on OncoKB™ (http://www.oncokb.org/).
Complete NGS analysis is reported in Supplementary Data 1.

Supplementary Table S6 (related to Fig. 3A). Cohort 2, clinical characteristics.

Sample ID	Age	Gender	Diagnosis (WHO 2021)	Recurrent
MG1926	54	F	GBM IDH-wt	no
MG1927_rec	48	M	grade 4 IDH-mutant astrocytoma	yes
MG1928	51	M	grade 3 IDH-mutant astrocytoma	no
MG1938	74	F	GBM IDH-wt	no
MG1942_rec	47	F	GBM IDH-wt	yes
MG1943	32	F	grade 3 IDH-mutant astrocytoma	no
MG1944	63	F	GBM IDH-wt	no
MG1945	50	F	GBM IDH-wt	no
MG2046[a]	73	M	GBM IDH-wt	no
MG2047	68	M	GBM IDH-wt	no
MG2048	69	M	GBM IDH-wt	no
MG2049	72	M	GBM IDH-wt	no
MG2049_rec	72	M	GBM IDH-wt	yes
MG2050	21	M	GBM IDH-wt	no
MG2051	46	M	GBM IDH-wt	no
MG2052	80	M	GBM IDH-wt	no
MG2053	53	M	GBM IDH-wt	no
MG2054	47	M	GBM IDH-wt	no
MG2055_rec	55	M	GBM IDH-wt	yes
MG2056	33	M	GBM IDH-wt	no
MG2057	58	M	GBM IDH-wt	no
MG2058_rec	50	F	GBM IDH-wt	yes
MG2059	64	F	GBM IDH-wt	no
MG2060	74	F	GBM IDH-wt	no
MG2061	67	F	GBM IDH-wt	no
MG2062_rec	48	M	GBM IDH-wt	yes
MG2063	67	F	GBM IDH-wt	no
MG2064	43	M	grade 4 IDH2-mutant astrocytoma	no
MG2065	78	M	GBM IDH-wt	no
MG2166	39	F	GBM IDH-wt	no
MG2167	61	F	grade 3 supratentorial ependymoma	no
MG2168	48	F	grade 2 IDH-mutant co-deleted oligodendroglioma	no
MG2169	78	M	GBM IDH-wt	no
MG2170	81	F	GBM IDH-wt	no
MG2171	55	F	GBM IDH-wt	no
MG2172	71	F	GBM IDH-wt	no
MG2173	57	M	grade 3 IDH-mutant co-deleted oligodendroglioma	no
MG2174	50	F	grade 1 ganglioglioma	no
MG2177	74	M	GBM IDH-wt	no
MG2179	75	M	GBM IDH-wt	no

[a]: patient with LP-CSF collected 2 months after surgery.

Supplementary Table S7 (related to Fig. 3B). Cohort 2, genetic alterations in tumors (targeted analysis: sequencing and FISH).

	IDH1	*IDH2*	*pTERT*	*EGFR*
MG1926	wt	n.a.	c.1-124C>T	amp
MG1927_rec	p.R132H	n.a.	wt	wt
MG1928	p.R132H	n.a.	wt	wt
MG1938	wt	n.a.	c.1-124C>T	amp
MG1942_rec	wt	n.a.	c.1-124C>T	wt
MG1943	p.R132H	n.a.	wt	wt
MG1944	wt	n.a.	c.1-124C>T	n.a.
MG1945	wt	n.a.	c.1-124C>T	neg
MG2046[a]	wt	n.a.	c.1-124C>T	wt
MG2047	wt	n.a.	c.1-124C>T	amp
MG2048	wt	n.a.	c.1-124C>T	wt
MG2049	wt	n.a.	c.1-124C>T	amp
MG2049_rec	wt	n.a.	c.1-124C>T	amp
MG2050	wt	wt	c.1-146C>T	amp
MG2051	wt	wt	wt	n.a.
MG2052	wt	n.a.	c.1-124C>T	wt
MG2053	wt	wt	c.1-124C>T	amp
MG2054	wt	wt	c.1-146C>T	amp
MG2055_rec	wt	n.a.	c.1-124C>T	amp
MG2056	wt	wt	wt	n.a.
MG2057	wt	n.a.	wt	n.a.
MG2058_rec	wt	wt	c.1-124C>T	amp
MG2059	wt	n.a.	c.1-124C>T	amp
MG2060	wt	n.a.	c.1-146C>T	amp
MG2061	wt	n.a.	c.1-124C>T	amp
MG2062_rec	wt	n.a.	wt	wt
MG2063	wt	n.a.	c.1-124C>T	wt
MG2064	wt	p.R172K	wt	amp
MG2065	wt	n.a.	c.1-124C>T	wt
MG2166	wt	n.a.	c.1-146G>A	wt
MG2167	wt	wt	-124G>T e -149G>A	wt
MG2168	p.R132H	n.a.	wt	wt
MG2169	wt	n.a.	c.1-124C>T	wt
MG2170	wt	n.a.	c.1-124C>T	wt
MG2171	wt	n.a.	c.1-124C>T	amp
MG2172	wt	n.a.	c.1-146C>T	wt
MG2173	p.R132H	n.a.	c.1-124C>T	wt
MG2174	wt	n.a.	wt	wt
MG2177	wt	n.a.	c.1-124C>T	amp
MG2179	wt	n.a.	c.1-124C>T	wt

wt: wild-type; amp: amplification.
n.a.: not assessed.
[a]: patient with LP-CSF collected 2 months after surgery.

