Association of Supratotal Resection with Progression-Free Survival,

Malignant Transformation, and Overall Survival in Lower-Grade Gliomas

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2

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Abstract

Background: supratotal resection is advocated in lower-grade-gliomas (LGGs) based on theoretical advantages, but with limited verification of functional risk and data on oncological outcomes. We assessed the association of supratotal resection in molecular-defined LGGs with oncological outcomes.

Methods: 460 presumptive LGGs included; 404 resected; 347 were LGGs, 319 IDH-mutated, 28 wildtype. All patients had clinical, imaging, molecular data. Resection aimed at supratotal resection without any patient or tumor a-priori selection. The association of Extent-of-Resection (EOR), categorized on volumetric-FLAIR-images as residual-tumor-volume, along with post-surgical-management with Progression-free-survival (PFS), malignant-progression-free-survival (MPFS), and Overall-Survival (OS) assessed by univariate, multivariate, propensity-score-analysis. The study mainly focused on IDH-mutated-LGGs, the "typical LGGs".

Results: Median follow-up:6.8 years(IQR:5-8). Out of 319 IDH-mutated-LGGs, 190 (59.6%) progressed, median PFS:4.7 years(95%CI:4–5.3). Total and supratotal resection obtained in 39% and 35% of patients of IDH1-mutated tumors. In IDH-mutated, most patients in partial/subtotal group progressed, 82.4% in total, only 6 (5.4%) in supratotal. Median PFS was 29 months(95%CI:25-36) in subtotal, 46 months(95%CI:38-48) in total, while at 92 months, PFS in supratotal was 94.0%. There was no association with molecular-subtypes and grade. At random-forest-analysis, PFS strongly associated with EOR,RT, previous treatment. In the propensity-score analysis, EOR associated with PFS (HR,0.03;95%CI,0.01-0.13). MPFS occurred in 32.1% of subtotal-total groups; 1 event in supratotal. EOR, grade-III, previous treatment correlated to MPFS. At random-forest analysis, OS associated with EOR as well.

Conclusions: Supratotal resection strongly associated with PFS, MPFS and OS in LGGs, regardless of molecular subtypes and grade, right from the beginning of clinical presentation.

Key words: lower-grade gliomas, supratotal resection, Progression-free-survival, Malignant-progression-free-survival; Overall-Survival

Key points

Supratotal resection prolongs progression free survival and overall survival, and reduces malignant transformation in LGGs, regardless of molecular subtypes and tumor grade

Importance of the study

Supratotal resection is advocated in lower-grade-gliomas (LGGs) based on theoretical advantages, but available data on the oncological impact are limited or missing. For the first time, in a large series of LGGs we assessed the association of supratotal resection with oncological outcomes, using a univariate, multivariate and propensity-score analysis. In molecular defined LGGs supratotal resection was associated with a decreased rate of progression and a prolonged progression-free-survival, regardless of molecular subtypes and tumor grade. Similarly, supratotal resection associated with a reduced rate of malignant transformation, and a longer Overall-Survival. Our data supports the use of extensive resection outside of MR-visible margins in LGGs, regardless of molecular subtypes and tumor grade, right from the beginning of clinical presentation.

5

Introduction

Low-grade gliomas are intrinsic brain tumors, often accompanied by seizures in young patients, appearing on conventional magnetic resonance as highly infiltrative non-enhancing masses, well visible in FLAIR images, involving one to multiple lobes, and occasionally presenting small enhancing nodules inside the tumor mass[1,2]. More than 90% of these tumors have IDH1-mutation and share a common biological behaviour and similar clinical prognosis. They include diffuse low-grade (grade-II) and intermediate-grade (grade-III) gliomas, and have been recently defined as lower-grade-gliomas (LGGs), a term that has replaced low-grade in the current clinical practice. Although, there are no randomized studies providing definitive evidence for a strict relationship between Extent-of-Resection (EOR) and survival in LGGs, "a imaging (FLAIR) complete resection of a suspected LGG is the currently favored approach, when feasible" [3,5–9]; massive resection improves survival also after correcting for molecular status[8,9]. However, the goal of complete resection must be balanced against potential damage, due to the strong infiltrative behavior of LGGs and their frequent involvement of highly functional cortical and subcortical regions. To achieve a maximal safe resection, the use of brain mapping techniques and a "functional approach" is beneficial combining an increase in the percentage of total/near-total resection and a decrease in the incidence of post-operative permanent deficits[2,11–13]. In the functional approach, resection is performed until functional boundaries are encountered[14,15], which can be found within, at, or outside FLAIR-visible tumor borders[16]. In the first case only a partial, or subtotal, removal is possible. In the second, a Gross-Total-Resection (GTR) is achieved. But, when functional boundaries are found outside the tumor area, and part of apparently normal brain parenchyma is removed along with the tumor itself, a supratotal resection is achieved[16,17]. The rationale for a supratotal resection stands on the finding that LGGs infiltrate the parenchyma far beyond conventional MR abnormalities, suggesting that their relapse after surgery is due to undetected glioma cells growing beyond MRI-defined abnormalities[18]. However, to date, the reports on resection going beyond the imaging targets as a strategy for surgical treatment of LGGs are very few, with limited verification of functional risk or survival benefits. Feasibility in clinical routine and global safety by the functional approach has been recently reported by our group, with

6

very limited morbidity and comparable neuropsychological and quality of life impact to total resection[16]. Although limited encouraging studies suggest an effect of supratotal resection on tumor growth control and histological transformation[19], the oncological impact of this approach on a large scale is still unknown and many important questions are still open. Its impact on rate and time of recurrence and of malignant transformation needs to be investigated in a larger sample of patients, to possibly reduce selection bias; the influence exerted by the different histo-molecular subtypes or tumor-grade, or by previous treatments, is still not known; nor is the effect of the association with adjuvant treatments.

In this study, all patients with a presumptive radiological diagnosis of LGGs consecutively admitted to our care in a five-year period were studied. In all of them, surgery was performed according to the functional boundaries, aiming at achieving a supratotal resection whenever possible. These patients were followed for a long-time (median 6.8 years,IQR:5-8) with the primary aim of assessing whether extending resection outside the MR-visible tumor border was associated with progression-free-survival: moreover, the association of supratotal resection with the occurrence and time of malignant transformation along with Overall-Survival was investigated as well.

Material and Methods

We included all patients admitted from May 2009-April 2014 harboring a radiological diagnosis of presumptive LGGs, and candidates for resective surgery. According to the radiological definition of lower-grade gliomas, we included intrinsic brain tumors appearing on conventional magnetic resonance as highly infiltrative non-enhancing masses, well visible in FLAIR images, involving one to multiple lobes, occasionally presenting small enhancing nodules inside the tumor mass. Patients harboring a FLAIR lesion involving multiple lobes, possibly of both hemispheres (gliomatosis-like) were excluded (Fig.1). Patients gave informed consent to the procedures which followed the Declaration of Helsinki for human experiments and were approved by Ethical-Committee-1299. All patients included in the study have full pre-operative, post-operative, and at follow-up clinical, imaging, and tumor data. Patients without full materials were excluded.

MR protocol includes volumetric FLAIR and T1, pre and post-GD weighted images, performed pre-operatively, post-operatively (48-hrs), 1 month, and every four months during the first four years, and every six months afterwards (eMethods.1).

Resection was accomplished according to functional boundaries aiming at achieving a supratotal resection whenever possible, without any patient or tumor (size/location/extension) *a priori* selection. Brain Mapping and Monitoring technique[14,16,20], in asleep-awake-asleep anesthesia, were used to locate functional boundaries. Resection continued till functional boundaries were reached, independent of where they located to MR-visible tumor borders.

Patients were submitted to clinical, neuropsychological and imaging (MR) follow-up every four months during the first four years, and every six months afterwards. Clinical, neuropsychological and imaging data for each follow-up time point are available for each patient.

