The impact of surgery in molecularly defined low-grade glioma: an integrated clinical, radiological and molecular analysis.

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ABSTRACT

Background

Extensive resections in low-grade glioma are associated with improved overall survival. However, WHO classification of gliomas has been completely revised and is now predominantly based on molecular criteria. This requires re-evaluation of the impact of surgery in molecularly defined low-grade glioma subtypes.

Methods

We included 228 adults who underwent surgery since 2003 for a supratentorial low-grade glioma. Pre-and postoperative tumor volumes were assessed with semi-automatic software on T2-weighted images. Targeted Next-Generation Sequencing was used to classify samples according to current WHO classification. Impact of postoperative volume on overall survival, corrected for molecular profile, was assessed using a Cox proportional-hazards model.

Results

Median follow-up was 5.79 years. In 39 (17.1%) of histopathologically classified glioma the subtype was revised after molecular analysis. Complete resection was achieved in 35 patients (15.4%), and in 54 patients (23.7%) only small residue (0.1-5.0 cm^3) remained. In multivariable analysis, postoperative volume was associated with overall survival with a HR of 1.01 (95% CI 1.002-1.02; p=0.016) per cm^3 increase in volume. The impact of postoperative volume was particularly strong in IDH mutated astrocytoma patients, where even very small postoperative volumes (0.1-5.0 cm) already negatively affected overall survival.

Conclusions

Our data provides the necessary re-evaluation of the impact of surgery in molecularly defined low-grade glioma and supports maximal resection as first-line treatment for molecularly defined low-grade glioma.

INTRODUCTION

Low-grade (WHO grade II) gliomas (LGG) are primary brain tumors that grow relatively slow but with a diffuse infiltrative pattern, which makes them impossible to fully eradicate. Recurrences always occur, and despite all advances in the field of oncology, the treatment modalities for LGG remain to be surgery, radiotherapy, and chemotherapy.[1,2] The optimum use of these regimens has been controversial for many years, though the use of chemo-and radiotherapy for LGG has been studied in several randomized controlled clinical trials.[3-7] The role of surgery has never been investigated in a randomized manner, and it is unlikely this ever will be due to ethical reasons in view of the assumptions about equipoise and the duration of such a trial. However, more extensive surgery was associated with an increased overall survival (OS) in several retrospective series.[8-13] Unfortunately, interpretation of these studies is not straightforward, due to probable selection bias. For example, the resectability of a brain tumor and the decision to operate depends on its location, size, and delineation: small and superficially located tumors in non-eloquent areas are more likely to be extensively resected. However, location, eloquency and size are major prognostic factors by themselves.[14] In addition, previous studies were based on the histopathological diagnosis of gliomas which is subject to a large degree of interobserver variability, particularly for LGG.[15] Nowadays, gliomas can also be classified based on a limited set of molecular markers (isocitrate dehydrogenase 1/2 (*IDH1/2)* gene mutation, chromosome 1p and 19q co-deletion), and this classification outperforms the prognostic value of the previous histopathological classification of gliomas. [16,17] This molecular approach to glioma diagnosis is now incorporated in the 2016 WHO Classification of tumors of the Central Nervous System (WHO 2016).[15,18,19] Since some mutations preferentially manifest in distinct brain regions, the impact of extent of resection in the different molecular subtypes remains to be determined, as well as to what extent previous studies are confounded by this major prognostic factor. [20,21] To address this issue, we examined the impact of surgery on OS in molecularly defined LGG in a large retrospective cohort.

METHODS

Patient selection and clinical data acquisition

We studied a cohort of adult patients with a supratentorial LGG (age ≥ 18 years) who underwent a resection or biopsy in one of two neurosurgical centers (Erasmus MC Cancer Institute, Rotterdam, The Netherlands (Erasmus MC); Elisabeth-TweeSteden Hospital, Tilburg, The Netherlands (ETZ)), with tumor material (formalin-fixed-paraffin-embedded (FFPE) tissue) available for tissue analysis and pre- and post-operative MR

scans (T2-weighted and/or T2-weighted Fluid Attenuated Inversion Recovery (FLAIR); either 2D or 3D) available for radiological review. We included only histopathologically confirmed LGG (grade II) diagnosed by a dedicated neuropathologist (J.M.K.). As the extent of resection can be improved with the advent of awake craniotomy for tumors in eloquent areas, the time-window of patient inclusion was based on the year of introduction of awake craniotomies in the respective institutes (2003-2016 for Erasmus MC and 2008-2016 for ETZ).[22] Patients that underwent their first resection or biopsy in this period were included, even when radiological diagnosis was made before this time window. Patients only undergoing a biopsy were also included, since those samples potentially reflect a distinct subset of patients with tumors that are more difficult to resect and/or with a distinct molecular profile. We excluded patients with WHO grade II histology who had a radiological appearance (extensive tumor enhancement) suggestive of a high-grade lesion. Clinical data were collected from patient records. All included patients were followed until death or censored at the date of last follow-up. Date of death was provided by patient records or the Municipal Personal Records Database. OS was defined as time between date of diagnostic scan and death or censorship. Progression free survival (PFS) was defined as time between date of first surgical intervention and date of first clinical or radiological progression as indicated by the treating clinician. The database was developed and maintained at Erasmus MC, and locked on January 30th 2017. This study was approved by the medical ethics committee of Erasmus MC.

Volumetric analysis and acquisition of MRI characteristics

Pre-and postoperative tumor volumes were assessed in a semi-automatic fashion using the SmartBrush tool in Brainlab Elements (version 2.1.0.15; see supplemental figure 1A-C). A 3D-volume-of-interest can be created by first manually segmenting the tumor on one MRI slice of a chosen plane. Then, a second manual segmentation is carried out on one perpendicular slice. Next, the software calculates the full 3D-volume-of-interest, which can be easily manually adjusted where necessary. If available, the T2-weighted FLAIR sequence (3D where possible) was used for pre-and postoperative volumetric assessment, otherwise the T2-weighted (T2w) sequence was used. All T2w and T2w-FLAIR signal abnormalities were included in the segmentations. Within single patients the same MRI sequence was used for pre-and postoperative analysis. Preferentially, we used the postoperative scan ≥ 3 months to minimize overestimation of postoperative volume due to postoperative edema or ischemia. Proportion of resection was calculated as (preoperative volume – postoperative volume) / preoperative volume. Localization of tumor in/near eloquent area was assessed using the criteria of Chang et al.[14]

Mutation analysis, copy number analysis and molecular classification

We used a targeted Next-Generation Sequencing panel to assess mutational and copy number status as described in detail elsewhere, using an Ion Torrent Personal Genome Machine or Ion S5XL (Life Technologies).[16,23] DNA was isolated from selected tissue areas composed of a high percentage of neoplastic cells on 10µm FFPE sections using Proteinase K digestion in presence of 5% Chelex 100 resin (Bio-Rad). TERT promoter mutations (C228T & C250T) were assessed in separate assays (SnaPshot) as described.[16]

The following criteria for molecular classification were used (consistent with the current WHO 2016 classification for brain tumors):

- Oligodendroglioma: *IDH1* or *IDH2* mutated and loss of heterozygosity consistent with co-deletion of the entire 1p and 19q chromosomal arms.
- Astrocytoma IDH mutated (IDHmt): *IDH1* or *IDH2* mutated.
- Glioblastoma-like (GBM-like): *IDH1* or *IDH2* wildtype and; *TERT* promoter mutation without 1p19q co-deletion, or loss of heterozygosity of chromosome 10q and imbalance of chromosome 7, or *EGFR* amplified (in the WHO 2016 classification described as astrocytoma IDHwt)[18]