Supplementary Table S8 (related to Fig. 3C-E). Cohort 2, features of CSF obtained by lumbar puncture (LP-CSF) and its DNA content.

Sample ID	Blood traces		LP-CSF volume (ml)	Total DNA amount (ng)		DNA concentration (ng/ml)		DNA molecular weight (MW)	
	Supernatant	Pellet		cfDNA[a]	cellular DNA[b]	cfDNA[a]	cellular DNA[b]	cfDNA[a]	cellular DNA[b]
MG1926	no	no	5.00	0.10	.	0.02	.	.	.
MG1927_rec	no	no	9.00	1224.00	.	136.00	.	MIX-MW	.
MG1928	no	no	3.00	0.10	.	0.03	.	.	.
MG1938	no	no	3.00	0.10	0.00	0.03	0.00	.	n.d.
MG1942_rec	no	no	12.00	36.18	0.00	3.02	0.00	MIX-MW	n.d.
MG1943	no	no	3.00	0.10	0.00	0.03	0.00	.	n.d.
MG1944	no	no	.	0.10	0.00	0.00	0.00	.	n.d.
MG1945	no	no	1.50	0.10	0.00	0.07	0.00	.	n.d.
MG2046[c,d]	no	no	5.00	0.10	140.00	0.02	28.00	.	H-MW
MG2047	no	no	3.00	0.10	25.92	0.03	8.64	.	H-MW
MG2048	no	no	0.30	0.10	8.75	0.33	29.17	.	n.d.
MG2049	no	no	10.00	0.10	9.96	0.01	0.99	.	n.d.
MG2049_rec	no	no	4.00	4.65	12.84	1.16	3.21	n.d.	H-MW
MG2050	no	no	5.00	0.10	11.16	0.02	2.23	.	H-MW
MG2051	no	no	5.00	0.10	0.00	0.02	0.00	L-MW	n.d.
MG2052	no	no	5.00	0.10	26.16	0.02	5.23	.	H-MW
MG2053	no	no	3.00	0.10	30.36	0.03	10.12	L-MW	H-MW
MG2054	no	no	2.00	0.10	76.80	0.05	38.40	.	H-MW
MG2055_rec	no	no	2.50	0.10	10.20	0.04	4.08	.	n.d.
MG2056[c]	no	no	1.50	12.96	851.50	8.64	567.67	L-MW	H-MW
MG2057	no	no	5.00	0.10	0.00	0.02	0.00	.	n.d.
MG2058_rec	no	no	3.00	0.10	17.52	0.03	5.84	.	H-MW
MG2059	no	no	3.00	0.10	1.56	0.03	0.52	n.d.	n.d.
MG2060	no	yes	3.00	0.10	6.99	0.03	2.33	n.d.	H-MW
MG2061	no	no	2.50	0.10	0.00	0.04	0.00	n.d.	n.d.
MG2062_rec	no	yes	3.50	1.59	23.85	0.46	6.81	n.d.	H-MW
MG2063	no	no	5.50	81.20	108.30	14.76	19.69	L-MW	H-MW
MG2064	no	no	5.00	5.82	1.56	1.16	0.31	n.d.	n.d.
MG2065	no	yes	3.00	4.86	7.50	1.62	2.50	n.d.	H-MW
MG2166	no	yes	4.00	4.80	63.30	1.20	15.83	n.d.	H-MW
MG2168	no	no	2.50	0.10	5.07	0.04	2.03	n.d.	n.d.
MG2169	no	no	4.00	5.37	4.62	1.34	1.15	n.d.	H-MW
MG2170	no	yes	5.00	30.90	10.23	6.18	2.05	L-MW	H-MW
MG2171	no	yes	3.00	8.16	8.91	2.72	2.97	H-MW	n.d.
MG2172	no	no	3.00	3.27	2.34	1.09	0.78	n.d.	n.d.
MG2173	no	no	2.00	0.10	0.00	0.05	0.00	n.d.	n.d.
MG2177	no	yes	3.50	3.78	7.50	1.08	2.14	n.d.	n.d.
MG2179	no	no	1.80	0.10	3.09	0.06	1.72	n.d.	n.d.

