Predetermined statistical analysis plan

Data source and analysis software
The dataset to be analyzed is a Microsoft Office Access database called "Grade II glioma and extent of resection". This database consists of ~250 grade II glioma patients from Erasmus MC and ETZ Tilburg. The complete dataset consists of in total 4 different data tables, which are linked via a patient identifier.

Data tables
tbl_Identifier: table with patient identifier, age, gender
tbl_ClinicalData: table with all clinical characteristics of the patients.
tbl_MRIData: table with all the MRI parameters (volume of tumor, location, etc.).
tbl_NGSdata: table containing all the sequencing data.

Analysis software
Data will be exported from Microsoft Access as .txt files and imported and merged in R. All analyses are processed in R (Version 3.3.2) and RStudio (Version 1.0.44).

Analysis objectives
1.Assessment of the interaction between molecular subtype and extent of resection in low-grade gliomas.
2.Assessment of the interaction between molecular subtype and outcome in relation to extent of resection in low-grade gliomas.

Handling of missing values
Missing extent of resection
If extent of resection is missing, this will be due to unavailability of perioperative MRI scans. In general sequencing of the tumors was canceled in these patients, so molecular classification will be missed in these patients as well. Therefore patients will be excluded from analysis.
Lost to follow-up
Patient will be censored at last date of follow-up
Missing molecular classification
Patients with missing molecular classification (due to failure of sequencing) will be excluded from analysis of the study objectives.

Outline for tables and figures and the underlying statistical procedures

Table 1: Descriptives of clinical characteristics, tumor and treatment characteristics

Characteristic	All patients	1p19q codeleted pts	Non-codeleted pts	IDHwt pts
Variable 1	N (%)	N (%)	N (%)	N (%)
Variable 2	N (%)	N (%)	N (%)	N (%)

For categorical variables (all variables except age) frequencies will be shown as N (%). Frequency distributions will be compared between the 3 molecular subtypes (codeleted/non-codeleted/IDHwt).
Chi-squared test will be used for this. If assumptions for chi-square distribution are violated (this will be indicated by R), then Fisher's exact test will be used.

The continuous variables will be checked for normal distribution with Shapiro-Wilks normality test. Depending on distribution mean with sd(normal distribution) or median with IQR will be reported. Distribution of age and homogeneity of variance (levene's test) within the three molecular groups will be explored and ANOVA (normal distribution and homogeneity of variance) or Kruskall-Wallis will be used for comparing between groups.

Variables to be included in table 1:
Gender; Age at diagnosis (median or mean, but also categorized as <40/40-60/>60); Presenting symptom; Watch-and-wait policy before first surgical intervention?; Type of first surgical intervention; Preoperative KPS (frequencies of categories 100/90/<80, as well as median with range); Histopathological diagnosis; Molecular diagnosis; Therapy after initial treatment; Tumor location; Eloquent area; Side of lesion; Preoperative tumor volume; Postoperative tumor volume; Resection percentage (categorized in groups, and mean/median); Median follow up
If convenient this table might be split up into 2 tables (clinical characteristics & tumor/treatment characteristics)

Table 2: Assessment of the interaction between molecular subtype and extent of resection/postoperative volume
Extent of surgery can be reported in two ways:
-percentage of resection (preoperative volume-postoperative volume/preoperative volume *100)
-postoperative volume in cm3
We will explore if there is a correlation between the molecular subtype and postoperative volume (model 1) and percentage of resection (model 2).

We will perform a multiple linear regression using the ENTER method. To avoid overfitting the input variables are chosen beforehand and stated below:

The following covariates will be put in the model (because they are likely to influence the dependent variable based on literature):
- molecular subtype (this is the main variable of interest)
- preoperative volume
- eloquency (this factor is known to influence extent of resection)
- tumor location
- age

Type of surgery will not be included, since this is probably strongly correlated with eloquency
- We will carry out 2 analyses: one with all the patients included, and one with exclusion of the biopsy patients (since in these patients there is no intent to get an extensive resection). If there is no difference, we will show the analysis with all patients included.