Statistical analysis

All primary analyses were carried out according to a predetermined analysis protocol; all other analyses are indicated as post-hoc. Categorical data were analyzed with Pearson's chi-square test or Fisher's exact test when assumptions of the chi-square test were violated (as indicated in the respective tables). Kruskal-Wallis test was used for continuous data. We used postoperative tumor volume as primary measure of extent of resection. The same calculations were made using resection percentage as alternative measure, which can be found in the supplementary material. Multiple linear regression models were used to explore correlations of molecular subtype with postoperative tumor volume and resection percentage. To meet the assumption of normal distribution of residual errors in multiple linear regression, postoperative volume was log10 transformed, and resection percentage was arcsin square root transformed. To investigate impact of surgery, univariable and multivariable analyses of overall survival were performed using a Cox proportional-hazards model. In this model pre- and postoperative tumor volumes were treated as continuous variable, and postoperative tumor volume was log transformed to prevent inappropriate weighting of results by very large preoperative tumor volumes. The assumption of proportional hazards was tested based on the scaled Schoenfeld residuals. All calculations were two-sided tests, with a p-value <0.05 considered as statistically significant. All analyses were performed using R (3.3.2) and RStudio (1.0.44) using ggplot2 and CRAN survival packages.[24,25]

RESULTS

Our initial cohort included 246 patients with confirmed LGG, and with MR scans and FFPE material available. Of these, 18 patients were excluded from analyses; two due to insufficient amount of DNA for sequencing, 14 due to sequencing failure, and two due to a preoperative radiological appearance suggestive of glioblastoma (both stereotactic biopsy samples, molecularly classified as GBM-like). 228 patients were included in final analyses. Median follow-up was 5.79 years (range 0.3 – 20.4). Clinical characteristics are shown in table 1. When comparing histopathological classification with molecular subtype, there was a change of diagnosis in 39 patients (17.1%, mixed oligo-astrocytomas not included); all mixed oligo-astrocytomas were reclassified according to the WHO 2016 update. There were no significant differences in clinical characteristics between oligodendroglioma and astrocytoma IDHmt, except for age and type of first surgery: oligodendroglioma patients were significantly older (median age 45 vs. 37 years; p<0.0001) and type of first surgery was more often a biopsy (p=0.006). We observed however several statistically significant different characteristics in the GBM-like group. Patients in this group were significantly older (median age 61 years), had a different presentation (only 11 (47.8%) patients presenting with epileptic seizures, vs. 77 (82.8%) and 82 (73.2%) in oligodendroglioma and astrocytoma IDHmt respectively), and a different tumor localization (more often situated in eloquent areas, N=16; 69.6%). Interestingly, the majority of GBM-like patients (N=19; 82.6%) underwent a biopsy, compared to only a small fraction in the oligodendroglioma and astrocytoma IDHmt group (N=20 (21.5%) and N=9 (8%) respectively; p<0.0001). This is also reflected in postoperative tumor volume: although preoperative tumor volume did not differ between the molecular groups, the postoperative tumor volume was significantly higher in the GBM-like tumors compared to oligodendroglioma and astrocytoma IDHmt (median volume 30.0 cm^3; 8.0 cm^3; 8.9 cm^3 respectively, p=0.005). Postoperative tumor volume did not differ between oligodendroglioma and astrocytoma IDHmt (p=0.553). An overview of salvage treatments is shown in supplementary table 3 and an overview of surgical outcome is shown in supplementary table 4.

Factors influencing postoperative tumor volume

We used multiple linear regression to explore which factors influence the amount of postoperative tumor volume. Results are shown in table 2. Factors such as increasing age, higher preoperative tumor volume (see also supplementary figure 2) and insular localization were significantly correlated with a higher postoperative tumor volume (p=0.002; p<0.0001; p<0.0001 respectively). Tumors located in eloquent areas were also significantly correlated with a higher postoperative volume (p<0.0001). Interestingly, corrected for the factors mentioned above, the molecular subtype of the tumor

Table 1. Clinical characteristics

	All patients		Oligodendroglioma		Astrocytoma IDHmt			GBM-like		
Characteristics	N	%	N	%	N	%	P*	N	%	P**
Patients (n)	228		93		112			23		
Sex							0.119			0.078
Male	136	59.6 %	48	51.6 %	71	63.4 %		17	73.9 %	
Female	92	40.4 %	45	48.4 %	41	36.6 %		6	26.1 %	
Age							<0.0001			<0.0001
Median (IQR)	42	(34 - 51)	45	(37 - 52)	37	(29 - 45)		61	(52 - 65)	
< 40	99	43.4 %	32	34.4 %	67	59.8 %		0	0.0 %	
40-60	104	45.6 %	50	53.8 %	43	38.4 %		11	47.8 %	
> 60	25	11.0 %	11	11.8 %	2	1.8 %		12	52.2 %	
Presenting symptom							0.239†			<0.0001†
Epilepsy	170	74.6 %	77	82.8 %	82	73.2 %		11	47.8 %	
Incidental	24	10.5 %	8	8.6 %	15	13.4 %		1	4.3 %	
Headache	8	3.5 %	1	1.1 %	6	5.4 %		1	4.3 %	
Miscellaneous neurologic complaints	26	11.4 %	7	7.5 %	9	8.0 %		10	43.5 %	
Watch-and-wait before first surgery? Yes	66	29.1 %	35	38.0 %	28	25.0 %	0.064	3	13.0 %	0.025
Type of 1st surgery							0.006†			<0.0001†
Awake craniotomy	105	46.1 %	50	53.8 %	54	48.2 %		1	4.3 %	
Normal resection	75	32.9 %	23	24.7 %	49	43.8 %		3	13.0 %	
Open biopsy	15	6.6 %	7	7.5 %	2	1.8 %		6	26.1 %	
Stereotactic biopsy	33	14.5 %	13	14.0 %	7	6.2 %		13	56.5 %	
Preoperative KPS							0.055			<0.0001
Median (IQR)	100	(90 - 100)	100	(100 - 100)	100	(90 - 100)		90	(85 - 95)	
100	148	64.9 %	71	76.3 %	71	63.4 %		6	26.1 %	

Table 1. Clinical characteristics (continued)

	All patients		Oligodendroglioma		Astrocytoma IDHmt			GBM-like		
Characteristics	N	%	N	%	N	%	P*	N	%	P**
90	62	27.2 %	17	18.3 %	34	30.4 %		11	47.8 %	
<=80	18	7.9 %	5	5.4 %	7	6.2 %		6	26.1 %	
Histopathological diagnosis										
Grade II Astrocytoma	112	49.1 %	7	7.5 %	87	77.7 %		18	78.3 %	
Grade II Oligodendroglioma	86	37.7 %	76	81.7 %	9	8.0 %		1	4.3 %	
Grade II Oligo-astrocytoma	30	13.2 %	10	10.8 %	16	14.3 %		4	17.4 %	
Molecular diagnosis										
Oligodendroglioma	93	40.8 %	93	100 %	0	0.0 %		0	0.0 %	
Astrocytoma IDHmt	112	49.1 %	0	0.0 %	112	100.0 %		0	0.0 %	
GBM-like	23	10.1 %	0	0.0 %	0	0.0 %		23	100.0 %	
Tumor location							0.299†			<0.0001†
Frontal	121	53.1 %	62	66.7 %	58	51.8 %		1	4.3 %	
Parietal	19	8.3 %	8	8.6 %	10	8.9 %		1	4.3 %	
Temporal	37	16.2 %	9	9.7 %	19	17.0 %		9	39.1 %	
Occipital	8	3.5 %	3	3.2 %	4	3.6 %		1	4.3 %	
Insula	28	12.3 %	9	9.7 %	19	17.0 %		0	0.0 %	
Basal Ganglia	4	1.8 %	0	0.0 %	0	0.0 %		4	17.4 %	
Gliomatosis Cerebri	11	4.8 %	2	2.2 %	2	1.8 %		7	30.4 %	
Eloquent Area: yes	90	39.5 %	35	37.6 %	39	34.8 %	0.786	16	69.6 %	0.007
Side of lesion										
Right	98	43.0 %	38	40.9 %	52	46.4 %		8	34.8 %	
Left	118	51.8 %	50	53.8 %	58	51.8 %		10	43.5 %	
Bilateral	12	5.3 %	5	5.4 %	2	1.8 %		5	21.7 %	