DNAs from LP-CSFs assessed in targeted NGS and corresponding patients are highlighted in blue.
DNAs from LP-CSFs not assessed in NGS despite quantity > 10 ng are highlighted in grey.
DNA molecular weight: H-MW, high molecular weight; L-MW, low molecular weight; MIX-MW, mixed high and low molecular weight.
n.d.: not detectable.
[a]: cfDNA extracted from LP-CSF supernatant.
[b]: cellular DNA extracted from LP-CSF pellet.
[c]: cfDNA not assessed in NGS despite quantity > 10 ng as corresponding tumor tissue was exhausted.
[d]: patient with LP-CSF collected 2 months after surgery.

Supplementary Table S9 (related to Fig. 3E). Cohort 2, tumor imaging features.

Sample ID	Tumor location	Tumor area D (mm) x d (mm)	Contrast enhancement	Tumor abutting CSF space	CSF space
MG1926	F left	8x8	yes	no	
MG1927_rec	F left	63x35	yes	yes	ventricles
MG1928	F right	30x22	yes	no	
MG1938	TO left	33x18	yes	yes	ventricles
MG1942_rec	F right	30x30	yes	yes	ventricles
MG1943	TP right	38x29	no	no	
MG1944	FI right	42x40	yes	no	
MG1945	F right	35x27	yes	yes	cisterns
MG2046	TO left	47x28	yes	yes	ventricles
MG2047	P left	27x25	yes	no	
MG2048	PO left	30x29	yes	yes	ventricles
MG2049	F left	56x43	yes	yes	ventricles
MG2049_rec	F left	49x32	yes	no	
MG2050	FT left	65x56	yes	yes	ventricles
MG2051	FT left	55x26	yes	no	
MG2052	T right	46x33	yes	no	
MG2053	F left	N/A	yes	no	
MG2054	T right	67x38	yes	yes	ventricles
MG2055_rec	T right	67x45	yes	yes	cisterns
MG2056	F right	84x18	yes	yes	ventricles
MG2057	P left	39x38	yes	yes	ventricles
MG2058_rec	F right	48x20	yes	no	
MG2059	T right	24x12	yes	yes	ventricles
MG2060	T right	36x28	yes	no	
MG2061	P left	55x48	yes	yes	ventricles
MG2062_rec	PO right	37x34	yes	yes	ventricles
MG2063	T left	49x16	yes	yes	cisterns
MG2064	P right	44x27	yes	no	
MG2065	O left	32x24	yes	no	
MG2166	FI left	67x43	no	yes	cisterns
MG2168	TI left	67x22	no	no	
MG2169	PO left	56x37	yes	yes	ventricles
MG2170	T right	57x38	yes	yes	ventricles
MG2171	P left	10x9	yes	no	
MG2172	PO left	42x24	yes	yes	ventricles
MG2173	F right	27x23	no	no	
MG2177	F left	38x30	yes	no	
MG2179	T left	36x22	yes	no	

Tumor location: F, frontal; T, temporal; P, parietal; I, insular; O, occipital.
N/A: not available.

Supplementary Table S10 (related to Fig. 3G and Supplementary Fig. S3). Cohort 2, NGS analysis of tumors and LP-CSFs.

Sample ID		SNV[a]				CNV[b]		
		Gene	cDNA Variant	Amino Acid Variant	VAF (%)	Gene	Amp/Del	CN
MG2063	Tumor DNA	*POLD1_a*	c.G2455T	p.D819Y	11.80	*CDKN2A*	del	0.36
		CIC_a	c.C2641T	p.Q881*	10.25	*CDKN2B*	del	0.44
						PTEN	del	0.60
						MLH3		1.41
						CIC_b	del	0.87
						POLD1_b	del	0.94
						NOTCH1	del	1.03
	CSF cfDNA	*POLD1_a*	c.G2455T	p.D819Y	0.00	*CDKN2A*	del	0.89
		CIC_a	c.C2641T	p.Q881*	0.00	*CDKN2B*	del	0.72
						PTEN	del	0.46
						MLH3	del	0.95
						CIC_b	del	0.89
						POLD1_b		1.69
						NOTCH1		1.65
	CSF cellular DNA	*POLD1_a*	c.G2455T	p.D819Y	0.00	*CDKN2A*		1.50
		CIC_a	c.C2641T	p.Q881*	0.00	*CDKN2B*		1.56
						PTEN		2.00
						MLH3		1.89
						CIC		1.83
						POLD1		1.78
						NOTCH1		1.45
MG1927_rec	Tumor DNA	*IDH1*	c.G395A	p.R132H	44.00	*CDK6*	amp	43.40
		TP53_a	c.T1022G	p.F341C	48.00	*PDGFRA*	amp	15.75
		TP53_b	c.T590G	p.V197G	45.00	*MYC*	amp	19.90
						MLH3	del	1.11
						PMS2	del	1.33
						ATRX		1.45
						CDKN2A	del	1.16
						CDKN2B	del	0.94
	CSF cfDNA	*IDH1*	c.G395A	p.R132H	47.70	*CDK6*	amp	58.90
		TP53_a	c.T1022G	p.F341C	40.30	*PDGFRA*	amp	17.17
		TP53_b	c.T590G	p.V197G	49.30	*MYC*	gain/amp	5.33
						MLH3	del	0.78
						PMS2	del	0.80
						ATRX	del	1.01
						CDKN2A		1.65
						CDKN2B		1.97