Univariate and multivariate analyses of effect of clinical and tumor parameters on outcome
Univariate analyses will be done using cox-regression for each variable.
Overall survival: Time between first diagnostic scan and death or censorship.
Variables to be tested: Age at diagnosis; Gender; KPS; Tumor location; Side of tumor; Eloquency; preoperative volume; postoperative volume; resection percentage; molecular subtype; postoperative radiotherapy (y/n); postoperative chemotherapy.
We will create 2 separate models with 2 different input variables that both reflect a volumetric measure of tumor burden: postoperative tumor volume and resection percentage. A cox-proportional hazard model will be used for this. Variables will be checked for proportional hazards assumption. Molecular subtype, age at diagnosis, volumetric measure of tumor burden (resection percentage or postoperative tumor volume), tumor location and eloquency will be put in the model regardless of significance in univariate analysis, since they are known prognostic factors in literature. Variables with significant impact in univariate analysis will be added to the model as well.

Several Kaplan-Meier's of overall survival grouped by different variables will be created:
- extent of resection (4 categories 0-40%/41-89%/90-99%/100%) 4 figures will be generated, one for all patients together and three for the different molecular subtypes
- Postoperative volume (categories 0cm3/0.1-5.0cm3/5-15cm3/>15 cm3) 4 figures will be generated, one for all patients together and three for the different molecular subtypes

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

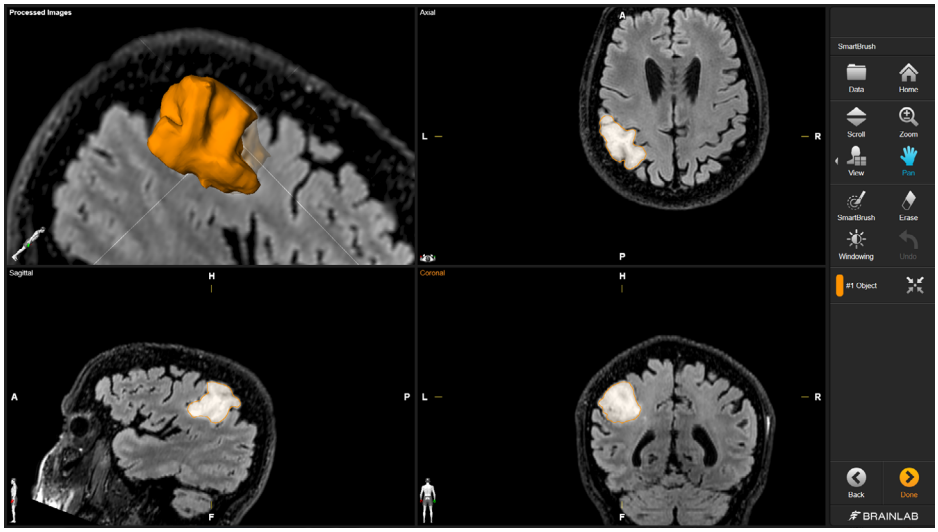
Table S3. Overview of salvage treatments

	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%)

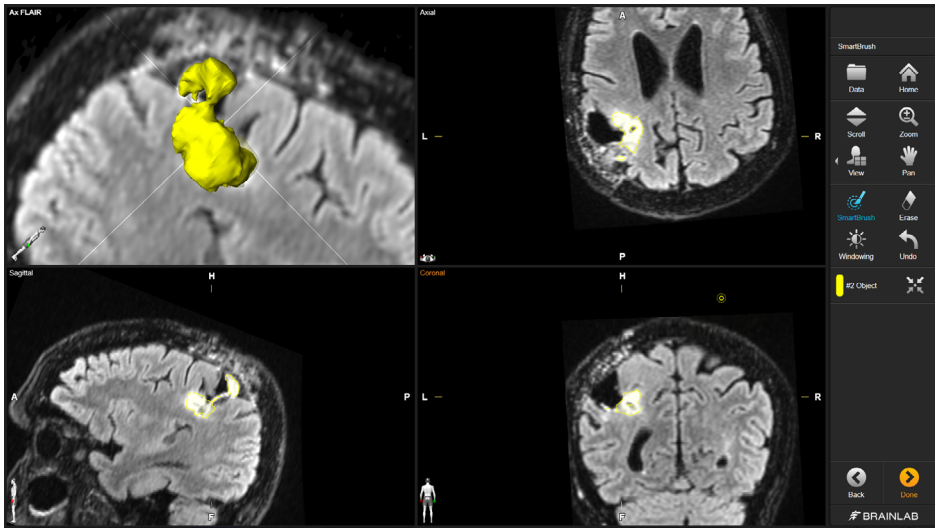
Table S4. Overview of surgical outcome

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%)