Table 1. Clinical characteristics (continued)

	All patients		Oligodendroglioma		Astrocytoma IDHmt			GBM-like		
Characteristics	N	%	N	%	N	%	P*	N	%	P**
Pre-operative tumor volume, cm3							0.785			0.891
<25.0	66	28.9 %	26	28.0 %	32	28.6 %		8	34.8 %	
25.1-50.0	54	23.7 %	24	25.8 %	24	21.4 %		6	26.1 %	
50.1-100.0	67	29.4 %	24	25.8 %	39	34.8 %		4	17.4 %	
100.1-250.0	38	16.7 %	17	18.3 %	16	14.3 %		5	21.7 %	
250.1-351.0	3	1.3 %	2	2.2 %	1	0.9 %		0	0.0 %	
Median (range)	47.3	(3.01 - 350.5)	46.1	(4.29 - 350.5)	50.95	(3.01 - 302.8)		33	(9.05 - 213.1)	
Postoperative tumor volume, cm3							0.553			0.005
0.0	35	15.4 %	15	16.1 %	19	17.0 %		1	4.3 %	
0.1-5.0	54	23.7 %	27	29.0 %	26	23.2 %		1	4.3 %	
5.1-15.0	41	18.0 %	12	12.9 %	23	20.5 %		6	26.1 %	
> 15.0	98	43.0 %	39	41.9 %	44	39.3 %		15	65.2 %	
Median (range)	10.95	(0 - 263.6)	8.02	(0 - 263.6)	8.9	(0 - 232.7)		30	(0 - 213.1)	
Extent of tumor resection (%)							0.166			<0.0001
0-40	67	29.4 %	28	30.1 %	20	17.9 %		19	82.6 %	
41-89	90	39.5 %	35	37.6 %	53	47.3 %		2	8.7 %	
90-94	22	9.6 %	10	10.8 %	11	9.8 %		1	4.3 %	
95-99	14	6.1 %	5	5.4 %	9	8.0 %		0	0.0 %	
100	35	15.4 %	15	16.1 %	19	17.0 %		1	4.3 %	
Median (range)	76.1	(0 - 100)	75.4	(0 - 100)	82.5	(0 - 100)		0	(0 - 100)	
Treatment after 1st surgery							<0.0001			<0.0001
Wait & Scan	104	45.6 %	51	54.8 %	52	46.4 %		1	4.3 %	
Chemotherapy	35	15.4 %	24	25.8 %	5	4.5 %		6	26.1 %	

Table 1. Clinical characteristics (continued)

	All patients		Oligodendroglioma		Astrocytoma IDHmt			GBM-like		
Characteristics	N	%	N	%	N	%	P*	N	%	P**
Radiotherapy	71	31.1 %	15	16.1 %	42	37.5 %		14	60.9 %	
Chemoradiation	18	7.9 %	3	3.2 %	13	11.6 %		2	8.7 %	
Follow-up (years)										
Median (range)	5.8	(0.3 - 20.4)	7.3	(0.8 - 20.4)	5.7	(0.3 - 15)		2.1	(0.3 - 4.7)	

* Comparison between Oligodendroglioma and Astrocytoma IDHmt

** Three group comparison (Oligodendroglioma vs. Astrocytoma IDHmt vs. GBM-like)

† Fisher's exact test

did not correlate with postoperative tumor volume. A similar model but using resection percentage instead of postoperative tumor volume is shown in supplementary table 1.

Table 2. Multiple linear regression of factors influencing postoperative tumor volume

	Estimate	SE	t	p-value
Intercept	0.307	0.134	2.291	0.023
WHO 2016 classification				
Oligodendroglioma	*	*	*	*
Astrocytoma IDHmt	0.007	0.062	0.107	0.915
GBM-like	0.183	0.127	1.438	0.152
Age	0.008	0.003	3.064	0.002
Preoperative tumor volume	0.008	0.001	14.831	0.000
Eloquency				
Eloquent location	*	*	*	*
Non-eloquent location	-0.320	0.061	-5.230	0.000
Tumor location				
Frontal	*	*	*	*
Parietal	-0.005	0.106	-0.047	0.963
Temporal	0.074	0.082	0.894	0.372
Occipital	0.078	0.151	0.515	0.607
Insula	0.482	0.086	5.577	0.000
Basal ganglia	0.316	0.240	1.319	0.189
Gliomatosis cerebri	0.161	0.157	1.027	0.305

Multiple linear regression model with log10 postoperative tumor volume as dependent variable.
* = Reference variable

Patient outcome per molecular subtype and extent of resection

Projected median OS was 13.1 years at a median follow-up of 5.79 years. At time of analysis 69 patients had died and the remaining 159 patients had a median follow-up of 6.3 years (IQR 3.6 - 9.6 years). There were no surgery related mortalities. GBM-like tumors had a significantly shorter median OS (2.1 years) compared to astrocytoma IDHmt (10.2 years) and oligodendroglioma (not reached) (three group comparison; p = 0.0001, GBM-like vs astrocytoma IDHmt; p = 0.0001, astrocytoma IDHmt vs oligodendroglioma; p =0.0001) (supplementary figure 3). The factors age, Karnofsky Performance Status (KPS), log preoperative volume, resection percentage and eloquency were also significantly correlated with OS in univariable analysis (Table 3, supplementary table 2).

Table 3. Univariable and multivariable Cox regression with postoperative volume as measure of extent of resection

	Univariable			Multivariable		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.04	1.02 - 1.06	0.0002	1.01	0.98 - 1.04	0.527
Gender						
Male	*					
Female	0.68	0.41 - 1.12	0.129			
KPS	0.94	0.91 - 0.96	<0.0001	1.01	0.97 - 1.06	0.666
Eloquency						
Yes	*			*		
No	0.49	0.3 - 0.79	0.004	1.59	0.90 - 2.82	0.110
Log preoperative volume	1.66	1.22 - 2.26	0.001	1.70	1.06-2.75	0.029
Postoperative volume	1.01	1 - 1.01	<0.0001	1.01	1.002 - 1.02	0.016
Molecular diagnosis						
Oligodendroglioma	*			*		
Astrocytoma IDHmt	3.77	1.9 - 7.46	0.0001	5.31	2.40 - 11.75	<0.0001
GBM-like	112.9	45.93 - 277.55	<0.0001	218.81	68.75 - 696.38	<0.0001
RTx after surgery						
No	*			*		
Yes	2.45	1.52 - 3.94	0.0002	1.13	0.59 - 2.13	0.716
Chemo after surgery						
No	*			*		
Yes	1.19	0.68 - 2.09	0.545	0.64	0.27 - 1.52	0.315
* = Reference category						

In univariable analysis the amount of postoperative tumor volume (as a continuous variable) was significantly associated with OS with a Hazard Ratio (HR) of 1.01 per 1 cm^3 increase in volume (95% CI 1.01 – 1.01; $p < 0.0001$). A particularly strong effect on OS was seen in patients with no detectable tumor after resection (Figure 1A). Interestingly, any residual volume negatively affects OS, but this effect was most notable in astrocytoma IDHmt (Figure 1B & 1C). In this group, even if residual volume was only 0.1-5.0 cm^3, OS was impaired compared to 0.0 cm^3 residue. No difference in OS was seen between 5.1-15.0 cm^3 and >15.0 cm^3 residue. In oligodendroglioma patients, a trend towards better OS with more extensive resection was observed, though the difference in OS benefit of no detectable tumor versus small residues was less impressive than in astrocytoma IDHmt patients. This analysis was not feasible in GBM-like tumors, due to small sample size. A similar pattern was observed when looking at resection percentages rather than postoperative tumor volume (supplementary figure 4). Progression free survival stratified by postoperative volume is shown in supplementary figure 5,