continued

Sample ID		SNV[a]				CNV[b]		
		Gene	cDNA Variant	Amino Acid Variant	VAF (%)	Gene	Amp/Del	CN
MG1942_rec	tumor DNA	*NOTCH1*	c.T1526A	p.I509N	63.57			
		PTEN	c.A536T	p.K179I	33.56			
		MLH1	c.C2250A	p.Y750*	20.90			
		NF1_a	c.G5108A	p.S1703N	17.29			
		NF1_b	c.C808T	p.Q270*	14.28			
		TP53	c.C847A	p.R283S	17.24			
		PIK3R1_a	c.T1063C	p.F355L	14.86			
		PIK3R1_b	c.697_711del	p.P233_W237del	0.00			
		POLD1	c.C1188A	p.Y396*	12.00			
		IDH2[c]	c.G355A	p.D119N	11.76			
		MET	c.C1067A	p.P356Q	11.11			
		FGFR1	c.G2303T	p.W768L	10.59			
		PMS2	c.A89C	p.Q30p	10.23			
	CSF cfDNA	*NOTCH1*	c.T1526A	p.I509N	51.16			
		PTEN	c.A536T	p.K179I	0.00			
		MLH1	c.C2250A	p.Y750*	0.00			
		NF1_a	c.G5108A	p.S1703N	0.00			
		NF1_b	c.C808T	p.Q270*	0.00			
		TP53	c.C847A	p.R283S	0.00			
		PIK3R1_a	c.T1063C	p.F355L	0.00			
		PIK3R1_b	c.697_711del	p.P233_W237del	12.66			
		POLD1	c.C1188A	p.Y396*	0.00			
		IDH2[c]	c.G355A	p.D119N	0.00			
		MET	c.C1067A	p.P356Q	0.00			
		FGFR1	c.G2303T	p.W768L	0.00			
		PMS2	c.A89C	p.Q30p	0.00			
MG2170	tumor DNA	*TP53_a*	c.G818A	p.R273H	31.50	*CDKN2A*	del	0.57
		TP53_b	c.C817T	p.R273C	30.00	*CDKN2B*	del	0.47
		TP53_c	c.G845A	p.R282Q	0.00	*MYCN*		2.08
		TP53_d	c.A740T	p.N247I	0.00	*PTEN*	del	0.68
		TP53_e	c.A701G	p.Y234C	0.00	*RB1*	del	1.16
		PIK3CA	c.A1G	p.M1V	33.90	*MLH3*	del	1.17
	CSF cfDNA	*TP53_a*	c.G818A	p.R273H	2.00	*CDKN2A*		2.17
		TP53_b	c.C817T	p.R273C	0.00	*CDKN2B*		1.65
		TP53_c	c.G845A	p.R282Q	30.70	*MYCN*	amp	62.11
		TP53_d	c.A740T	p.N247I	40.70	*PTEN*	del	1.09
		TP53_e	c.A701G	p.Y234C	17.60	*RB1*	del	1.04
		PIK3CA	c.A1G	p.M1V	0.00	*MLH3*	del	1.36
MG2166	tumor DNA	*PTEN*	c.C517T	p.R173C	51.70	*EGFR*	amp	13.60
		EGFR	c.C866A	p.A289D	92.40	*CDKN2A*	del	0.50
						CDKN2B	del	0.46
						PMS2	del	1.15
						HIST1H3B	del	1.12
	CSF cellular DNA	*PTEN*	c.C517T	p.R173C	0.00	*EGFR*		2.40
		EGFR	c.C866A	p.A289D	0.00	*CDKN2A*		1.40
						CDKN2B		1.36
						PMS2		1.52
						HIST1H3B		1.45
MG2062_rec	tumor DNA	*NF1*	c.7229_7232del	p.V2410Efs*7	15.10	*HIST1H3B*		3.30
						ATRX	del	1.10
	CSF cellular DNA	*NF1*	c.7229_7232del	p.V2410Efs*7	0.00	*HIST1H3B*	del	1.19
						ATRX	del	0.98

SNV: single nucleotide variant; CNV: copy number variation; VAF: variant allele frequency; CN: copy number.
[a]: variants predicted to be pathogenic based on MutationTaster (https://www.mutationtaster.org/), COSMIC (https://cancer.sanger.ac.uk/cosmic) and IARC (https://tp53.isb-cgc.org/) databases.
[b]: CNV predicted to be pathogenic based on OncoKB™ (http://www.oncokb.org/).
[c]: non-canonical IDH2 variant, not used to define IDH2-mutant tumors.
Complete NGS analysis is reported in Supplementary Data 1.