EOR was calculated on post-operative (2 months) volumetric FLAIR and classified on the basis of Residual-Tumor-Volume (RTV) as Total (RTV=0), Subtotal (0<RTV≤5ml), Partial (RTV>5ml). Tumor volumes were computed onto volumetric pre-operative FLAIR with manual segmentation using iPlanCranial-software (BrainLab,Germany) by five blinded investigators (M.R.,M.R,L.G.,T.S.,M.C.N). A supratotal resection was defined as the complete removal of any signal abnormalities, with the volume of the post-operative cavity larger than pre-operative tumor volume[20]. The degree of supratotal resection is the ratio between the volume of the surgical cavity and of the tumor (as a percentage)[16].

Progression-Free-Survival (PFS) was calculated from the date of first surgery till the occurrence of true progression. Progression was defined (according to RANO criteria) by any of the following: 1) a 25% increase of the T2/ FLAIR non-enhancing lesion on stable or increasing doses of steroids: 2) development of new lesions or the appearance of enhancement (suggesting radiological evidence of malignant transformation), that require the start of a new treatment as judged by the treating physician[8,21,22]. Malignant progression–free survival (MPFS) was defined as the time between initial surgery and demonstration of malignant progression[7]. Malignant progression was based on biopsy confirmation of a high(er) grade (in case of surgery) or new or enhanced contrast enhancement on imaging with a multidisciplinary (board) consensus opinion that the findings represented progression

8

to a higher grade[7]. No deaths were observed without progression or MPFS. Overall Survival (OS) was calculated from the date of first surgery until death.

Among factors possibly associated with PFS, MPFS, and OS the following were investigated: 1) factors relating to the patient (age, sex, clinical history duration, incidental discovery, epilepsy history, handedness, dominance); 2) factors related to the tumor that were deductible by evaluating pre-operative conventional MR (location, volume, side, involvement of functional sites at cortical or subcortical level; involvement of corpus callosum); 3) immediate and permanent post-operative deficits; 4) previous treatments (biopsy or surgery, pre-operative chemotherapy); 5) integrated molecular/histological diagnosis, histological grade; 6) EOR, categorized as partial, subtotal, total, supratotal; 7) Post-surgical management: wait and see, chemotherapy, radiotherapy (for definition see eMethods.1).

The type of post-surgical management was evaluated by a multidisciplinary-board: adjuvant treatments were reserved to grade II-III tumors which underwent to subtotal or partial resection; observation to grade II tumors underwent to total or supratotal resection; grade III tumors underwent to supratotal resection were submitted to observation or to adjuvant treatment depending on the histo-molecular profile and pre-operative tumor volume; generally only large size tumors (with limited degree of supratotal resection) were submitted to post-surgical treatment. Chemotherapy included Temozolomide (standard-regimen) or less frequently PCV; radiotherapy included 54 Gy in 2Gy per fractions (eMethods.1,Tab.5S).

IDH1-status was determined by immunohistochemistry with mouse monoclonal anti-IDH1 p.R132H(DIA-H09)(Dianova,Hamburg,Germany); in case of negative expression by mutational analysis (sequencing for alternate mutations in IDH1 or IDH2). Co-deletion was determined by FISH; ATRX loss,p53 mutation,Ki67 by immunohistochemistry. Tumors were reviewed to confirm diagnostic classification and grading based on WHO-2016 criteria. Grade III tumors include all tumors with any sign of anaplasia, inclusive of those with small anaplastic foci embedded within a specimen of grade-II tumor. According to WHO-2016 classification, patients included in the analysis were IDH-mutated and IDH-wildtype grade II-III tumors[2](Fig.1); however, most of the analysis were performed on IDH-mutated-LGGs, the "typical LGGs".

Summary of Statistics

Continuous variables are summarized as median with interquartile range (IQR) and differences among groups were assessed by ANOVA. Categorical and ordinal variables are presented as frequencies and percentages and differences were assessed with Fisher-exact-test. P-value was considered significant when<0.05. The association between the considered variables with time to event outcome was analyzed with univariate and multivariable Cox-regression with Firth's-correction, accounting for possible problems of monotone likelihood, when needed. Significant variables in univariate analysis were included in the multivariable model. The proportionality of hazards was checked by inspection of the Schoenfeld-residuals. Kaplan-Meier was used for the analysis of PFS,MPFS, and OS as all deaths occurred after progression. In IDH-mutated-LGGs, the comparison between supratotal and total resection in terms of PFS was studied with a propensity-score-matched analysis. For propensity-score calculation, a logistic regression was used with EOR as dependent variable as a function of age, sex, duration of clinical history, reason for diagnosis, seizures, integrated diagnosis, previous treatment, location, side, volume, dominance, corpus callosum, grade and immediate deficits. A nearest neighbor matching with 0.2 caliper width was used. A multivariable-clustered-Cox-regression was used to account for matched data. Variable importance was evaluated using two measures computed by fitting a random-forest: VIMP and minimal depth. Cutoff used for variable importance correspond to the average level of the calculated variable importance measures over all the trees. Analysis was conducted using R-software.(for details:eMethods.2).

Results

Patients

In the study period, 460 patients harboring a FLAIR lesion were admitted. Four patients were excluded because of partial follow-up data; 51 were submitted to stereotactic biopsy only because of tumor involving multiple lobes, possibly of both hemispheres; 404 presumptive LGGs patients (249 males, 155 females,Tab.1S) were submitted to resection; 347 were LGGs, 319 with IDH-mutation, 28 without (Fig.1). Clinical, imaging and histo-molecular features of both series are detailed in Tab.1A(IDH-mutated) and Tab.2S(IDH-wildtype). They recapitulate all the clinical and imaging

10

features of LGGs. All patients and tumors were treated with a standard functional approach, aimed at achieving a supratotal resection, with no patient or tumor (location/size/extension) "a priori" selection. 290 patients had a first and 57 a second surgery, which followed a previous treatment. Previous treatments were partial resection followed by observation (32 patients) or chemotherapy (10 patients), stereotactic biopsy followed by observation (9 patients) or chemotherapy (6 patients). Median time interval between previous diagnosis and second surgery was 13.4 months (4-38 months). In the IDH-mutated LGGs, a partial resection was achieved in 10 patients (3%), a subtotal in 74 (23%), a total in 125 (39%) and a supratotal in 110 (35%)(for clinical, imaging, histo-molecular and post-surgical management details of EOR-classes:Tab.3SA). In the wildtype group, a subtotal was obtained in 3 (10.7%), a total in 13 (46.4%) and a supratotal in 12 (42.8%)(for clinical, imaging, histo-molecular and post-surgical management details of EOR-classes:Tab.3SB).

Progression-Free-Survival and Associated Factors

As of February 2020, median follow-up was 6.8 years (IQR:5-8); out of 404 patients included, 257 (63.6%) progressed; median PFS was 4 years (IQR:3.7-4.7 years). Analyzing only the 319 IDH-mutated-LGGs ("typical LGGs"), 190 (59.6%) progressed, median PFS: 4.7 years (95%CI: 4–5.3 years). All deaths were observed after progression. Clinical and imaging features of progressions are in Tab.1B. Most of progression occurred in cases with previous treatments, and were located within the previous surgical cavity. We presented here data on IDH-mutated tumors; data on IDH-wt are in Suppl.R1 and Fig.11S .