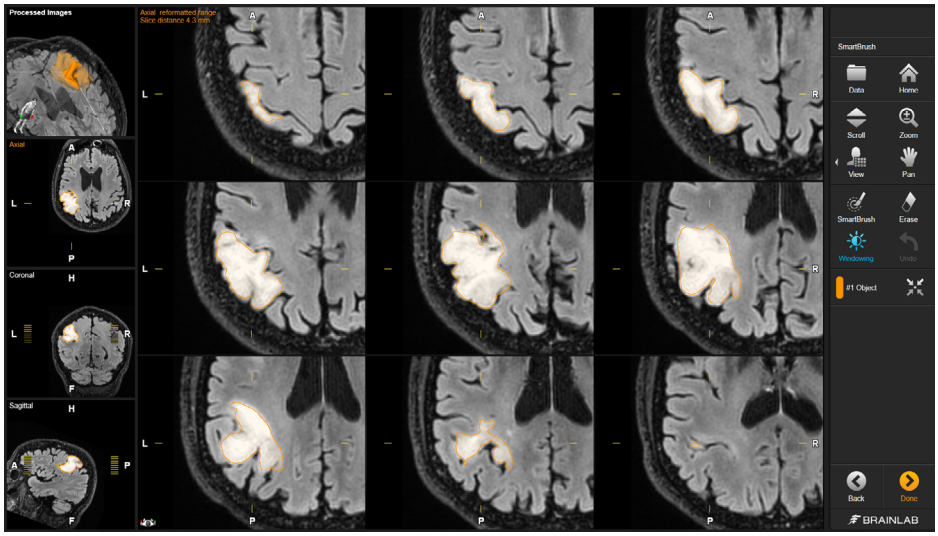
Figure S1 A-C. Example of semi-automatic assessment of pre- and postoperative tumour volume



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.

Figure S2. Quartiles of preoperative tumour volume vs postoperative tumour volume