Supplementary Table S11 (related to Fig. 4C). Cohort 2, genetic alterations in LP-CSF cfDNA and cellular DNA (ITEC protocol).

Sample ID	VAF (%)						CN			
	IDH1 p.R132H		*pTERT* c.1-124C>T		*pTERT* c.1-146C>T		*EGFR*		*CDKN2A*	
	cfDNA[a]	cellular DNA[b]	cfDNA[a]	cellular DNA[b]	cfDNA[a]	cellular DNA[b]	cfDNA[a]	cellular DNA[b]	cfDNA[a]	cellular DNA[b]
MG1926	failed	N/A	n.a.	N/A	n.a.	N/A	n.a.	N/A	n.a.	N/A
MG1927_rec	48.00	N/A	-	N/A	-	N/A	-	N/A	1.06	N/A
MG1928	failed	N/A	n.a.	N/A	n.a.	N/A	n.a.	N/A	n.a.	N/A
MG1938	0.00	0.00	17.00	0.00	-	-	-	-	-	-
MG1942_rec	0.00	0.00	11.80	0.00	-	-	-	-	-	-
MG1943	0.00	0.00	failed	0.00	n.a.	0.00	n.a.	1.84	-	-
MG1944	0.00	0.00	failed	0.00	n.a.	0.00	n.a.	2.67	-	-
MG1945	0.00	0.00	11.43	failed	-	n.a.	-	n.a.	-	-
MG2046[c]	0.00	0.00	9.62	0.00	-	-	-	-	-	-
MG2047	0.00	0.00	0.00	0.00	0.00	0.00	failed	1.83	-	-
MG2048	0.00	0.00	12.49	5.57	-	-	-	-	-	-
MG2049	0.00	0.00	0.00	0.00	failed	0.00	n.a.	1.95	-	-
MG2049_rec	0.00	0.00	16.40	0.00	-	-	-	-	-	-
MG2050	0.00	0.00	0.00	0.00	11.75	0.00	-	-	-	-
MG2051	0.00	0.00	0.00	0.00	0.00	0.00	1.97	2.05	-	-
MG2052	0.00	0.00	0.00	0.00	failed	failed	n.a.	n.a.	-	-
MG2053	0.00	0.00	5.87	0.00	-	-	-	-	-	-
MG2054	0.00	0.00	0.00	0.00	7.90	0.00	-	-	-	-
MG2055_rec	0.00	0.00	failed	0.00	n.a.	0.00	n.a.	1.83	-	-
MG2056	0.00	0.00	0.00	0.00	0.00	0.00	1.88	1.94	-	-
MG2057	0.00	0.00	8.68	0.00	-	-	-	-	-	-
MG2058_rec	0.00	0.00	failed	0.00	n.a.	0.00	n.a.	-	-	-
MG2059	0.00	0.00	failed	0.00	n.a.	0.00	n.a.	-	-	-
MG2060	0.00	0.00	failed	0.00	n.a.	0.00	n.a.	2.05	-	-
MG2061	0.00	0.00	34.20	0.00	-	-	-	-	-	-
MG2062_rec	0.00	0.00	10.90	0.00	-	-	-	-	-	-
MG2063	0.00	0.00	24.70	0.00	-	-	-	-	-	-
MG2064	0.00	0.00	1.14	0.00	-	-	-	-	-	-
MG2065	0.00	0.00	38.31	0.00	-	-	-	-	-	-
MG2166	0.00	0.00	5.26	0.14	-	-	-	-	-	-
MG2168	failed	0.00	n.a.	0.00	n.a.	0.00	n.a.	1.93	-	-
MG2169	0.00	0.00	6.24	0.00	-	-	-	-	-	-
MG2170	0.00	0.00	35.00	3.37	-	-	-	-	-	-
MG2171	0.00	0.00	0.00	0.00	0.00	0.00	2.14	2.53	-	-
MG2172	0.00	0.00	5.71	0.00	-	-	-	-	-	-
MG2173	0.00	0.00	failed	39.60	-	-	-	-	-	-
MG2177	0.00	0.00	9.52	0.00	-	-	-	-	-	-
MG2179	0.00	0.00	8.07	0.00	-	-	-	-	-	-

VAF: variant allele frequency; CN: copy number.
-: not assessed, after protocol termination.
n.a.: not assessed after protocol failure.
N/A: not available sample.
[a]: cfDNA extracted from LP-CSF supernatant.
[b]: cellular DNA extracted from LP-CSF pellet.
[c]: patient with LP-CSF collected 2 months after surgery.

Supplementary Table S12 (related to Fig. 4F and G). Cohort 2, progression free survival and overall survival of first diagnosis GBM IDH-wt.