In the 319 IDH-mutated-LGGs, at univariate analysis (Fig.1S), among pre-operative clinical features, only duration of clinical history >6 months, reasons for diagnosis, dominance and previous treatments associated with PFS, while sex, age at diagnosis, seizure history, and handedness were not; among imaging features, tumor location, side, vicinity to eloquent cortical/subcortical sites, corpus callosum involvement and tumor volume >44 ml associated with PFS (Fig.2S). There was no association with occurrence of immediate deficits while permanent post-operative deficits were associated (condition related to 6 patients only)(Fig.3S). Among histo-molecular factors, integrated diagnosis

11

(oligodendroglioma better than astrocytoma), tumor grade as a whole (grade-II better than grade-III), and for molecular subtypes, associated with PFS (Fig.4S). In the IDH-mutated-LGGs, at univariate analysis, EOR strongly associated with PFS. All patients (100%) in the partial group progressed, 71 (95.9%) in the subtotal group, 103 (82.4%) in the total group, and only 6 (5.4%) in the supratotal (Tab.4S). Median PFS was 27.5 months (95%CI: 23-NA) in partial group, 29 months (95%CI: 25-36) in subtotal, 46 months (95%CI: 38-48) in total, while at 92 months, PFS in the supratotal group was 94% (95%CI: 86.2%-100%)(Fig.2A). The association was independent of molecular subtypes and tumor grade (Fig.2B,C,D,E)(Fig.5S). As for adjuvant treatment, about 90% of the patients in the partial or subtotal groups were submitted to adjuvant treatments. In total and supratotal groups, 68% and 86.4% of the patients were not submitted to any therapy, respectively. In the total group, 93.8% of patients with a grade-III received chemotherapy or radiotherapy; 41.2% of patients with a grade-III in the supratotal group were not submitted to any treatment (Tab.3S-A,Tab.5S). When all variables (clinical, imaging, treatments) were considered together by a random-forest analysis, PFS strongly associated with treatments, particularly EOR and RT (Fig.3A).

Progression-Free-Survival and Supratotal Resection

To investigate whether extending resection beyond the MR-visible margins was associated with PFS we further restricted the analysis to IDH-mutated-LGGs patients belonging to the EOR-classes total and supratotal. These two groups were different for some pre-operative clinical and imaging factors, and integrated diagnosis. The adjusted Cox-regression considered EOR (Total-Supratotal), integrated diagnosis (Astrocytoma-Oligodendroglioma), grade at first diagnosis (III vs II) and the factors that resulted significant in the univariate analysis, namely duration of clinical history, previous treatments, location, side, volume, dominance, corpus callosum. Other variables were not considered as they involved a too restricted number of patients. EOR and grade-III at first diagnosis resulted associated with PFS also after adjusting for the other variables (Tab.1C). PFS was significantly associated with EOR in each molecular subgroup. Specifically, PFS was significantly longer in astrocytoma or oligodendroglioma patients who received a supratotal resection in comparison to those who had a

12

total resection only (Fig.3B,C), and the association was observed in both grade-II or III tumors, for each molecular subgroup (Fig.6S). In each resection group, PFS didn't differ according to molecular subgroups (Fig.7S-A,B,C,D,E,F).

Previous treatment resulted also significantly associated with PFS (Fig.7S-G,H). To further explore this, the adjusted Cox-regression-analysis was repeated in patients who were submitted to primary surgery (no previous treatment) alone (Tab.6S). Again, PFS strongly associated with EOR and grade-III, independent of molecular factors. In patients who underwent to a previous treatment, the association was still evident, again independent of molecular factors and tumor grade.

The comparison of supratotal vs total resection on PFS was further investigated by a propensity-score-matched analysis. As in fact, patients in the total and supratotal groups have different distributions for many risk factors, and by using a propensity-score-matched analysis, two groups with similar distributions were created (Tab.7S). In the 74 matched IDH-mutated-patients, the standardized differences were reduced without any significant difference between groups:>90% of patients have a clinical history>6 months, with seizures, left handedness, 95% are grade-II, median tumor volume of 32.1ml(range: 2.4-240)(Supratotal) and 33.9ml(range: 2.73-259)(Total). Median follow-up was 6 years(IQR, 4.5-8), median PFS 8 years(IQR, 3.8-8). According to Cox-regression with robust standard errors, the HR was 0.03(p<0.001). The result was maintained after adjusting for age (HR,0.03;95%CI,0.01-0.13)(Fig.3D).

Supratotal resection and post-surgical management

To investigate the association between supratotal resection and the access to further treatments, we looked at differences in the number and type of post-surgical treatments in IDH-mutated-grade-III tumor subgroup (Tab.5S). Globally considered, EOR associated with further treatment. 41.2% of patients with a grade-III tumor in the Supratotal group were not submitted to any treatment, in comparison to 6.2% in the total group. In the latter, 75% of patients were submitted to RT and CHT, and 18.8% to CHT only; in the supratotal, only 17.6% of patients received RT and CHT, and 41.2% CHT only. According to molecular subtypes, all patients with a grade-III astrocytoma in the total

13

group received CHT, 21.1% alone, whereas 78.9% RT followed by CHT; in the supratotal group, 41.7% of grade-III astrocytoma received no treatment, 41.7% CHT alone, and 16.7% RT followed by CHT. Out of patients with a grade-III oligodendroglioma in the total resection group, 15.4% refused treatment, 84% had CHT (15.4% alone), and 69.2% combined RT and CHT; in grade-III oligodendrogliomas in the supratotal group, 40% had no treatment, 40% CHT only, and 20% RT and CHT.

Malignant-Transformation and Supratotal resection

Radiological and/or histological findings of Malignant-Transformation was documented in 63 (33.2%) out of 190 patients who progressed in the IDH-mutated-LGGs group (Tab.2A). To investigate the association between EOR and MPFS, we performed a multivariate analysis in which MPFS was analyzed considering the effect of pre-operative clinical, imaging and molecular factors. EOR, grade-III at first diagnosis, integrated diagnosis (astrocytoma) and previous treatments resulted significantly associated with MPFS (Tab.2B); EOR was strongly associated, in both grade-II and grade-III tumors (Fig.4A,B). While malignant transformation occurred in 34.6% of IDH-mutated-LGGs in the subtotal/partial resection groups and in 33% of those in the total group, no events were recorded in grade-II tumors that underwent to supratotal resection, and only one in grade-III tumors (Fig.4A,B). The association was evident in astrocytomas (independent of grade) while only in grade-II oligodendrogliomas was significant (Fig.8S,A,B,C,D). While in the total resection groups, MPFS associated with tumor grade, in those who underwent to supratotal was independent (Fig.8S,E,F).

Recurrences and Degree of Supratotal Resection

Of the 6 IDH-mutated-LGGs patients in the supratotal group who progressed, 4 had previous treatments (partial surgery followed by CHT); most had tumors in the frontal lobe, of large volume. The mean degree of supratotal resection in all 110 cases was 262.98%,median 143%. The degree of supratotal was lower in the 6 recurrent patients (mean 111.3%,median 107.41%) than in the 104 cases who didn't recur (mean 271.11%,median 148.775%)(Fig.4C). The mean degree in the 12 IDH-wt-LGGs who didn't recur, was 818.20%,median 197.08%(Fig.4C).

14

Overall-Survival and Supratotal resection

In the 319-IDH-mutated LGGs the median OS at 72 months (median follow-up duration) was 0.77(95%CI:0.72-0.83). At univariate analysis, among pre-operative clinical features, only age, duration of clinical history >6 months, reasons for diagnosis and previous treatments were associated with overall survival, while for gender, seizure history, and handedness there was no evidence of association, as for occurrence of immediate and permanent deficits. Among imaging features, only tumor volume was associated. Among histo-molecular factors, tumor grade as a whole (grade-II better than grade-III), and molecular subtypes (oligodendroglioma better than astrocytoma) were associated (Fig.9S.A,B). EOR was strongly associated with OS; at 72 months, PFS in the subtotal and total group was 65.8%(95%CI:55.5%-78%) and 68.5%(95%CI:60.4%-78.3%) respectively, while no events were recorded in grade II-III tumors underwent to supratotal resection (Fig.4D). OS was significantly associated with EOR in each molecular subgroup (Fig.9S.C,D-Fig.10S,A,B,C,D). When all the variables were considered together by random-forest analysis, OS was mainly associated with treatments, RT and EOR (Fig.4E).