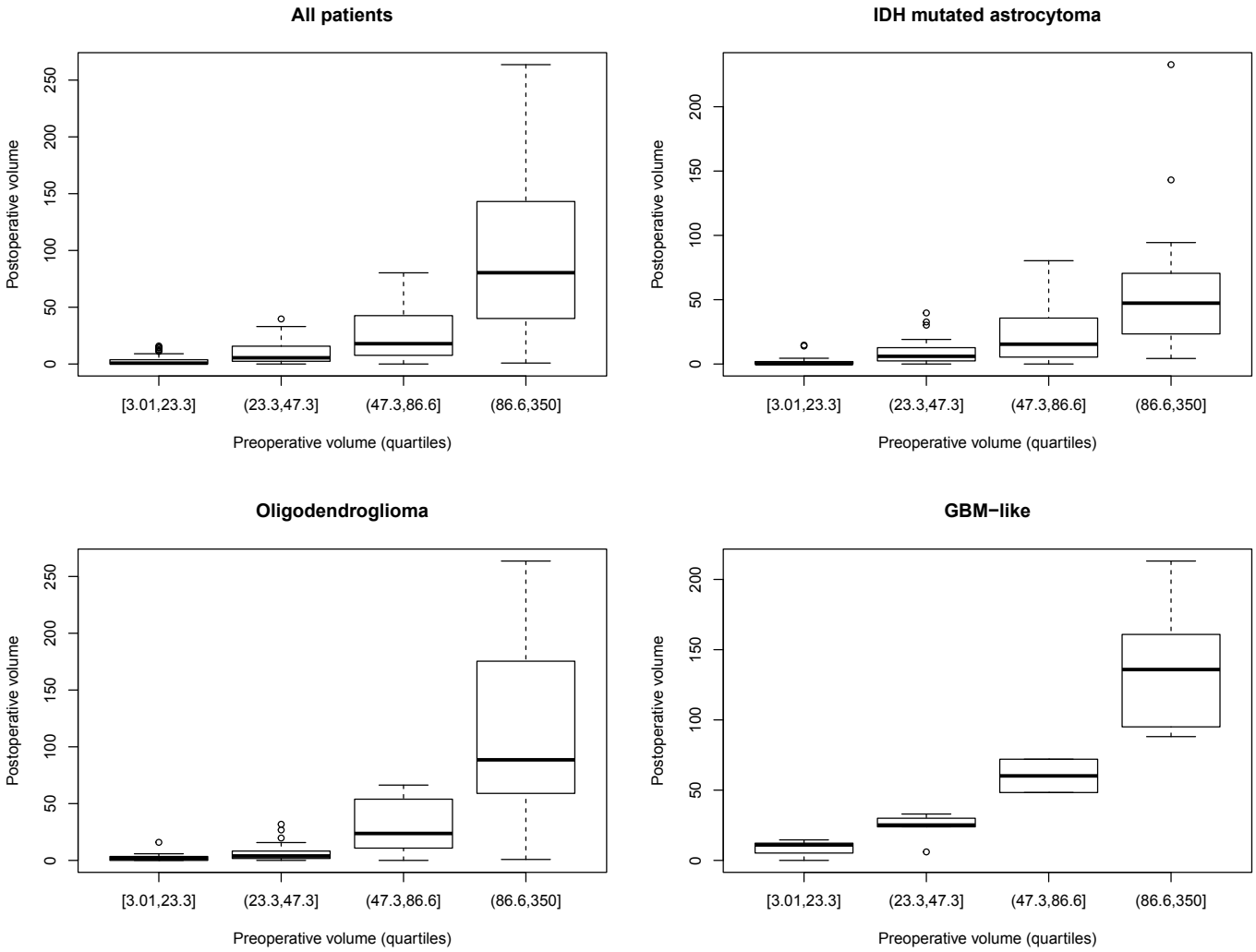
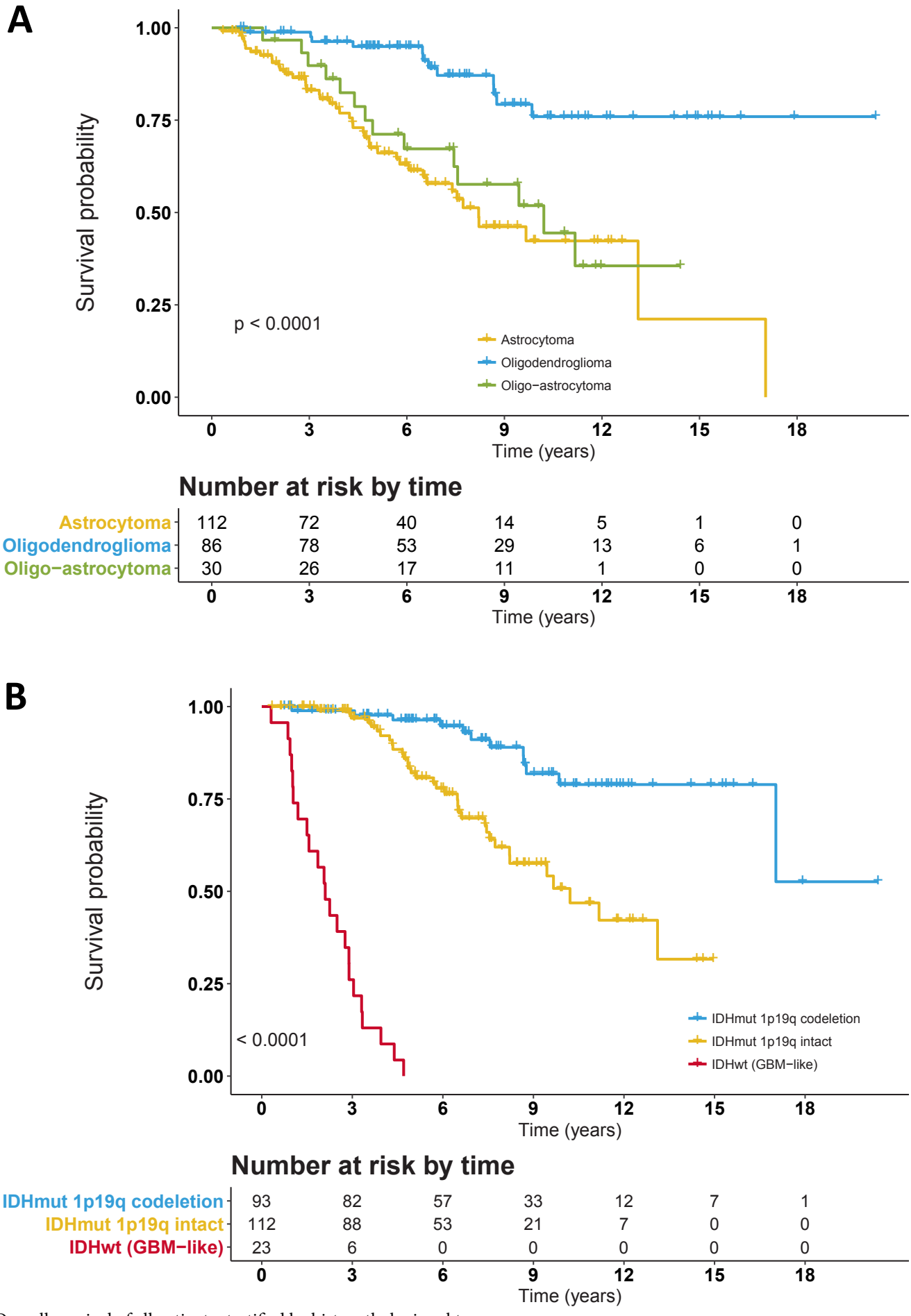