Sample ID	PFS (days)	OS (days)	Alive[a]	CSF DNA informative
MG1926	411	710	no	no
MG1938	289	255	no	yes
MG1944	525	646	no	no
MG1945	126	856	yes	yes
MG2046[b]	0	28	no	yes
MG2047	391	410	no	no
MG2048	107	239	no	yes
MG2049	263	438	no	no
MG2050	95	500	no	yes
MG2051	N/A	725	yes	no
MG2052	90	350	no	no
MG2053	102	102	no	yes
MG2054	N/A	18	no	yes
MG2056	282	689	no	no
MG2057	276	304	no	yes
MG2059	132	206	no	no
MG2060	175	462	no	no
MG2061	N/A	86	no	yes
MG2063	261	505	yes	yes
MG2065	249	459	no	yes
MG2166	240	374	no	yes
MG2169	234	418	no	yes
MG2170	23	64	no	yes
MG2171	N/A	431	yes	no
MG2172	127	257	no	yes
MG2177	306	380	yes	yes
MG2179	166	317	yes	yes

PFS: progression free survival; OS: overall survival.
N/A: not available.
[a]: July 1, 2022.
[b]: patient excluded from PFS and OS analysis as he underwent LP-CSF after surgery.

Supplementary Table S13 (related to Fig. 5B and C). Cohort 1 and 2, *MGMT* promoter methylation analysis in tumor and CSF.

Sample ID		*MGMT* methylation (%)	
		Tumor DNA[a]	cfDNA[b]
Cohort 1 (peritumoral CSF)	**CT025**	37.90	53.30
	CT027	14.40	74.10
	CT046	5.10	5.50
	CT047	0.00	0.10
	CT053	0.30	0.00
	CT062	2.40	7.30
	CT076	0.30	0.20
	CT082	1.20	3.10
	CT085	87.70	35.00
	CT087	43.60	30.70
Cohort 2 (LP-CSF)	**MG1927_rec**	45.00	49.90
	MG1942_rec	6.00	2.08
	MG2046	2.00	0.74
	MG2048	3.00	0.00
	MG2049_rec	6.00	2.50
	MG2061	4.00	0.00
	MG2063	4.00	0.03
	MG2065	43.00	3.10
	MG2177	4.00	11.60

[a]: *MGMT* methylation measured by methyl-beaming PCR in Cohort 1 and by pyrosequencing in Cohort 2.
[b]: *MGMT* methylation measured by methyl-beaming in both Cohorts.

Description of Supplementary Data 1-3 (separate .xlsx files).

Supplementary Data 1 (related to Materials and Methods)

Primers and probes used during this study.

Sheet 1 – primers for Sanger sequencing; for each sequenced amplicon, columns report: (A) gene name; (B) gene accession number; (C) oligo ID; (D) reference transcript; (E) oligonucleotide sequence (5'-3'); (F) template.

Sheet 2 – Custom primer-probes for CNV; for each target gene, columns report: (A) gene name; (B) primer 1 sequences (5'-3'); (C) primer 2 sequences (5'-3'); (D) probe sequence reporter and quencher; (E) distributor.

Sheet 3 – Methyl beaming oligos: oligonucleotide sequences used during the multiple stages of DNA amplification for the detection of *MGMT* methylation by Beaming PCR. (A) gene name; (B) oligo ID; (C) oligonucleotide sequence (5'-3').

Supplementary Data 2 (related to Fig. 2I and 3G, Supplementary Fig. S3)

Mutations (SNVs and InDels) found in tumor tissues and CSF by NGS Analysis – Complete Datasets (Cohort 1 and 2).

Sheet 1 – NGS panel design: list of genes included in the design.

Sheets 2 and 3 – Variants identified in Cohort 1 (sheet 2) and in Cohort 2 (sheet 3). Columns report: (A) the case in which the mutation has been detected; (B) the altered gene; (C) the reference transcript used for annotation of identified variants; (D) the nucleotide change mapped on the coding sequence; (E) the corresponding amino acid change mutation; (F-H) observed VAF in different biological samples (cfDNA, cellular DNA, and tumor DNA); (I-J) COSMIC identifier according to COSMIC v96, released 31-MAY-22 and number of mutant cases; (K-L) variant classification according to MutationTaster2021 and relative permanent link generated after prediction.

Supplementary Data 3 (related to Fig. 3G)

Single Nucleotide Polymorphism (SNP) for each matched tumor and LP-CSF (Cohort 2).

Sheet 1 – Haplotypes: for each SNP columns report observed haplotypes in every sequenced DNA sample. Data were used to evaluate concordance between samples as shown in Sheet 2.

Sheet 2 – Percentage of correlation between samples.

Supplementary Materials and Methods.