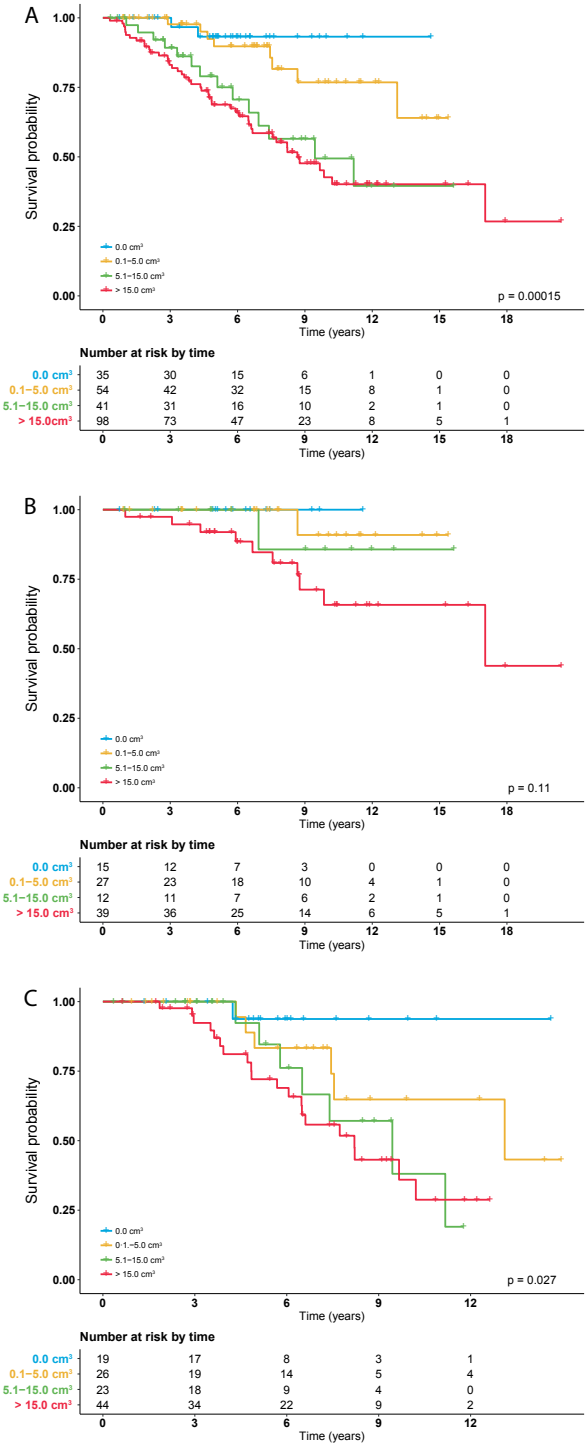


Figure 1. OS stratified by subgroups of postoperative volume for all patients (A), oligodendroglioma patients (B), and astrocytoma IDHmt patients (C).

which shows similar patterns. Postoperative tumor volume remained an independent prognostic factor in a multivariable analysis (HR 1.01; 95% CI 1.002-1.02; p=0.016; per 1 cm^3 increase in volume). Univariable and multivariable Cox-regression with resection percentage as measure of extent of resection is shown in supplementary table 2.

In a post-hoc analysis we dichotomized postoperative volume with different cut-offs to explore at what maximal postoperative volume a resection is still associated with improved OS. In astrocytoma IDHmt, a postoperative volume of up to 25 cm^3 still showed a significant longer OS compared to > 25cm^3. More importantly though, any residual tumor (i.e. greater than 0.0 cm^3) already impacts OS negatively (Figure 2 & supplementary Figure 6). In oligodendroglioma patients such cut-off points for postoperative tumor volume and patient benefit could not be defined (supplementary Figure 7).

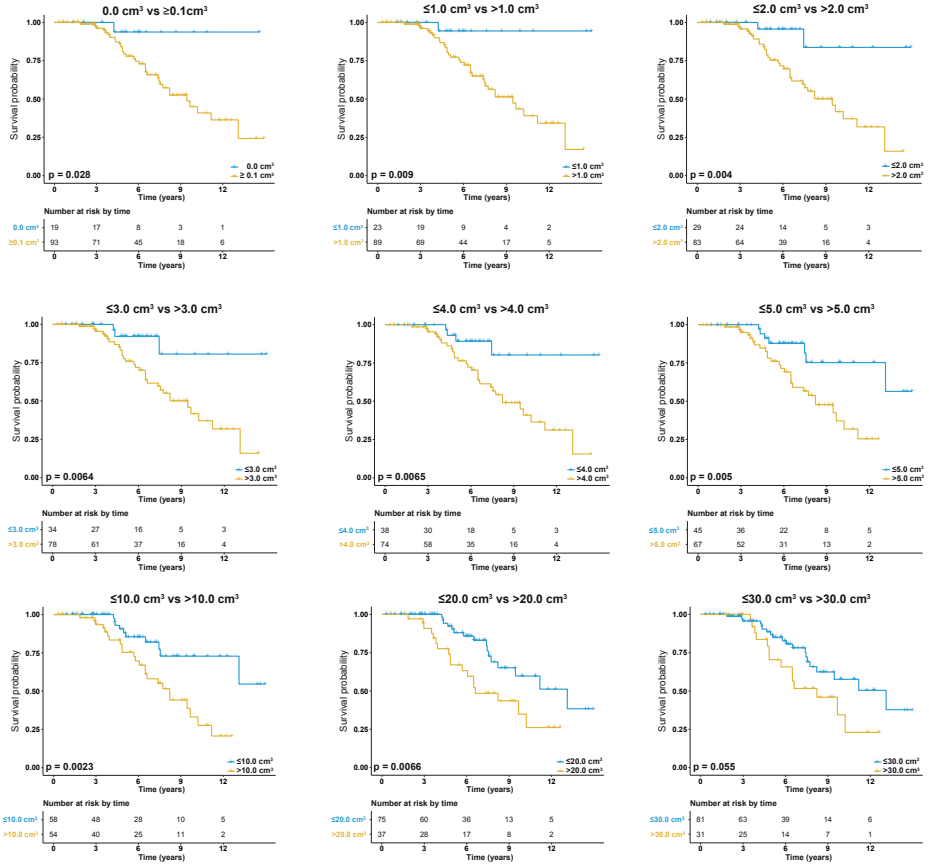


Figure 2. OS in astrocytoma IDHmt stratified by different cut-offs of postoperative tumor volume to explore at what maximal postoperative volume a resection is still beneficial. A postoperative volume of up to 25 cm^3 still shows a significant better OS compared to >25cm^3. However any residual tumor negatively impacts survival. The same figure with more cut-offs is shown in supplementary figure 6.

DISCUSSION

With the incorporation of a molecular classification for glioma, WHO classification of tumors of the central nervous system has been completely revised and molecular markers now overrule the histopathological diagnosis.[18,19] Differences in sensitivity and overall outcome to chemo- and/or radiotherapy according to molecular subgroup have emerged in past years, but the assessment of the role of surgery in the molecularly defined subgroups of low grade glioma was lacking so far. Since the molecular markers in the WHO 2016 update are prognostically very powerful, a re-evaluation of the impact of surgery is important to validate and evaluate current clinical practice.