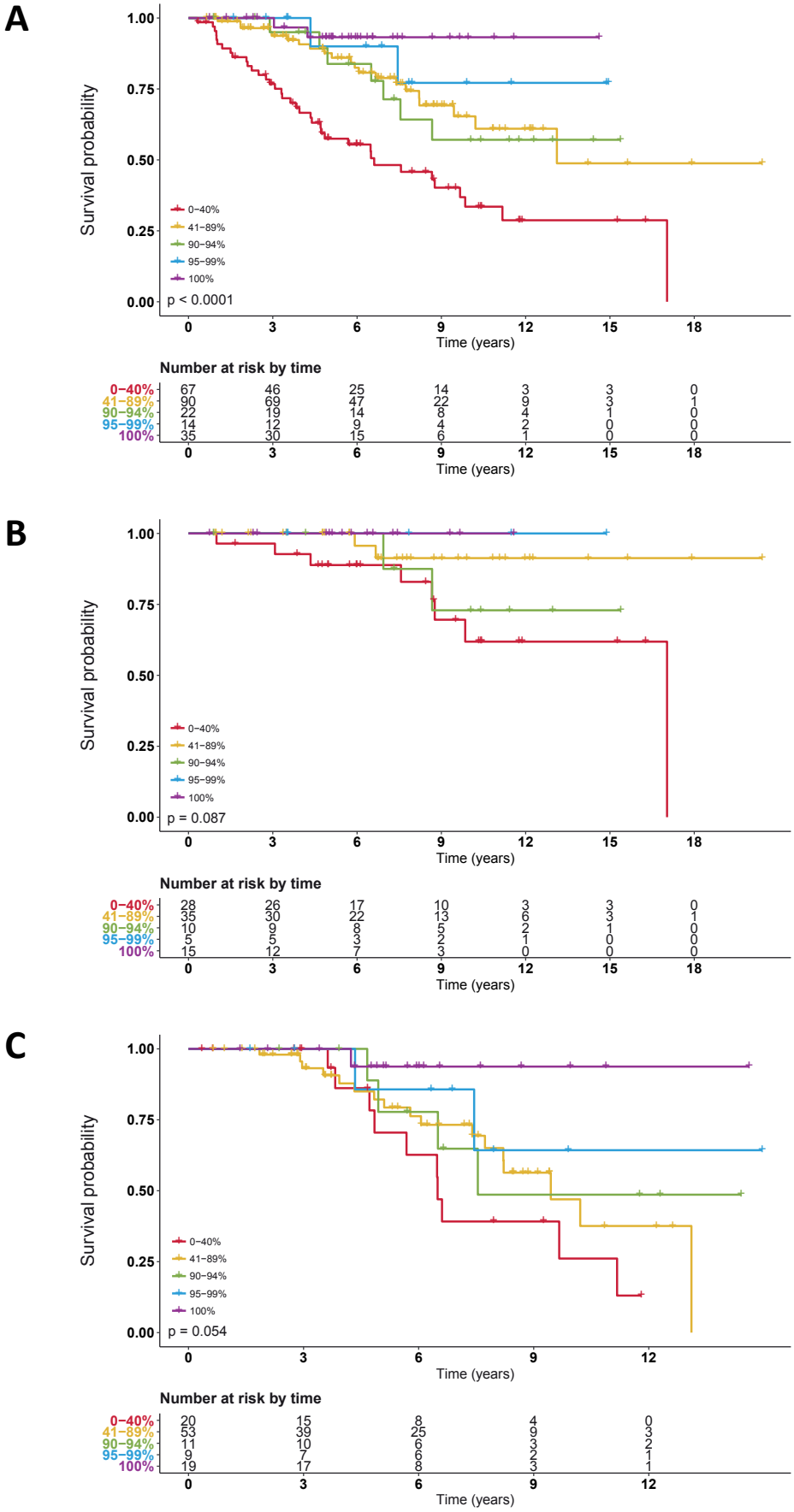