PCR and Sanger Sequencing conditions

In tumor gDNA samples (Cohort 1), *IDH1/2* and *TERT* promoter (*pTERT*) hotspot mutations, and *TP53* and *PTEN* full-length sequences were analyzed by Sanger Sequencing. *IDH1/2*, *TP53*, *PTEN* were amplified using Platinum® Taq DNA Polymerase (ThermoFisher) and specific primer pairs (Supplementary Data 3). PCR conditions were as follows: 95°C for 3' ; 3 × [95°C for 15'', 64°C for 30'', and 70°C for 1']; 3 × [95°C for 15'', 61°C for 30'', and 70°C for 1']; 3 × [95°C for 15'', 58°C for 30'', and 70°C for 1']; 37 × [95°C for 15'', 57°C for 30'', and 70°C for 1']; and 70°C for 5'. *pTERT* was amplified using 2x Phanta Max Master Mix (Vazyme) and specific primer pairs (Supplementary Data 3). PCR conditions were as follows: 95°C for 3'; 40 × [95°C for 15'', 63.9°C for 15'', and 72°C for 30'']; and 72°C for 3'. PCR products were purified using illustra™ ExoProStar 1-Step (Merck) according to manufacturer's instructions. Cycle sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing kit (ThermoFisher). Sequencing products were purified using CleanSEQ Dye-Terminator Removal Kit (Beckman Coulter) and analyzed with a 3730 DNA Analyzer ABI capillary electrophoresis system (ThermoFisher).

Primary cell cultures and artificial CSF composition

Neurospheres (BT205, BT328, CT025 and CT151) were derived from surgical tissue samples of primary GBMs (according to a protocol approved by the institutional Ethical Committee) as previously described (1). BT205 and BT328 were grown in standard NS medium containing human EGF (20 ng/ml, Merck) and FGF2 (20 ng/ml, PeproTech); for CT025 and CT151 HGF (20 ng/ml), PDGF/BB (20 ng/ml, PeproTech) were added to standard medium. Neurospheres were tested monthly for being Mycoplasma free and were used between p10 and p20. For assessing cell viability in CSF, an artificial cerebrospinal fluid (aCSF) was prepared mimicking the Elliott's B solution with addition of BSA [(NaCl 73 mg + $NaHCO_3$ 19 mg + D-Glucose 8 mg + $MgSO_4 \times H_2O$ 3.45 mg + KCl 3 mg + $CaCl_2 \times 2H_2O$ 2.65 mg + K_2HPO_4 2 mg+ BSA 2.5 mg)/10 ml; pH7.3] (2), and NS were plated in either NS medium or aCSF. Cell viability was measured using Tripan Blue Stain (0.4%; ThermoFisher) at the indicated time points following manufacturer's instructions. Medium or aCSF were collected at the indicated time points and were assessed as described in the Materials and Methods session.

Assessment of ddPCR linearity

In order to asses ddPCR linearity of *IDH1* (p.R132H), *TERT* c.1-124C>T and c.1-146 C>T assays in the presence of reduced DNA concentrations, we performed serial dilutions of DNA template starting from 5 ng DNA/reaction. ddPCR reaction were performed as described in the Materials and Methods section. Linearity has been measured (i) comparing observed vs. expected DNA concentration of a control DNA (wild-type for the searched alterations) and (ii) comparing VAF consistency across multiple serial DNA dilutions.

Analysis of MGMT promoter methylation

Bisulfite conversion of DNA extracted from tumors or CSF was performed by EZ DNA methylation Gold kit (Zymo Research). *MGMT* methylation was assessed via methyl-BEAMing digital PCR according to Li et al. (3). A first amplification allows the enrichment of the locus of interest and is carried out using tagged primers (see Supplementary Data 3). Amplicons are then diluted (1/16000) and resubjected in emulsion to PCR amplification using the tag and tag-coated-beads, allowing the physical separation and independent amplification of the different templates. PCR mixes are prepared according to (4). Next, amplicons are hybridized with fluorescent probes specific for the methylated or unmethylated bisulfite-converted templates and fluorescence is assessed by an Accuri C6 flow cytometer (BD). The percentage of methylation is calculated by dividing the number of methylated specific events by the sum of methylated plus unmethylated specific events. A minimum of 200 cumulative events (methylated + unmethylated) are required. *MGMT* promoter methylation in Cohort 2 tumor DNA was performed on 10 CpG islands located in the first exon of the gene by pyrosequencing using the *MGMT* plus kit (Diatech Pharmacogenetics) according to manufacturer's instructions. The biological samples is considered methylated if the CpG island methylation average is >10%.