Several studies have suggested that early and extensive surgery has a positive impact on survival for LGG patients. Unfortunately, most of these studies used qualitative measures for extent of resection or used tumor diameter to calculate tumor volume.[9-13,26,27] These approaches do not allow accurate assessment of extent of resection. A recent report by Duffau et al. incorporated volumetric measurements in a large molecularly defined LGG cohort. The authors showed that resectability was independent of molecular markers, however, survival data were not reported.[28] One report that used a volumetric approach and included survival analysis was the study by Smith et al.[8] These authors showed that a larger extent of resection predicts significant longer OS and that even small postoperative tumor volumes negatively influence OS. Their 2008 study, however, was based on histopathologically diagnosed LGG (WHO 2007) without molecular classification. A recent paper by Wahl et al. reported on the impact of postoperative tumor volumes on OS corrected for molecular subtype. Every 10cm^3 increase in postoperative tumor volume was associated with shorter OS. However, definite conclusions cannot be drawn since this was an exploratory, post-hoc analysis in a selected cohort of 71 patients with only significant residual disease after first surgical treatment.[29]

In this study we show that postoperative volume remains a prognostic factor for OS in molecularly defined LGG. This is an important finding, since it supports the policy of maximal safe resection in all molecular subtypes of LGG. Our data also shows that even very small tumor residues already negatively impact OS in astrocytoma IDHmt tumors. This is exemplified by the clear survival difference between tumor residues as little as 0.1-5.0 cm^3 and 0.0 cm^3, and the absence of a survival difference between 5.1-15.0 cm^3 and >15.0 cm^3 residue. Also, in a post-hoc analysis with dichotomization of postoperative volume we show that up to 25 cm^3 residue is still associated with a significant better OS compared to > 25cm^3. Since this is a post-hoc analysis, we should interpret this specific cut-off value with caution. More importantly though, this analysis shows that any residual tumor (i.e. greater than 0.0 cm^3) already impacts survival negatively in astrocytoma IDHmt. In oligodendroglioma it seems that a small residue

does not have that strong impact on OS as is observed in astrocytoma IDHmt. The absence of a strong relationship between OS and limited amounts of residual tumors might be explained by the more indolent natural course of these tumors and their increased sensitivity to treatment which may have more impact on survival than surgical intervention.[19,30] Although residual tumor probably is located in eloquent areas in the majority of cases, one may argue that a second-look look operation if safely possible might be something to consider in the few astrocytoma IDHmt patients with minor residual tumor located in non-eloquent area. On the other hand, our data also encourages further efforts to implement new methods that aid in safely maximizing extent of resection. Imaging techniques like intraoperative MRI or ultrasound to assess extent of resection during surgery might be valuable in this setting. At current these techniques are not available in most clinics however.

Our data suggest that some of the findings in previous studies may have been impacted by the presence of GBM-like tumors, which were more likely to undergo a biopsy only in this series. This might be explained by the more frequent location of GBM-like tumors in eloquent areas (N=16; 69.6%) and thus a higher frequency of biopsy or limited resections.

Our study has limitations that need to be addressed. The main limitation is the retrospective nature. However, due to ethical and practical reasons a randomized trial on the impact of early and of extent of resection is considered not feasible, and retrospective data with detailed clinical and molecular annotation is the best available data to address this issue.[31] Clearly, the preoperative volume of LGG has a major impact on outcome, and this also influences the postoperative volume. A larger series is necessary to further study this. Due to the retrospective nature of this study the follow-up, postoperative treatment and the used MRI protocol were heterogeneous. We corrected OS for administration of chemo- and/or radiotherapy, but correcting for different timing and sequence of these treatments is not informative due to the large variety of possible combinations. T2w-FLAIR sequences were not available for both pre- and postoperative measure for some patients. When this was the case, T2-weighted images were used. We used the late postoperative scan to minimize overestimation of postoperative tumor volume due to edema or ischemia and to minimize potential differences between sequences.[32] Also, non-standardized MRI follow-up limits the reliability of evaluation of PFS in terms of the importance of the extent of resection. Another important limitation that comes with the retrospective nature of this study is the inclusion based on histology. This has to be taken into account when generalizing results, since histology is only known after surgery.

Also, several recent studies have shown that final conclusions in studies on LGG require lengthy follow-up for definitive conclusions.[33] Further follow-up and more importantly expanding this dataset is very important. This especially concerns the

oligodendroglioma group, since the impact of small tumor residues in this commonly slow-growing tumor might become more clear with longer follow-up. Lastly, confirmation of our findings in an independent dataset is needed before definitive clinical conclusions are drawn.

In conclusion, we validated and added substantial and necessary evidence to current practice of early maximal resection for LGG. Importantly, we show that even very small tumor residues in astrocytoma IDHmt patients already have negative impact on OS.

Table s1. Multiple linear regression of factors influencing resection percentage[a]

	Estimate	SE	t	p-value
Intercept	1.384	0.124	11.177	<0.0001
WHO 2016 classification				
Oligodendroglioma	*	*	*	*
IDH mutated, 1p19q intact	0.049	0.057	0.850	0.396
IDH wildtype, TERT mutated	-0.400	0.117	-3.405	0.001
Age	-0.008	0.002	-3.545	0.001
Preoperative tumor volume	-0.003	0.000	-5.802	<0.0001
Eloquency				
Eloquent location	*	*	*	*
Non-eloquent location	0.256	0.057	4.531	<0.0001
Tumor location				
Frontal	*	*	*	*
Parietal	-0.039	0.098	-0.396	0.693
Temporal	-0.031	0.076	-0.412	0.681
Occipital	-0.120	0.139	-0.862	0.390
Insula	-0.269	0.080	-3.364	0.001
Basal ganglia	-0.363	0.222	-1.637	0.103
Gliomatosis cerebri	-0.260	0.145	-1.794	0.074

[a]Linear regression model with resection percentage as dependent variable. To meet the assumption of normal
distribution of residual errors, resection percentage was arcsin square root transformed.
* = Reference category

Table s2. Univariable and multivariable Cox-regression with resection percentage as measure of extent of resection

	Univariable			Multivariable		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.04	1.02 - 1.06	0.0002	1.00	0.98 - 1.03	0.734
Gender						
Male	*					
Female	0.68	0.41 - 1.12	0.129			
KPS	0.94	0.91 - 0.96	<0.0001	0.97	0.93 - 1.01	0.112
Eloquency						
Yes	*			*		
No	0.49	0.3 - 0.79	0.004	1.40	0.77 - 2.54	0.273
Resection percentage	0.16	0.08 - 0.3	<0.0001	0.41	0.16 - 1.05	0.062
Molecular diagnosis						
Oligodendroglioma	*			*		
Astrocytoma IDHmt	3.77	1.9 - 7.46	<0.0001	4.23	2.03 - 8.81	0.0001
GBM-like	112.9	45.93 - 277.55	<0.0001	63.77	22.35 - 181.99	<0.0001
RTx after surgery						
No	*			*		
Yes	2.45	1.52 - 3.94	0.0002	1.26	0.93 - 1.01	0.481
Chemo after surgery						
No	*			*		
Yes	1.19	0.68 - 2.09	0.545	1.19	0.56 - 2.51	0.652

