Student Name: Julie Semenchuk **Date:** 08/03/2017

Project Title: Extent of Resection in Glioblastoma: Incorporating IDH Status and Clinical Factors to Predict Outcome

Primary Supervisor Name: Dr. Marshall Pitz
Department: Internal Medicine

Co-Supervisor Name: Dr. Marco Essig
Department: Radiology

Summary (250 words max single spaced):

Background: The benefits of increasing extent of resection (EOR) for both overall survival and progression-free survival(PFS) in glioblastoma has been well documented. However, models predicting surgical outcomes have failed to incorporate a patient's IDH status, a known prognostic factor.

Objective: We isolate the impact of IDH and the interaction between IDH and tumor volume while adjusting for known prognostic variables. Both overall survival and time to progression-free survival (PFS) are analyzed.

Methods: We performed a retrospective cohort study of 98 patients with glioblastoma who had undergone either biopsy or surgical resection. Tumor volumes were determined by volumetric analysis. Univariable and multivariable Cox PH Regression models were built using overall survival and PFS as endpoints.

Results: Increasing EOR and decreasing residual tumor volume (RTV) were both associated with prolonged overall survival and PFS. When IDH status was added to multivariable models, the model utilizing RTV provided a slightly better fit compared to EOR. An interaction term between RTV and IDH status was characterized, such that at low RTVs the prognosis of an IDH mutant is significantly better than that of an IDH wild-type, an effect that is less important as RTV increases. The significance of this term was confirmed by improved fit upon insertion into multivariable models .

Conclusion: Minimizing RTV and increasing EOR are important prognostic factors for both IDH wild-type and IDH mutant glioblastoma. The protective benefit of the IDH mutation at lower RTVs suggests these patients are the best candidates for aggressive surgical resection.

Student Signature

Primary Supervisor Signature

Digitally signed by M Pitz
Date: 2017.08.05 23:56:28 -05'00'

Acknowledgments: I gratefully acknowledge the sole or partial funding support from:

H.T. Thorlakson Foundation
Dean, College of Medicine
Research Manitoba

Manitoba Medical Service Foundation (MMSF)
Vice-Dean, Research Rady FHS
Health Sciences Centre Research Foundation
Heart and Stroke Foundation

Sponsorship if different than above;
Additionally, CancerCare MB and the Research Institute in Oncology and Hematology

Introduction and Background

Glioblastoma is the most common and most lethal primary brain tumor in adults. It is associated with short survival, deterioration in neurocognitive function, decreased functional independence and a progressive decrease in health-related quality of life.[1] After diagnosis and treatment, the median survival is 15 months with a 5-year survival rate of only 5%.[1,2] Given the aggressive nature of the tumor, the current standard of care involves maximal surgical resection, followed by concurrent radiotherapy and temozolomide.[3.] Independent clinical factors that have been prospectively validated as indicators for shorter survival include advanced age, aggressive histological features and low Karnofsky performance score at presentation.[1,4] The approach to glioblastoma has evolved from the principal belief that maximal safe resection also improves symptom management, quality of life and progression-free survival (PFS).[5] Surgical extent of resection (EOR) has been shown as an independent prognostic factor in numerous retrospective studies accounted for in a recent large meta-analysis.[6] In a few studies, it has been reported that residual enhancing tumor volume (RTV) is an even stronger predictor for overall survival than EOR.[2,7] However, the retrospective nature of the mentioned studies increases their risk of selection bias. Beyond this, the vast majority of studies fail to consider the effect of known molecular factors on surgical outcomes.[6] In an ever-evolving understanding of molecular heterogeneity, the benefits associated with maximizing tumor resection and minimizing tumor volume must be revisited.