Discussion

This is the first study evaluating the association of supratotal resection with PFS, MPFS, and OS in a large series of LGGs. To reduce selection bias constitutively present in each retrospective surgical study, we included in the analysis all patients harboring a radiological diagnosis of presumptive LGGs consecutively admitted in our care in the study period. The population studied was large and representative in term of clinical and imaging features of LGGs; only patients with a diffuse large tumor involving multiple lobes and both hemisphere were excluded. This reduced the influence of tumor size, location and delineation on decision to operate and resectability[3,8]. Most patients were at first diagnosis; all patients were submitted, without any tumor-patient a priori selection, to a functional surgical approach aimed to achieve a supratotal resection[14,16,20]. In all, the resection was pushed until functional boundaries were encountered, independent of their location to FLAIR-visible tumor margins. 85.9% were LGGs, 90.8% had IDH-mutation, of different molecular subtypes. EOR was categorized into volumetric FLAIR images, according to RTV[8].

15

In the IDH-mutated-LGGs -the "typical LGGs"- PFS was associated with very few pre-operative clinical factors, such as long duration of clinical history, tumor volume, or previous treatments. EOR, with post-surgical adjuvant treatments (radiotherapy), was strongly associated. In particular, the number of patients who progressed was very limited (5.4%) in the group in whom a supratotal resection was achieved, compared to that observed in the total (82.4%), subtotal (95.9%) and partial (100%) groups. The association of EOR with PFS was maintained in molecular defined subtypes, and according to tumor grade. It could be argued that these results are mitigated by the possible intrinsic patients selection, with those undergoing subtotal-partial resection carriers of unfavourable tumors, and those in supratotal with more easily resectable tumors. For this reason, we focused the analysis on the low-risk group patients, those in whom a complete resection was always achieved[3,8]. In fact, it is exactly in this low-risk group of patients that the question "why to stop resection when a supratotal is feasible" is of relevance. The benefit of extending removal outside MR-visible border was confirmed; the supratotal group was characterized by a very low number of late progression, a figure confirmed for each molecular subtype and tumor grade. The association was then finally demonstrated in the same groups by the propensity-score analysis: supratotal resection not only decreased the number of progression but also postponed progression over time. The impact of supratotal resection was higher in primary tumors than in those previously treated, confirming the benefit of performing an aggressive extensive treatment right from the beginning of clinical history or diagnosis.

In IDH-mutated-LGGs, malignant transformation was associated with grade-III at first diagnosis, molecular subtypes (astrocytoma), and EOR; EOR was a strong determinant. While 33.7% (average) of tumors in partial/subtotal-total groups transformed, no event was recorded in grade-II belonging to supratotal group, and only one was documented in grade-III tumors. This suggests that resecting the apparently normal tissue at the tumor periphery by decreasing the number of possibly remaining tumor cells, strongly decreases their propensity to acquire and progress toward a malignant phenotype[8,17-19].

The impact of supratotal resection was also evaluated in term of OS. Although considering the median duration of the follow-up period, OS associated with molecular subtypes (oligodendroglioma better

16

than astrocytoma), tumor grade (grade II better than grade III), tumor volume and EOR; at random-forest analysis, EOR was a strong determinant, supporting the benefit of supratotal resection.

This study was mainly focused on IDH-mutated tumors, the "typical LGGs". The 2016-WHO classification includes within the LGGs also IDH-wt-tumors; however, recent studies suggested that these are completely different entities, which deserve a separate management[25]. Interestingly, EOR associated with PFS also in this small group of LGGs: median PFS was 34 months in total resection group, and no event were recorded in the supratotal group.

It is of note that the achievement of a supratotal resection was associated to a different choice of post-surgical treatment. Interestingly, while most of grade-III tumors in the total group received an adjuvant treatment (radio or chemo, or a combination), 42% of those belonging to the supratotal group didn't receive any treatment. In addition, in all the other cases, chemotherapy was the treatment mainly administered.

The measurement of the degree of supratotal resection links to the question of how much the resection should be extended outside the MR-visible margin to impact on PFS. Analyzing the patients who underwent progression in the supratotal group, the degree of supratotal resection reached in these patients was lower than those achieved in those who didn't recur[16,20]; in addition most of these patients had a previous treatment and large volume tumor. This suggests that particularly in the presence of previous treatment, if the degree of supratotal doesn't exceed a certain value, the benefit in term of PFS is minimal to total resection.

The removal of apparently normal tissue outside the MR-visible borders raises the issue of safety. We addressed these issues in a previous work, analyzing the functional impact of supratotal resection in a series of 449 LGGS operated on by the same functional surgical approach: supratotal resection was safe and characterized by a very low functional risk, as demonstrated by the detailed neurological, neuropsychological and quality of life long-term examinations[16]. Part of the patients of that analysis was included in this study. When extended to the remaining part, the analysis of neurophychological

17

and quality of life profiles didn't show any differences in patients submitted to total or supratotal resection, further confirming the global safety of the procedure (Tab.8S).

This study has limitations. It is retrospective in nature, and therefore conclusions should be confirmed in further larger independent series. In addition, these data are originated from a single neuro-oncological network in Italy, strongly supporting the policy of early maximal resection[9]. Therefore, it is unclear whether these results are generalizable to all institutions given possible differences in patient referral and populations, treatment selection policy, and post-treatment management plans. Furthermore, the association between degree of supratotal and PFS opens to the assessment of number of infiltrative tumor cells or of tumor cell-free borders[17,18]. This raises new questions, all outside the scope of the study.

In conclusions, this study confirms the association of EOR with PFS,MPFS and OS in LGGs[5,7,8,10,23,24]; furthermore, this is the first study to our knowledge to explore the outcome of supratotal resection in a large cohort of LGGs, in light of clinical and molecular information. This study supports extensive resection outside of MR-visible margins, regardless of molecular subtypes, right from the beginning of clinical presentation.

18

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References

1   Reuss DE, Mamatjan Y, Schrimpf D, *et al.*IDH mutant diffuse and anaplastic astrocytomas

have similar age at presentation and little difference in survival: a grading problem for WHO.

Acta Neuropathol 2015,129:867-873.

2   Buckner J, Giannini C, Eckel-Passow J, *et al.*Management of diffuse low-grade gliomas in

adults - Use of molecular diagnostics.Nat.Rev.Neurol.2017;13(6):340-351

3   Schiff D, Van Den Bent M, Vogelbaum MA, *et al.*Recent developments and future directions

in adult lower-grade gliomas: Society for Neuro-Oncology (SNO) and European Association

of Neuro-Oncology (EANO) consensus.*Neuro Oncol* 2019; 21(7):837-853.

4   Tom MC, Cahill DP, Buckner JC, *et al.*Management for Different Glioma Subtypes: Are All

Low-Grade Gliomas Created Equal? *Am Soc Clin Oncol Educ B* (2019).133-145

5   Jakola AS, Myrmel KS, Kloster R, *et al.*Comparison of a strategy favoring early surgical

resection vs a strategy favoring watchful waiting in low-grade gliomas.*JAMA*

2012,308,18:1881-1889.

6   Jakola AS, Skjulsvik AJ, Myrmel KS, *et al.*Surgical resection versus watchful waiting in low-

grade gliomas.*Ann Oncol* 2017; 28:1942-1948

7   Smith JS, Chang EF, Lamborn KR, *et al.*Role of extent of resection in the long-term outcome

of low-grade hemispheric gliomas.*J Clin Oncol* .2008:26,8:1338-1345.

8   Wijnenga MMJ, French PJ, Dubbink HJ, *et al.*The impact of surgery in molecularly defined

low-grade glioma: An integrated clinical, radiological, and molecular analysis.*Neuro Oncol*

2018.,20(1):103-112

9   Rudá R, Angileri FF, Ius T, *et al*. Italian consensus and recommendations on diagnosis and

treatment of low-grade gliomas. An intersocieties (SINch/AINO/SIN) document. J Neurosurg

Sci.2020 Apr 29.

10 Choi J, Kim SH, Ahn SS, *et al.*Extent of resection and molecular pathologic subtype are potent prognostic factors of adult WHO grade II glioma.*Sci Rep* 2020;10-2086

11 Aghi MK, Nahed B V, Sloan AE, *et al.*The role of surgery in the management of patients with diffuse low grade glioma.*J Neurooncol* 2015;125:503–30.