* = Reference category

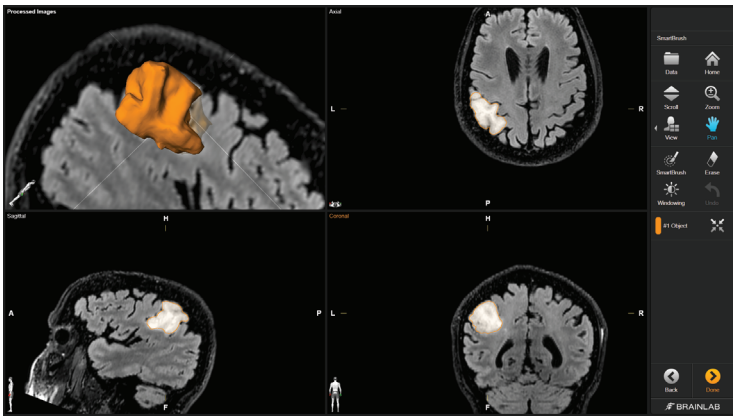
Table s3. Overview of salvage treatments

	All patients	Oligodendroglioma	Astrocytoma IDHmt	GBM-like
Number of re-resections				
0	182 (79.82%)	83 (89.25%)	78 (69.64%)	21 (91.30%)
1	43 (18.86%)	10 (10.75%)	31 (27.68%)	2 (8.70%)
2	3 (1.32%)	0 (0.0%)	3 (2.68%)	0 (0.0%)
Treatment after 1st surgery				
Wait & Scan	104 (45.61%)	51 (54.84%)	52 (46.43%)	1 (4.35%)
Chemotherapy	35 (15.35%)	24 (25.81%)	5 (4.46%)	6 (26.09%)
Radiotherapy	71 (31.14%)	15 (16.13%)	42 (37.50%)	14 (60.87%)
Chemoradiation	18 (7.89%)	3 (3.23%)	13 (11.61%)	2 (8.70%)
Ever radiotherapy				
Yes	155 (67.98%)	38 (40.86%)	97 (86.61%)	20 (86.96%)
No	73 (32.02%)	55 (59.14%)	15 (13.39%)	3 (13.04%)
Ever chemotherapy				
Yes	146 (64.04%)	54 (58.06%)	78 (69.64%)	14 (60.87%)
No	82 (35.96%)	39 (41.94%)	34 (30.36%)	9 (39.13%)
No progression during f/u yet				
N (%)	74 (32.46%)	37 (39.78%)	37 (33.04%)	0 (0.0%)

Table s4. Overview of surgical outcome

Frequencies of deficits after surgery/biopsy	N (%)
No deficitis	182 (79.8%)
Speech disorder	23 (10.1%)
Palsy	23 (10.1%)
Recovery of deficits	N (%)
Speech disorder	
Full recovery within 3 months	16 (69.6%)
Partial recovery	7 (30.4%)
Permanent deficit	0 (0.0%)
Palsy	
Full recovery within 3 months	11 (47.8%)
Partial recovery	8 (34.8%)
Permanent deficit	4 (17.4%)
Work resumption after surgery	N (%)
Yes	153 (67.1%)
No	17 (7.5%)
Unknown	58 (25.4%)

A



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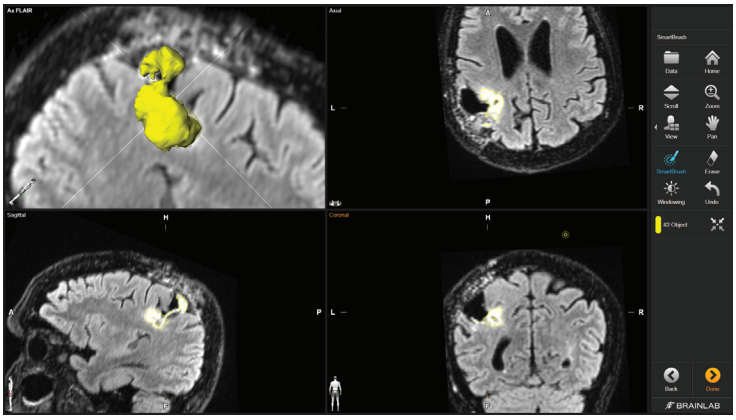

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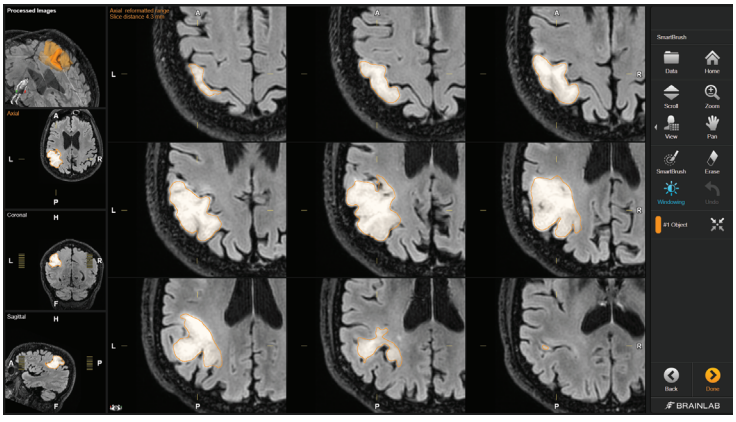
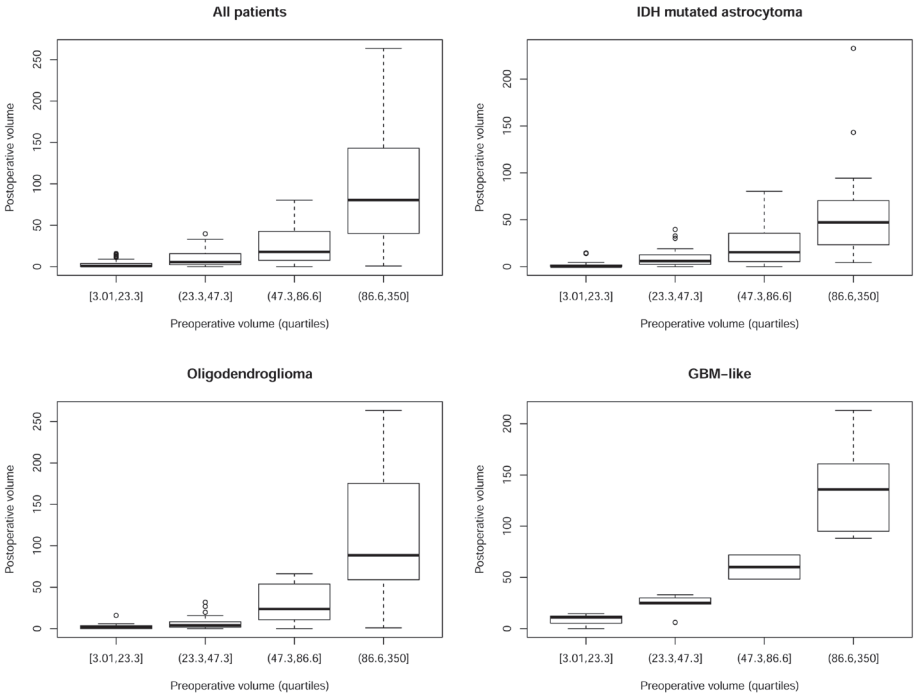
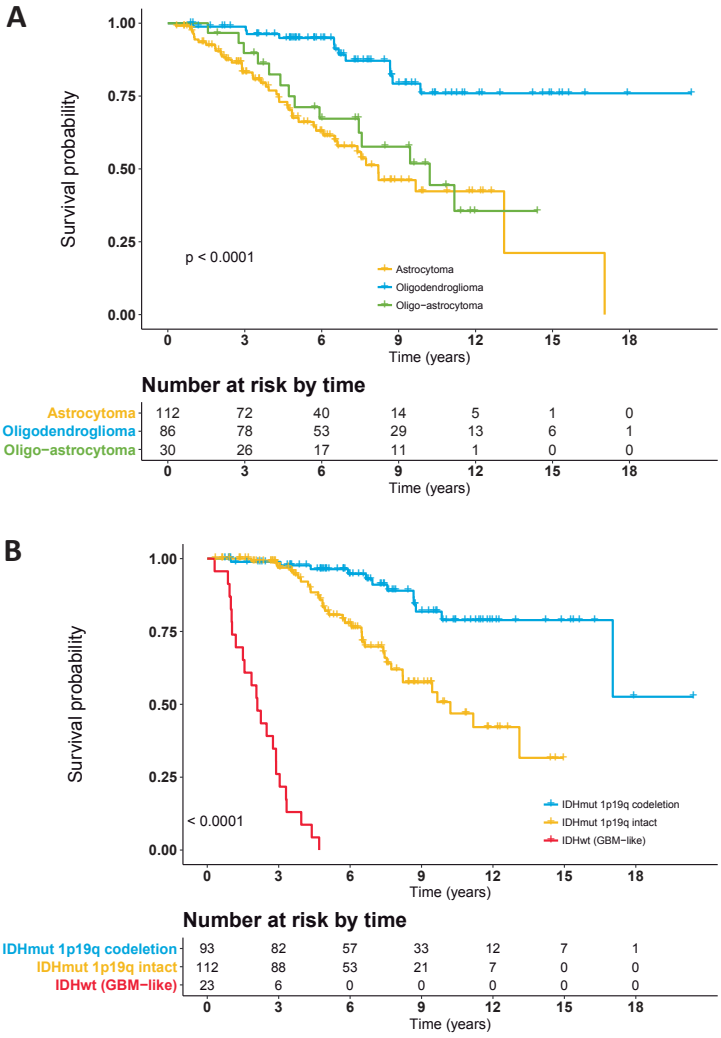