Key resource table

Reagent or resource	Source	Identifier
QIAamp® MinElute® ccfDNA Mini Kit	Qiagen	55284
ReliaPrep™ gDNA Tissue Miniprep System	Promega	A2051
Maxwell® RSC DNA FFPE Kit	Promega	AS1450
Qubit™ dsDNA HS Assay Kit	ThermoFisher	Q32854
Qubit™ dsDNA BR Assay Kit	ThermoFisher	Q32853
Agilent High Sensitivity DNA Kit	Agilent	5067-4626
IDH1/2 status kit	Diatech Pharmacogenetics	UP045
EGFR/CEN7 dual-color probes	ZytoVision GmbH	Z-2033
MGMT plus kit	Diatech Pharmacogenetics	UP050
Platinum® Taq DNA Polymerase	ThermoFisher	10966034
2x Phanta Max Master Mix	Vazyme	P515-01
illustra™ ExoProStar 1-Step	Merk	GEUS77705
BigDye Terminator v3.1 Cycle Sequencing kit	ThermoFisher	4337455
CleanSEQ Dye-Terminator Removal Kit	Beckman Coulter	A29151
TaqMan™ Universal PCR Master Mix	ThermoFisher	4304437
ddPCR Supermix for Probes (No dUTP)	Bio-Rad	1863023
Tripan Blue Stain	ThermoFisher	15250-61
anti-IDH1 R132H mouse monoclonal antibody (Clone H09)	Dianova	RRID: AB_2335716
Software and Algorithms		
Chromas Lite 2.01 software	Technelysium	http://www.technelysium.com.au/chromas_lite.html
COSMIC	Sanger Institute	http://cancer.sanger.ac.uk/cosmic
IARC TP53 DATABASE	De Andrade et al., 2022 (5)	https://tp53.isb-cgc.org/
MutationTaster2021	Steinhaus et al., 2021 (6)	https://www.genecascade.org/MutationTaster2021/
OncoDNA	Lombardi et al., 2020 (7)	http://www.oncodna.com
OncoKB™	Chakravarty et al., 2017 (8)	http://www.oncokb.org/
Deposited Data		
Raw NGS data are available at the European Nucleotide Archive under project accession number PRJEB55332, study ERP140225 (https://www.ebi.ac.uk/ena/browser/search).		
qPCR Probes for CNV analysis		
RNase P	ThermoFisher	4403328
PDGFRA	IDT	See Supplementary Data 3
CDK4	IDT	See Supplementary Data 3
CDKN2A	IDT	Hs.PT.58.14776964.g
EGFR	IDT	Hs.PT.58.27649789.g
APOA1	IDT	Hs.PT.56a.40574746.g
GREB1	IDT	Hs.PT.58.41000378.g
TERT	IDT	Hs.PT.58.24546260.g
ddPCR probes		
IDH1 p.R132H c.395G>A	Bio-Rad	dHsaMDV2010055
TERT C228T_113	Bio-Rad	dHsaEXD72405942
TERT C250T_113	Bio-Rad	dHsaEXD46675715

CDKN2A	Bio-Rad	dHsaCP1000581
EGFR	Bio-Rad	dHsaCP2500318
TP53 p.R306*	Bio-Rad	dHsaMDV2510552
PTEN p.R335* c.1003C>T	Bio-Rad	dHsaMDS593814798
PTEN p.T319fs*4 c. 955_958delACTT	Bio-Rad	dHsaMDS563006064
TP53 p.C275Y	Bio-Rad	dHsaMDS2511638
TP53 p.R282W c.844C>T	Bio-Rad	dHsaMDV2516902
VOPP1	Bio-Rad	dHsaCP2506684
ASL	Bio-Rad	dHsaCP2506678
AGO1 (EIF2C1)	Bio-Rad	dHsaCP2500349
RPP30	Bio-Rad	dHsaCP2500350
AP3B1	Bio-Rad	dHsaCP2500348

Supplementary References

1. De Bacco F, Casanova E, Medico E, Pellegatta S, Orzan F, Albano R, et al. The MET oncogene is a functional marker of a glioblastoma stem cell subtype. Cancer Res 2012;72:4537-50.
2. Olmos-Jiménez R, Espuny-Miró A, Díaz-Carrasco MS, Fernández-Varón E, Valderrey-Pulido M, Cárceles-Rodríguez C. Stability of four standardized preparations of methotrexate, cytarabine, and hydrocortisone for intrathecal use. J Oncol Pharm Pract 2016;22:659-65.
3. Li M, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laurberg S, et al. Sensitive digital quantification of DNA methylation in clinical samples. Nat Biotechnol 2009;27:858-63.
4. Barault L, Amatu A, Bleeker FE, Moutinho C, Falcomatà C, Fiano V, et al. Digital PCR quantification of MGMT methylation refines prediction of clinical benefit from alkylating agents in glioblastoma and metastatic colorectal cancer. Ann Oncol. 2015;26:1994-9.
5. De Andrade KC, Lee EE, Tookmanian EM, Kesserwan CA, Manfredi JJ, Hatton JN, et al. The TP53 Database: transition from the International Agency for Research on Cancer to the US National Cancer Institute. Cell Death Differ 2022;29:1071-1073.
6. Steinhaus R, Proft S, Schuelke M, Cooper DN, Schwarz JM, Seelow D. MutationTaster2021. Nucleic Acids Res 2021;49:W446-W51.
7. Lombardi G, Barresi V, Indraccolo S, Simbolo M, Fassan M, Mandruzzato S, et al. Pembrolizumab Activity in Recurrent High-Grade Gliomas with Partial or Complete Loss of Mismatch Repair Protein Expression: A Monocentric, Observational and Prospective Pilot Study. Cancers (Basel) 2020;12:2283.
8. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol 2017;1:1-16.