12 Duffau H. A new philosophy in surgery for diffuse low-grade glioma (DLGG): Oncological and functional outcomes.*Neurochirurgie*.2013;59(1):2-8

13 De Witt Hamer PC, Robles SG, Zwinderman AH, *et al.*Impact of intraoperative stimulation brain mapping on glioma surgery outcome: A meta-analysis.*J.Clin.Oncol*. 2012; 30(20):2559-65

14 Bello L, Riva M, Fava E, *et al.*Tailoring neurophysiological strategies with clinical context enhances resection and safety and expands indications in gliomas involving motor pathways. *Neuro Oncol* 2014;16(8):110-28

15 Bello L, Fava E, Carrabba G, *et al.*Present day's standards in microsurgery of low-grade gliomas.*Adv.Tech.Stand.Neurosurg*.2010; 35:113-57

16 Rossi M, Ambrogi F, Gay L, *et al.*Is supratotal resection achievable in low-grade gliomas? Feasibility, putative factors, safety, and functional outcome.*J Neurosurg* 2019;17:1-14.

17 Yordanova YN, Moritz-Gasser S, Duffau H.Awake surgery for WHO grade II gliomas within 'noneloquent' areas in the left dominant hemisphere:Toward a 'supratotal' resection - Clinical article.*J Neurosurg* .2011;115:232-239

18 Pallud J, Varlet P, Devaux B, *et al.*Diffuse low-grade oligodendrogliomas extend beyond MRI-defined abnormalities.*Neurology* 2010;74(21):1724-31

19 Duffau H.Long-term outcomes after supratotal resection of diffuse low-grade gliomas: a consecutive series with 11-year follow-up.*Acta Neurochir (Wien)*,2016.:158:51-58

20 Rossi M, Sani S, Nibali MC, *et al.*Mapping in Low-Grade Glioma Surgery: Low- and High-

Frequency Stimulation.*Neurosurg.Clin.N.Am*.2019;30(1):55-63

21   Van den Bent MJ, Wefel JS, Schiff D, *et al.*Response assessment in neuro-oncology (a report

of the RANO group):Assessment of outcome in trials of diffuse low-grade gliomas.*Lancet

Oncol*.2011;12(6):583-93

22.   Wen PY, Chang SM, Van den Bent MJ, et al.Response Assessment in Neuro-Oncology

Clinical Trials.*J Clin.Oncol.* 2017 Jul 20;35(21):2439-2449.

23.   Sanai N, Chang S, Berger MS.Low-grade gliomas in adults.*J Neurosurg.* 2011

Nov;115(5):948-65

24.   Capelle L, Fontaine D, Mandonnet E, *et al*.Spontaneous and therapeutic prognostic factors in

adult hemispheric World Health Organization Grade II gliomas: a series of 1097 cases: clinical

article*J Neurosurg*.2013 Jun;118(6):1157-68

25.   Weller M, Weber RG, Willscher E, *et al*.Molecular classification of diffuse cerebral WHO

grade II/III gliomas using genome. and trascriptome-wide profiling improves stratification of

prognostically distinct patient groups.*Acta neuropathol* 2015:129:679-693

Figure Legends

Figure 1: Data Flow Diagram for the retrospective cohort; the study mainly focuses on IDH-mutated LGGs (highlighted with a circle).

Figure 2: Kaplan-Meier curves for PFS for EOR classes, and in molecular and tumor grade defined subtypes.

A) Kaplan-Meier curves and number at risk for PFS and EOR classes. The analysis includes all 319 IDH-mutated-LGGs. Time is expressed as months. Patients were categorized according to EOR classes. In Partial (N=10)(Black line) median PFS was 27.5(95%CI:23-NA) months(univariable HR,1[reference]); in Subtotal (N=74)(Black small-dotted line) was 29(95%CI:29-25) months (univariable HR,0.51[95%CI:0.26-1]; p=0.05), in Total (N=125)(Black medium-dotted line) was 46(95%CI:38-48) months (univariable HR,0.27[95%CI:0.14-0.54];p<0.001); in supratotal (N=110)(Black large-dotted lined) at 92 months 94% of patients had no recurrences (univariable HR,0.01[95%CI:0.00-0.02];p<0.001). Shadings are the 95% confidence intervals.

B) Kaplan-Meier curves and number at risk for PFS and EOR classes in IDH-mutated grade-II Astrocytomas. Only grade-II IDH-mutated astrocytomas were included (N=89). Time is expressed as months. Patients were categorized according to EOR classes. In Partial (N=3)(Black line) median PFS was 24(95%CI:23-NA) months (univariable HR,1[reference]); in Subtotal (N=21)(Black small -dotted line) was 47(95%CI:28-72) months (univariable HR,0.26[95%CI:0.07-0.94];p=0.04), in Total (N=43)(Black medium-dotted line) was 48(95%CI:36-60) months (univariable HR, 0.21[95%CI: 0.06-0.73];p=0.01); in supratotal (N=22)(Black large-dotted lined) at 80 months 100% of patients had no recurrences months (univariable HR,0.01[95%CI:0.00-0.06];p<0.001). Shadings are the 95% confidence intervals.

23

C) Kaplan-Meier curves and number at risk for PFS and EOR classes in IDH-mutated grade-II Oligodendrogliomas. Only grade-II IDH-mutated oligodendrogliomas were included (N=159). Time is expressed as months. Patients were categorized according to EOR classes. In Partial (N=6)(Black line) median PFS was 32(95%CI:25-NA) months (univariable HR,1[reference]); in Subtotal (N=32)(Black small-dotted line) was 28.5(95%CI:24-41) months (univariable HR,0.60[95%CI:0.25-1.48];p=0.27), in Total (N=50)(Black medium-dotted line) was 48(95%CI:45-60) months (univariable HR,0.24[95%CI:0.10-0.60];p=0.002); in supratotal (N=71)(Black large-dotted lined) at 80 months 100% of patients had no recurrence, at 92 months 93.8% had no recurrence months (univariable HR,0.004[95%CI:0.00-0.02];p<0.001). Shadings are the 95% confidence intervals.

D) Kaplan-Meier curves and number at risk for PFS and EOR classes in IDH-mutated grade-III Astrocytomas. Only grade-III IDH-mutated astrocytomas were included (N=41). Time is expressed as months. Patients were categorized according to EOR classes. In Subtotal (N=10)(Black line) was 24.5(95%CI:23-NA) months (univariable HR,1[reference]), in Total (N=19)(Black small-dotted line) was 35(95%CI:34-46) months (univariable HR,0.35[95%CI: 0.15-0.81];p=0.01); in supratotal (N=12)(Black large-dotted lined) at 48 months 90% of patients had no recurrence (univariable HR,0.01[95%CI:0.00-0.11];p<0.001). Shadings are the 95% confidence intervals.

E) Kaplan-Meier curves and number at risk for PFS and EOR classes in IDH-mutated grade-III Oligodendrogliomas. Only grade-III IDH-mutated oligodendrogliomas were included (N=30). Time is expressed as months. Patients were categorized according to EOR classes. In Partial (1 case, not shown in figure and in the analysis) PFS was 23 months, in Subtotal (N=11)(Black dotted line) median PFS was 23(95%CI:22-NA) months (univariable HR,1[reference]), in Total (N=13)(Black small-dotted line) was 36(95%CI:34-NA) months (univariable HR,0.53[95%CI:0.23-1.25];p=0.15); in supratotal (N=5)(Black large-dotted lined) at 48 months 75% of patients had no recurrence (univariable HR,0.07[95%CI:0.01-0.58];p=0.01). Shadings are the 95% confidence intervals.

24

Figure 3

A) Variable importance for PFS using Random.Forest in the IDH-mut-LGGs. All the clinical, imaging, histo-molecular and treatments (EOR,RT,CHT) were considered together. VIMP and Minimal Depth are two criteria proposed in the context of random forests algorithms to evaluate variable importance in explaining the PFS. The variables on the diagonal red line are those ranked equally by the two methods. The vertical line divides variables with positive VIMP (left) from those with negative VIMP (right; unimportant). The horizontal line indicates the minimal depth threshold: important variables are below the line.