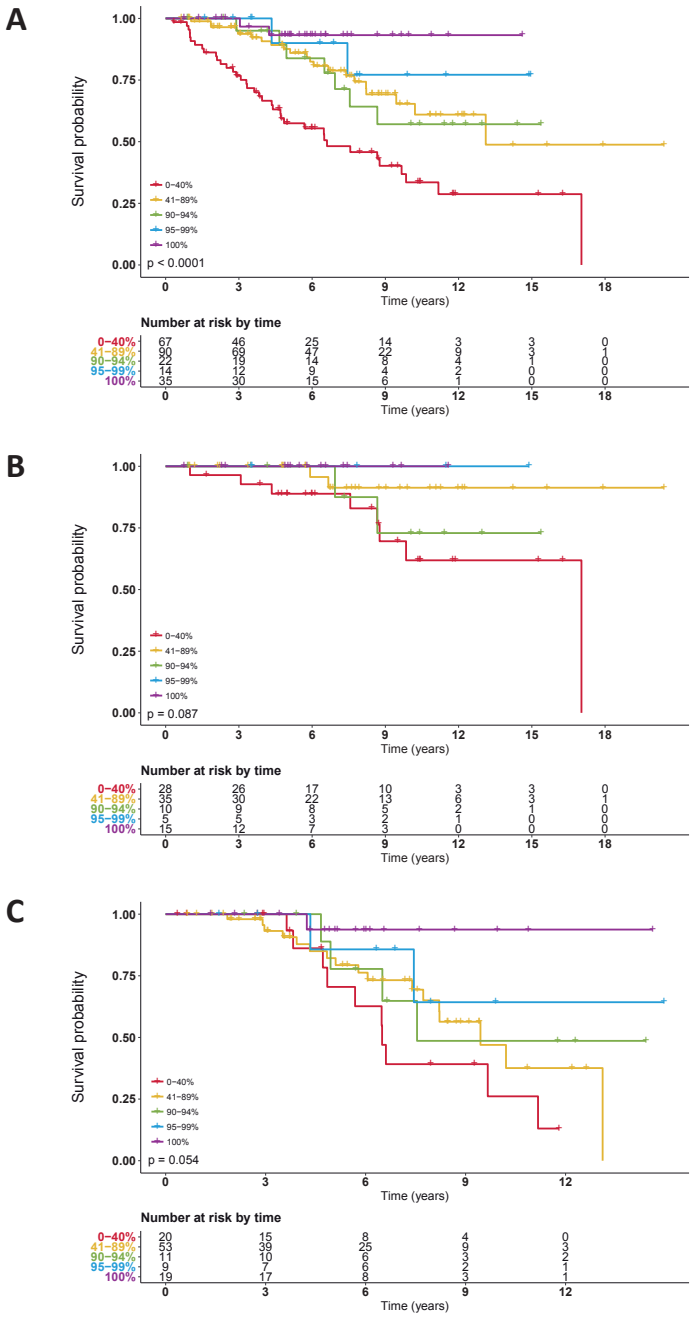
Supplementary figure 1. (A) Assessment of preoperative tumour volume. A 3D volume of interest can be created by first manually segmenting the tumour on one MRI slice of a chosen plane (i.e.the top-right panel of this figure). Then, a second manual segmentation is carried out on one perpendicular slice (i.e. the bottom-right panel). Next, the software automatically calculates the full 3D volume-of-interest (see top-left panel). (B) Assessment of postoperative tumour volume. (C) The volume of interest can be check slice by slice and where necessary, the volume can be easily manually adjusted.



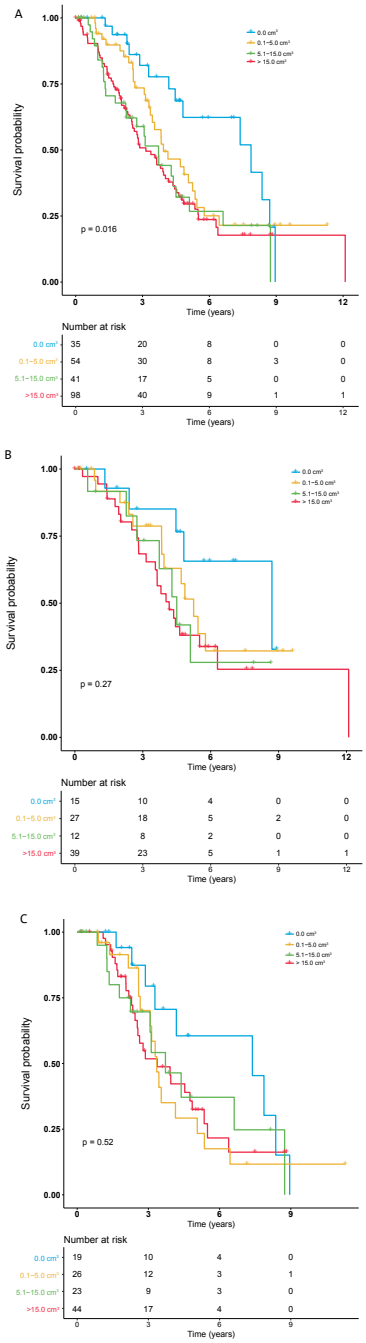
Supplementary figure 2. Quartiles of preoperative tumour volume vs postoperative tumour volume.