Figure S3 A-B. Overall survival stratified by histopathologic subtype (A) and molecular subtype (B)



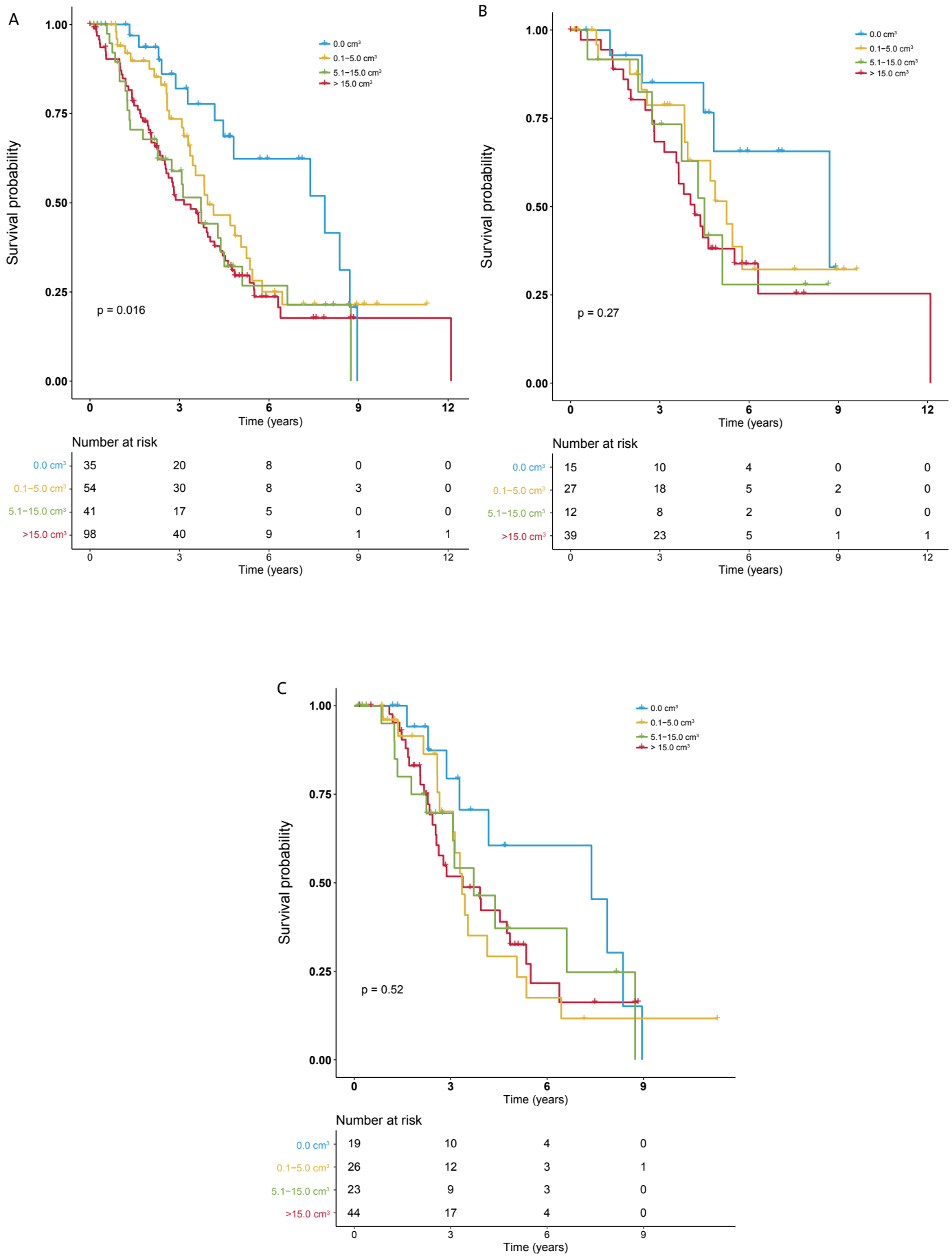
A) Overall survival of all patients stratified by histopathologic subtype.
B) Overall survival of all patients stratified by molecular subtype.