 PFS was mainly associated to treatments, particularly EOR and RT. The VIMP rank is reported in x-axis. The Minimal Depth (Rank Order) is in y-axis.

B) Kaplan Meier curve and number at risk for IDH-mutated grade-II-III astrocytomas underwent to total vs supratotal resection (N=96). Time is expressed as months. In Total (N=62)(Black line) was 38(95%CI:36-55) months (univariable HR,1[reference]); in Supratotal (N=34)(Black dotted line) at 48 and 96 months, 97% and 72.7% respectively, of patients had no recurrence (univariable HR,0.05[95%CI:0.02-0.16];p<0.001). Shading is the 5-95% range across distribution.

C) Kaplan Meier curve and number at risk for IDH-mutated grade-II-III oligodendrogliomas underwent to total vs supratotal resection (N=139). Time is expressed as months. In Total (N=63)(Black line) was 48(95%CI:40-48) months (univariable HR,1[reference]); in Supratotal (N=76)(Black dotted lined) at 48 and 96 months, 98.6% and 85.6% respectively, of patients had no recurrence (univariable HR,0.02[95%CI:0.01-0.07];p<0.001). Shadings are the 95% confidence intervals.

D) Kaplan Meier curve and number at risk for the 74 IDH-mut-LGGs patients of the propensity-score matched group. Time is expressed as months. In Total (N=37)(Black line) was 55(95%CI:40-72) months (univariable HR,1[reference]); in Supratotal (N=37)(Black dotted line) at 96 months 87.5% of patients had no recurrence (univariable HR,0.01[95%CI:0.00-0.11];p<0.001).

25

Figure 4: Malignant Progression-Free Survival in 319 IDH-mutated-LGGs patients, and Degree of supratotal resection.

A) Kaplan-Meier curve and number at risk for MPFS in IDH-mutated-grade-II tumors according to EOR classes. Grade-II tumors (N=248) are inclusive of grade-II astrocytomas and grade-II oligodendrogliomas. Partial and Subtotal resection groups were merged for the analysis. Time is expressed as months. In Partial+Subtotal resection group (N=62)(Black line) median PFS was 72(95%CI: 60-NA) months (univariable HR,1[reference]), in Total (N=93)(Black small-dotted line) at 48 and 72 months, 80% and 75% of patients had no transformation (univariable HR,0.59[95%CI:0.29-1.21];p=0.15); in supratotal (N=93)(Black large-dotted lined) no events were recorded during the observation period (univariable HR,0.01[95%CI:0.00-0.08];p<0.001). Shadings are the 95% confidence intervals.

B) Kaplan-Meier curve and number at risk for MPFS in IDH-mutated-grade-III tumors according to EOR classes. Grade-III tumors (N=71) are inclusive of grade-III astrocytomas and grade-III oligodendrogliomas. Partial and Subtotal resection groups were merged for the analysis. Time is expressed as months. In Partial+Subtotal resection group (N=22)(Black line) median PFS was 26 (95%CI:23-NA) months (univariable HR,1[reference]), in Total (N=32)(Black small-dotted line) was 39(95%CI:34-NA) months (univariable HR,0.46[95%CI:0.22-0.94];p=0.03); in supratotal (N=17)(Black large-dotted line) only one event at 48 months (grade-III oligodendroglioma; the patient refused post-surgical treatments) was recorded during the observation period (univariable HR,0.03[95%CI:0.00-0.21];p=0.001). Shadings are the 95% confidence intervals.

C) Degree of supratotal resection in patients who underwent progression (N=6,IDH-mutated) and in those who didn't recur, in the IDH-mutated group (N=104) and in the IDH-wt group (N=12). Data are reported as Mean and Median. The degree of supratotal was lower in recurrent patients (mean 111.3%,median 107.41%;) than in those who didn't recur (IDH-mutated: mean 271.11%,median 148.775%; IDH-wt: mean 818.20%,median 197.08%).

D) Kaplan-Meier curve and number at risk for OS in IDH-mutated- tumors according to EOR classes. Time is expressed as months. In Partial resection group (N=10)(Black line) median OS was 45(95%CI: 36-NA) months (univariable HR,1[reference]), in Subtotal (N=74)(Black small-dotted line) and in Total (N=125)(Black large dotted line) at 45 months, 83.8%(95%CI:0.75-0.92) and 83.2%(95%CI:0.76-0.90) of patients were alive; in supratotal (N=110)(Black large-dotted lined) no deaths were recorded during the observation period (univariable HR,0.01[95%CI:0.00-0.08];p<0.001). Shadings are the 95% confidence intervals.

E) Variable importance for OS using Random-Forest in the IDH-mutated-LGGs. All the clinical, imaging, histo-molecular and treatments (EOR,RT,CHT) were considered together. VIMP and Minimal Depth are two criteria proposed in the context of random-forests algorithms to evaluate variable importance in explaining the OS. The variables on the diagonal red line are those ranked equally by the two methods. The vertical line divides variables with positive VIMP (left) from those with negative VIMP (right; unimportant). The horizontal line indicates the minimal depth threshold: important variables are below the line.

OS was mainly associated to treatments, particularly RT and EOR. The VIMP rank is reported in x-axis. The Minimal Depth (Rank Order) is in y-axis.

1A) Clinical, Imaging, and Histo-molecular features of the 319 Lower-grade gliomas (IDH-mutated grade-II and III) patients included in the study

Clinical Features

Sex		Seizures	
-Male	195 (61.1%)	-Focal	72/306 (23.5%)
-Female	124 (38.9%)	-Generalized	234/306 (76.4%)
Age		**Handedness**	
Mean (SD)	38.9(11.8)	-Left	316 (99.1%)
Median [Min-Max]	38 [18-75]	-Right	3 (0.9%)
Duration of Clinical History		**Previous Treatments***	
<6 months	90 (28.2%)	-No	263 (82.4%)
>6 months	229 (71.8%)	-Yes	56 (17.6%)
Reason For diagnosis		**Dominance**	
-Incidental	13 (4.1%)	- Left	185 (58.0%)
-Symptoms	306 (95.9%)	- Right	134 (42.0%)

Pre-operative imaging Features

Location		Side	
-Frontal	140 (43.9%)	-Left	195 (61.1%)
-Temporal	56 (17.6%)	-Right	124 (38.9%)
-Parietal	45 (14.1%)		
-Insular	78 (24.5%)		
Involvement of eloquent sites	299 (93.7%)	**Involvement of CC**	84 (26.3%)
-Close	20 (6.3%)	-Yes	235 (73.7%)
-Distant		-No	
Volume (cm^3)			
-Mean (SD)	65.2 (61)		
-Median [Min-Max]	46 [0.39-386]		

Histo-molecular and Tumor Grade Features

Integrated Diagnosis		Tumor Grade	
-Astrocytoma	130 (40.8%)	-II	248 (77.7%)
-Oligodendroglioma	189 (59.2%)	-III	71 (22.3%)

Post-operative Neurological Conditions

Immediate Deficits		Permanent Deficits	
-No	26 (8.2%)	-No	313 (98.1%)
-Yes	293 (91.8%)	-Yes	6 (1.9%)

Dominance: indicates if the tumor was located in the dominant hemisphere for language; Involvement of eloquent sites considers the distance between the tumor margins and the location of cortical or subcortical sites anatomically related to main neurological functions (motor, language, visual, visuospatial, ..) as visible in pre-operative volumetric FLAIR; involvement of CC = involvement of corpus callosum as detectable in pre-operative volumetric FLAIR; previous treatments includes partial resection followed by observation (31 cases) or chemotherapy (10), stereotactic biopsy followed by observation (9) or chemotherapy (6). Immediate deficits (at 5 days post-surgery); permanent deficits (at 1 month). For details see suppl.Methods.1.