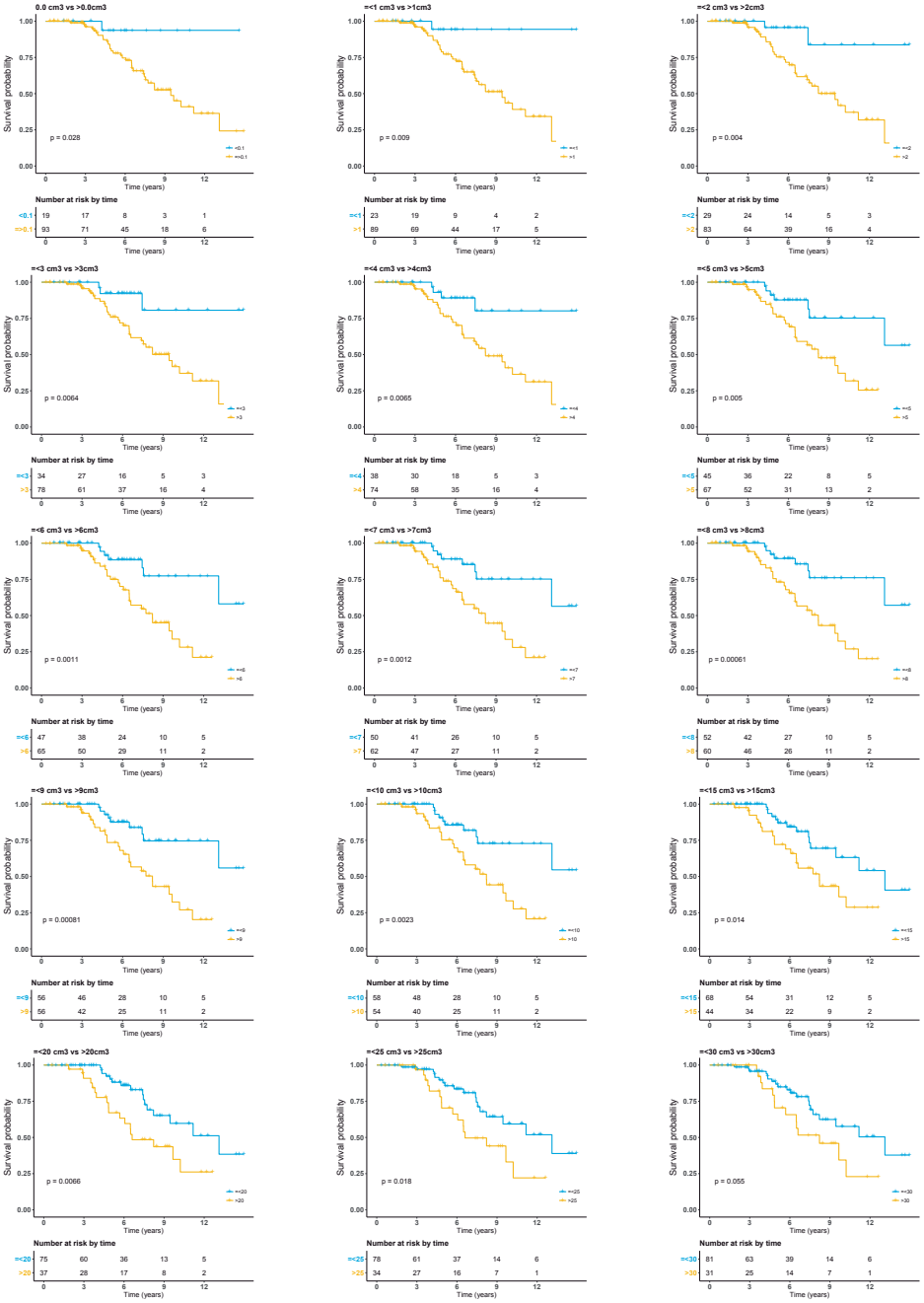
Supplementary figure 3. Overall survival stratified by histopathologic subtype (A) and molecular subtype (B).



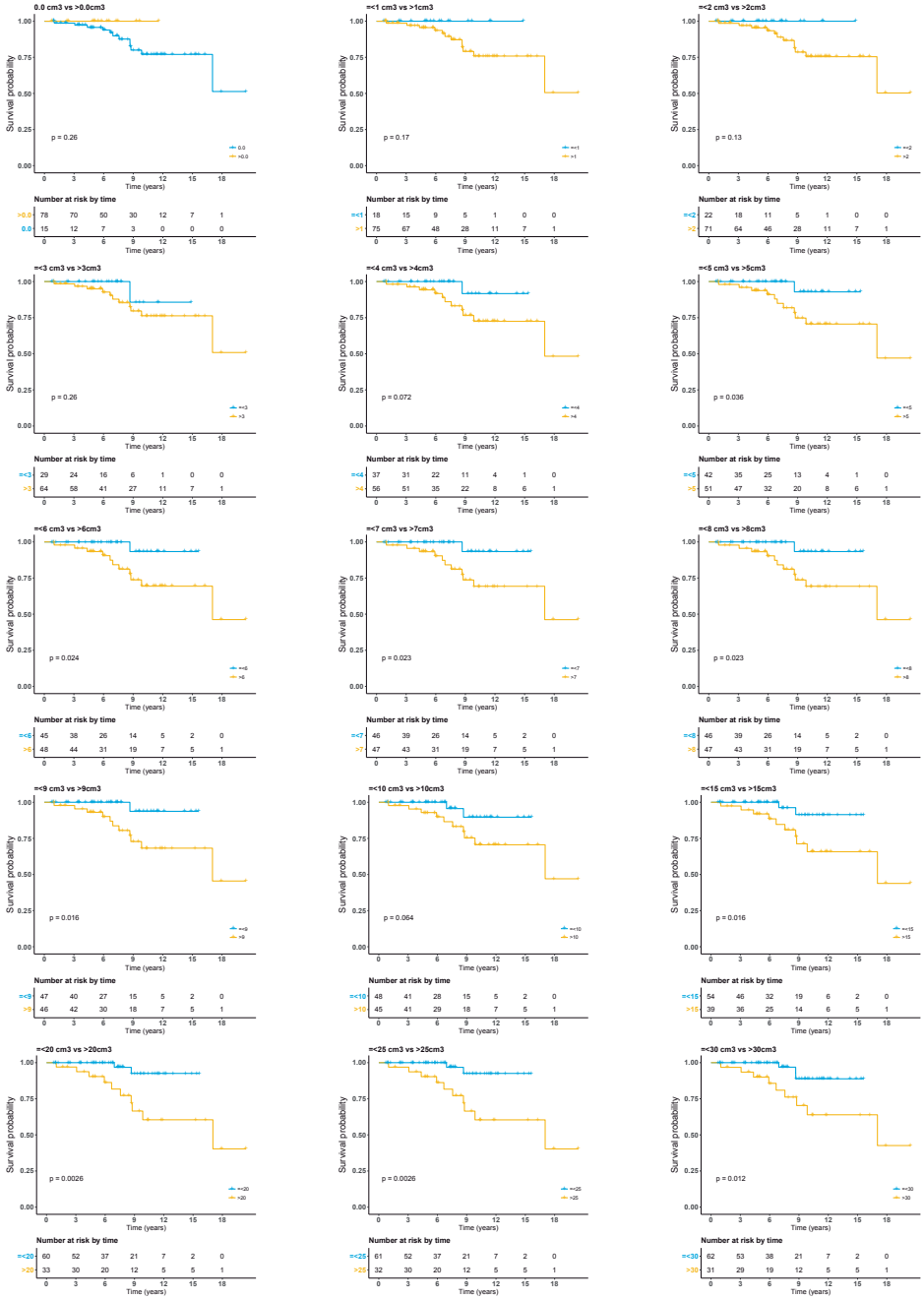
Supplementary figure 4. Overall survival stratified by resection percentage. (A) Overall survival of all patients stratified by resection percentage. (B) Overall survival of oligodendroglioma patients stratified by resection percentage. (C) Overall survival of IDH mutated astrocytoma stratified by resection percentage



Supplementary figure 5. Progression free survival stratified by postoperative tumor volume. (A) Progression free survival of all patients stratified by postoperative tumor volume. (B) Progression free survival of oligodendroglioma patients stratified by postoperative tumor volume. (C) Progression free survival of IDH mutated astrocytoma stratified by postoperative tumor volume.



Supplementary figure 6. Overall survival with dichotomisation of postoperative tumour volume in IDH mutated astrocytoma patients.



Supplementary figure 7. Overall survival with dichotomisation of postoperative tumour volume in oligodendroglioma.

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