Figure S4 A-c. 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

Figure S5 A-C. 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

Figure S6. Overall survival with dichotomisation of postoperative tumour volume in IDH mutated astrocytoma patients

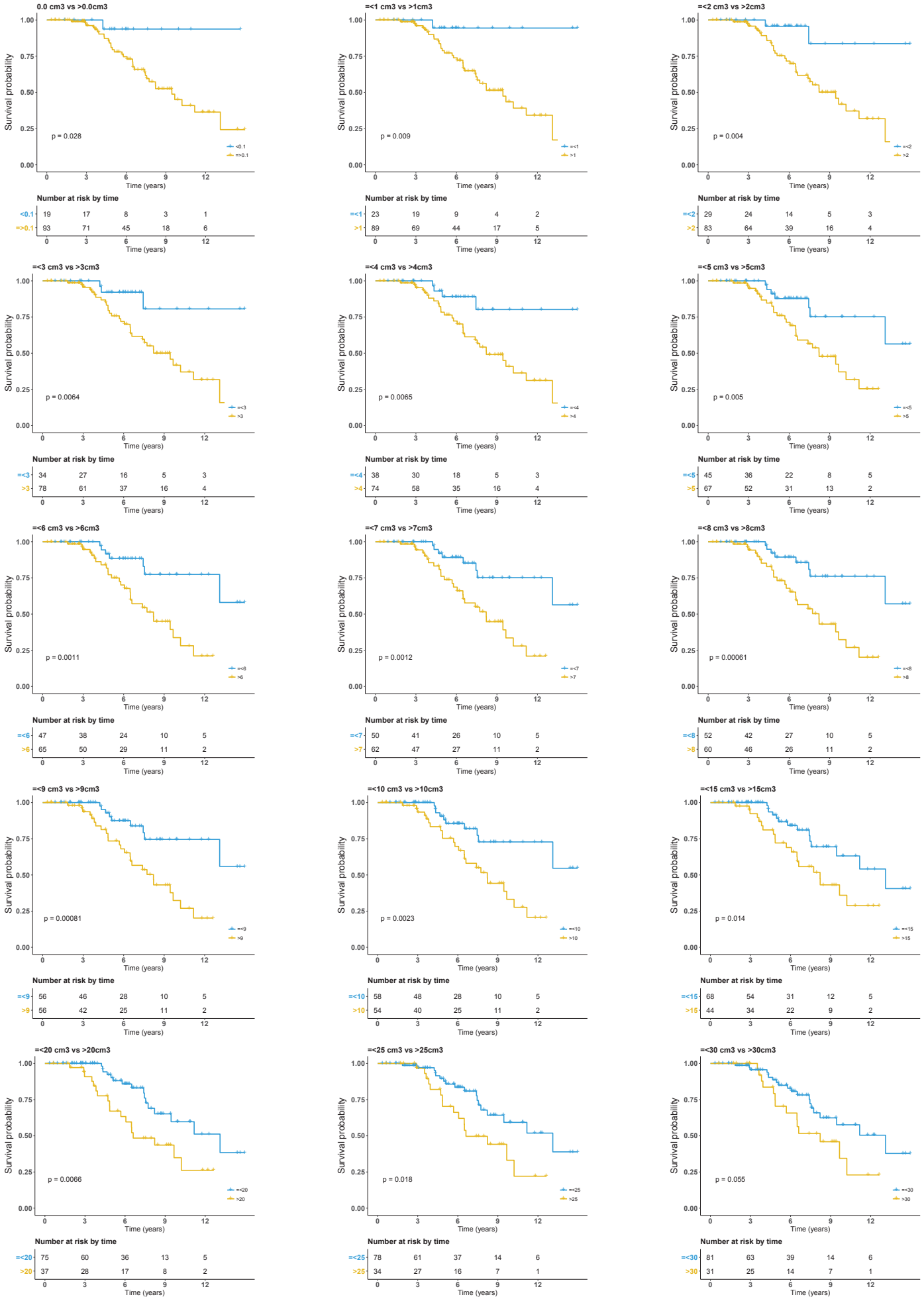


Figure S7. Overall survival with dichotomisation of postoperative tumour volume in oligodendroglioma