1B) Clinical and Imaging Features of Progression in the 319 IDH-mut LGGs

	Total Population N = 319	Progression N = 190
Gender		
Male	195	119 (61.0%)
Female	124	71 (57.0%)
Age (mean; median)	39; 38	40.2; 39
Previous Treatment		
Yes	56	53 (94.7%)
No	263	137 (53.6%)
Location		
Frontal	140	70 (50.0%)
Temporal	56	29 (51.7%)
Parietal	45	34 (75.6%)
Insular	78	57 (73.1%)
Side		
Left	195	127 (65.1%)
Right	124	63 (50.8%)
Involvement of eloquent sites		
Close	299	184 (61.5%)
Distant	20	6 (30.0%)
Astro II	89	61 (68.5%)
Oligo II	159	77 (48.4%)
Astro III	41	28 (68.3%)
Oligo III	30	24 (80.0%)
Histolog. Confirmation		74 (38.9%)
Site of		

Progression

Cavity	182 (95.8%)
Distant	8 (4.2%)

Involvement of CC = involvement of corpus callosum.. Astro = astrocytoma; Oligo = oligodendroglioma; Histolog. Confirmation = indicates the cases in which transformation was confirmed by histological analysis subsequent to second surgery. Site of progression indicates the location of the progression in respect to the pre-existing surgical cavity; Cavity = when the progression is within or at the border of the cavity; Distant = when the progression is far away from the pre-existing resection cavity. We did not observe any leptomeningeal spread.

Tab.1C) Adjusted Cox-regression analysis for PFS in Total and Supratotal groups.

The adjusted analysis considered Extent of Resection (Supratotal-ST vs Total-T), integrated diagnosis (Astrocytoma-Astro vs Oligodendroglioma-Oligo) and grade at first diagnosis (III vs II). The model was adjusted for the factors that resulted significant in the univariate analysis, namely duration of clinical history, previous treatments, location, side,volume, Dominance, Corpus callosum. Other variables were not considered as they involved a too restricted number of patients, namely reason for diagnosis, eloquent and permanent deficits. EOR and grade-III at first diagnosis resulted significantly associated with PFS also after adjusting for the other variables.

	HR	95% CI	P-value
ST vs T	0,04	0,02 - 0,09	<0,01
Grade III vs Grade II	1,94	1,19 - 3,14	0,01
Integr.diagn. oligo vs astro	0,74	0,48 - 1,14	0,18
Duration clinical history >6 vs <6 months	0,66	0,42 - 1,03	0,07
Previous Treats.	1,93	1,19 - 3,13	0,01
Location T. vs F.	0,74	0,40 - 1,38	0,34
Location P. vs F.	1,07	0,61 - 1,86	0,82
Location Ins. vs F	1,05	0,62 - 1,79	0,84
Righ vs Left	0,70	0,38 - 1,31	0,27
Volume ml	1,00	0,99 - 1,00	0,30
Dominant no vs yes	0,73	0,38 - 1,38	0,33
CC no vs yes	0,77	0,49 - 1,22	0,27

ST = supratotal; T = Total; Integr.Diagn. = integrated diagnosis; Oligo = oligodendroglioma; Astro = astrocytoma; Previous Treats. = previous treatments; Location: T = temporal, F = frontal, P = parietal, Ins. = insular. Dominant = dominance; CC = corpus callosum involvement.

Tab2.A) IDH-mutated LGGs: MPFS Features according to EOR classes

	Partial	Subtotal	Total	Supratotal
	N=10	N=74	N=125	N=110
Progression	10/10 (100.0%)	71/74 (95.9%)	103/125 (82.4%)	6/110 (5.5%)
-No malignant Transformation	4/10 (40.0%)	49/71 (69.1%)	69/103 (67.0%)	5/6 (83.4%)
-Malignant Transformation	6/10 (60.0%)	22/71 (30.9%)	34/103 (33.0%)	1/6 (16.6%)
-Gd Enhancing	6/6 (60.0%)	18/22 (25.3%)	32/34 (94.1%)	1/1 (100.0%)
-Histological confirmation	4/6 (40.0%)	18/22 (25.3%)	26/34 (76.4%)	1/1 (100.0%)
-Both	4/6 (66.7%)	14/22 (63.6%)	24/34 (70.6%)	1/1 (100%)

The table reports for each EOR class, the number of progression, the number of progression without and with malignant transformation, the number of progression with GD-enhancement (radiological sign of malignant transformation), and the cases in which the transformation was confirmed by histological analysis subsequent to second surgery; the number of cases with Gd enhancement followed by histological confirmation is indicated as both .
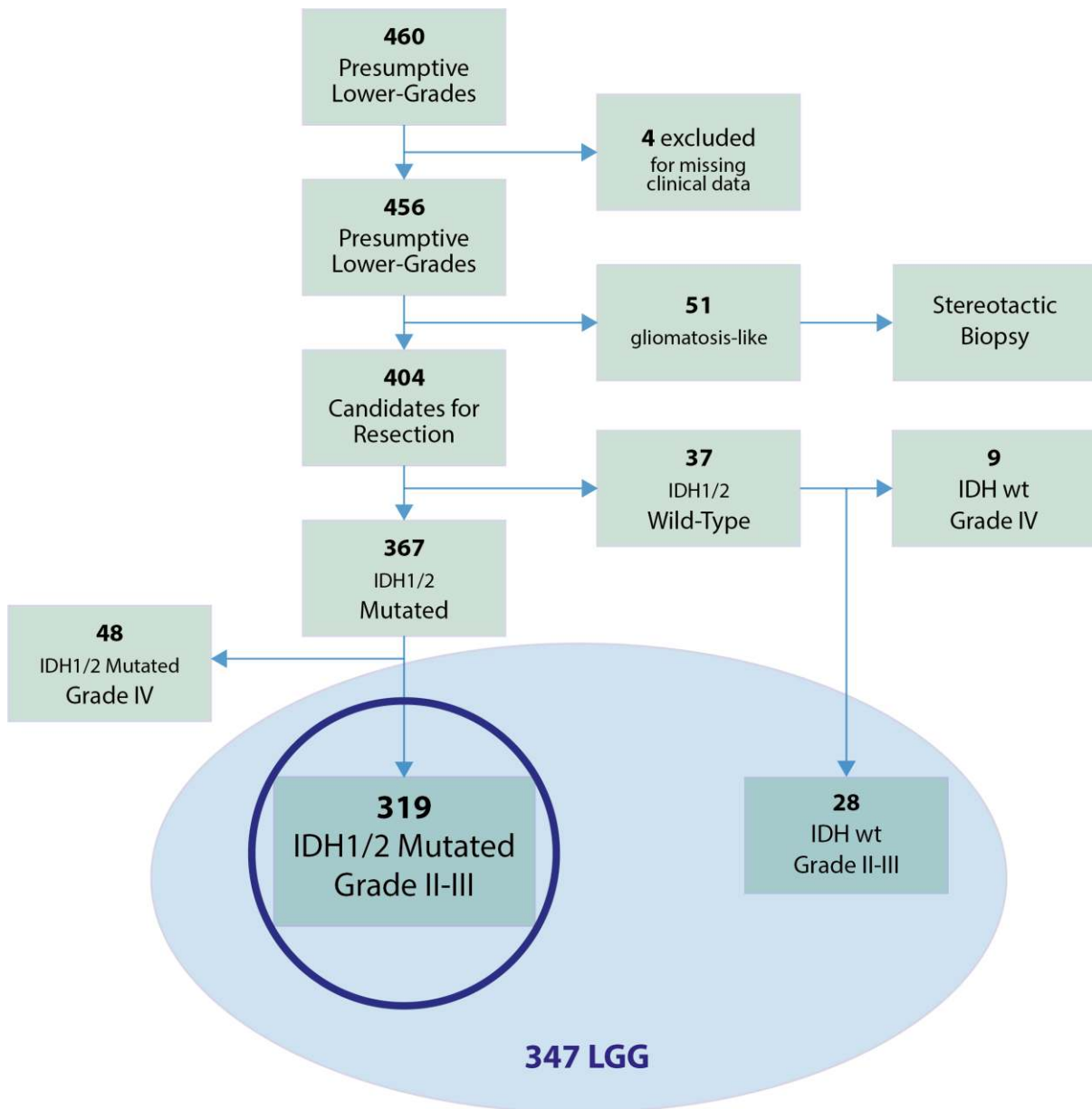
Tab2.B) IDH-mutated LGGs: Cox-regression analysis for the association of MPFS with pre-operative clinical, imaging and molecular factors: EOR, grade-III at first diagnosis, integrated diagnosis (astrocytoma) and previous treatment resulted significantly associated to MPFS

	HR	95% CI	Pr(>|z|)
EOR S vs P	0,53	0,12 - 2,41	0,41
EOR ST vs P	0,01	0,00 - 0,09	<0,01
EOR T vs P	0,25	0,05 - 1,17	0,08
Grade III vs Grade II	3,89	2,20 - 6,88	<0,01
Integr.diagn. Oligo vs Astro	0,51	0,29 - 0,92	0,02
Duration clinical history >6 vs <6 months	1,03	0,54 - 1,96	0,94
Previous Treat. yes vs no	2,58	1,43 - 4,63	<0,01
Location T vs F	0,84	0,34 - 2,11	0,72
Location P vs F	1,29	0,62 - 2,69	0,50
Location Ins. vs F	1,43	0,76 - 2,70	0,26
Right vs Left	0,44	0,17 - 1,10	0,08
Volume ml	1,00	0,99 - 1,00	0,16
Dominant no vs yes	1,42	0,55 - 3,68	0,47
CC no vs yes	0,63	0,32 - 1,24	0,18

S = Subtotal; P = Partial; ST = supratotal; T = Total; Integr.Diagn. = integrated diagnosis; Oligo = oligodendroglioma; Astro = astrocytoma; Previous Treats. = previous treatments; Location: T = temporal, F = frontal, P = parietal, Ins. = insular. Dominant = dominance; CC = corpus callosum involvement.
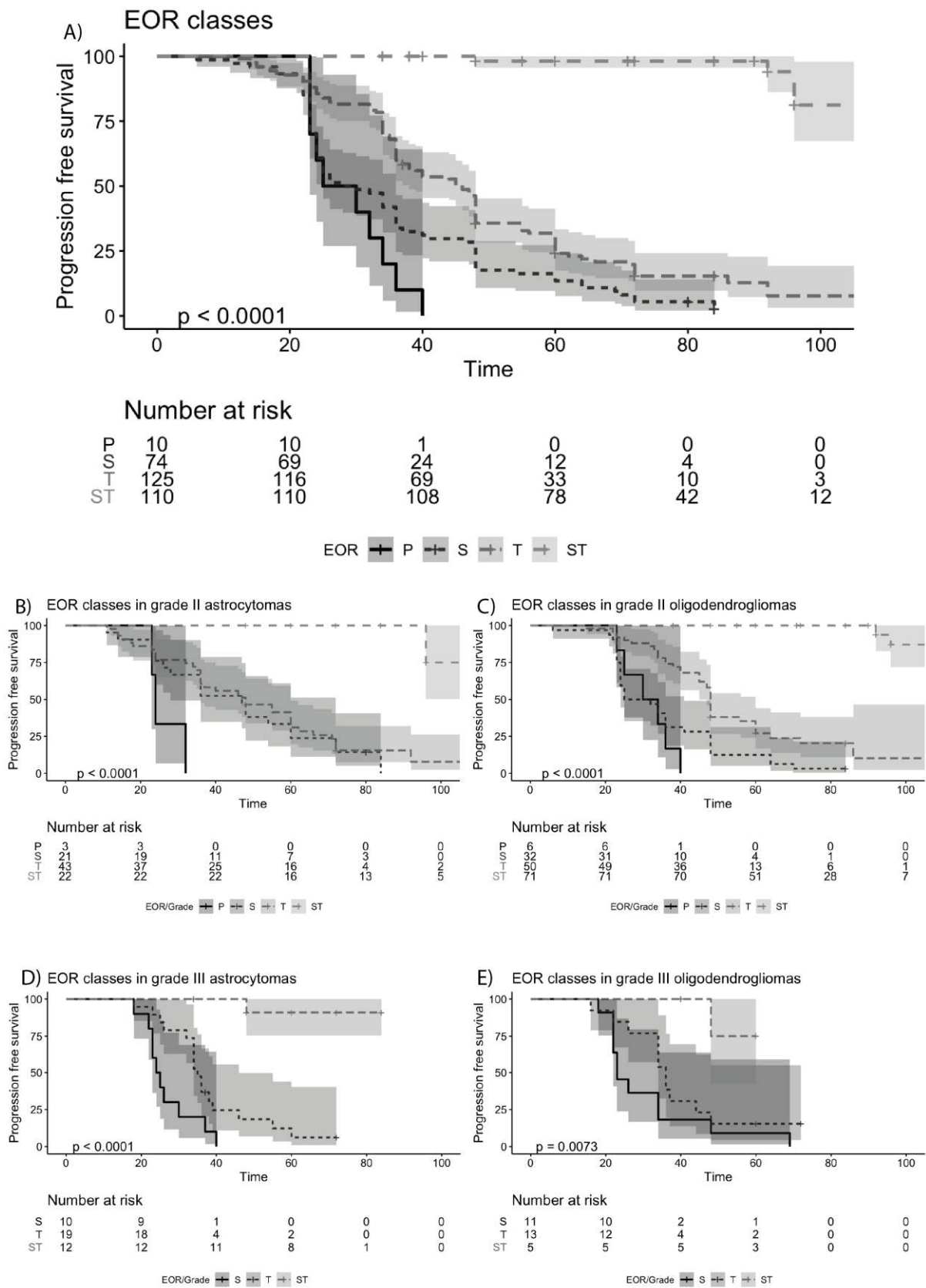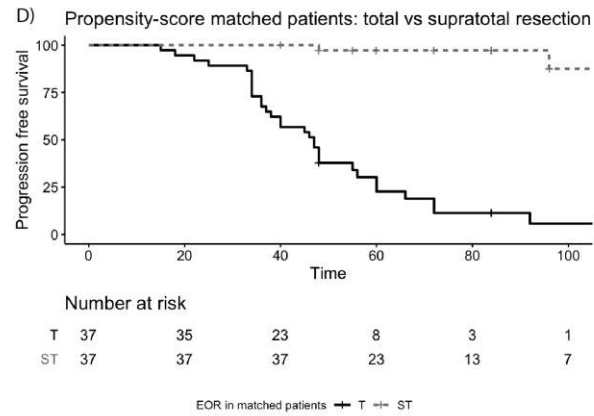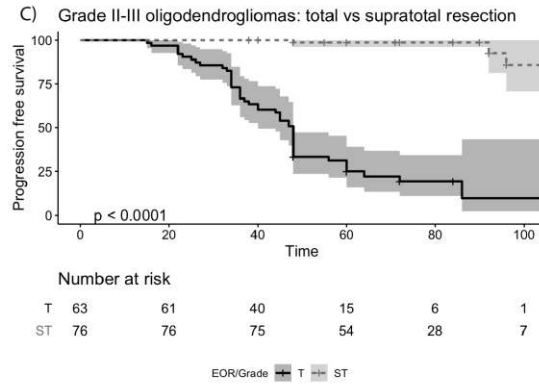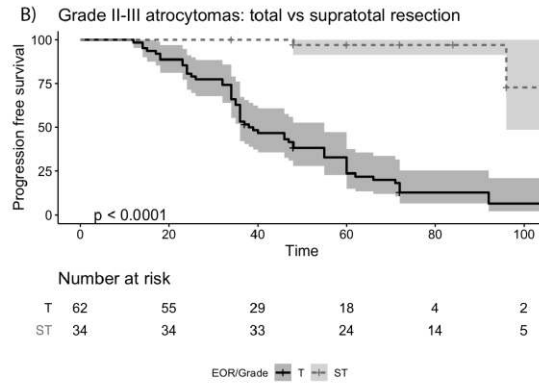
Figure 1

Figure 2

Figure 3

Figure 4