Although genome-wide studies have revolutionized our understanding of tumor biology, only a few molecular markers influence clinical decision-making and treatment.[8] For glioblastoma, one known prognostic factor is the presence of IDH1 or IDH2 mutations.[8,9] The newest classification criteria from the 2016 CNS WHO divides glioblastomas into IDH-wildtype (about 90% of cases) and IDH-mutant (about 10% of cases).[10] IDH-mutant glioblastoma is associated with a significantly different history and clinical presentation. IDH normally catalyzes the conversion of isocitrate to α-ketoglutarate (α-KG). IDH1 is responsible for this reaction in the cytoplasm while IDH2 acts mainly in the tri-carboxylic acid cycle in the mitochondria. The most common missense mutation in IDH-mutant tumors is found at R132 and results in the replacement of arginine with histidine at the enzyme's active site.[9.] Early on, it was found only a single copy of the mutation was present in tumors.[9] This led researchers to believe a new function was gained from the mutation and not simply a loss of function. Enzymatic studies have shown this mutation results in a new ability of the enzyme to catalyze the reduction of α-KG to R-2hydroxyglutarate (2HG).[11] Since 2-HG can be toxic to cells and evidence suggests IDH1 is often the first mutation to occur, the malfunction of this enzyme likely accounts for the initial tumorigenesis and its unique history.[12] IDH mutant tumors are associated with a precursor astrocytoma, a younger age at diagnosis and a significantly improved overall survival between 24 and 31 months.[10] IDH-wildtype glioblastoma usually develops de novo, is associated with an older age at diagnosis and a median survival between 10 and 15 months.[10] Another important molecular factor is methylation status of the DNA repair gene MGMT detected in about 40% of patients.[8] Methylation leads to epigenetic silencing of MGMT, inhibiting DNA repair in cancer cells. In general, the silencing of this promoter has been associated with longer survival as well as increased progression-free and overall survival in those treated with radiation and chemotherapy.[13] Despite the differences in history and survival, most studies on maximizing safe resection have failed to control for MGMT methylation and IDH mutation. One study has found that survival rates after surgery in malignant astrocytic gliomas differed based on IDH1 status.[14] IDH1 mutant astrocytomas were found to be more amenable to resection and had substantially improved survival associated with more aggressive resection compared to IDH1 wild-type tumors.[14] To date, this is still the only paper characterizing the relationship between IDH and resection; we seek to confirm this and further characterize it.

Before volumetric analysis, resection of a tumor was typically classified as gross-total, subtotal and partial based on estimations from experienced observers[15,16] The earliest of these methods was based on interpretation of CT scans.[15] There were no precise measurements taken to confirm these estimates. More accurate determination of EOR has been made possible using volumetric analysis of pre-operative and post-operative imaging MRIs.[17] With this approach, EOR as well as RTV can be measured as a continuous variable, as opposed to categorical, avoiding generalization and misinterpretation of results.[18] In studies using volumetric analysis, the prognostic value of resection has been confirmed, showing a stepwise improvement in survival with increasing EOR.[5] However, the majority of these studies failed to take into account molecular factors of the tumor.

Minimizing RTV requires a cautious approach, as failure to identify and preserve eloquent brain regions can dramatically alter neurological function and have serious consequences on quality of life. Though contrast-enhancing brain is targeted in high grade gliomas, even gross total resection (GTR) fails to completely remove microscopic residual tumor that may not appear on imaging[19], providing an important context within which to evaluate the benefits of maximal resection. Strategies for maximal resection are ongoing and an important topic for patient management. Various surgical intra-operative techniques have been utilized to enhance maximal resection while preserving function.[21] As a general rule, tumor located within functional cortical or subcortical sites on preoperative anatomical imaging is never an absolute contraindication to resection.[22] Variability of individual neuroanatomy, distortion due to mass lesions, and functional reorganization caused by plasticity make classic anatomic identification of functional areas insufficient for surgical planning.[21] In cases where a brain tumor is located within or adjacent to regions presumed to have language or sensorimotor function, patients can be considered for an awake craniotomy. During the procedure, stimulation of areas using a bipolar electrode can help identify areas of brain critical to language and motor function versus non-critical areas safe to remove.[23] Another surgical advance has come from the use of 5-aminoluvelinic acid (ALA) which can aid experienced surgeons in achieving a complete resection.[24,25.] 5-ALA is a non-fluorescent amino acid precursor that produces the accumulation of fluorescent porphyrins in high-grade glioma.[24] Exogenous 5-ALA administered before surgery will accumulate within tumor cells, peaking in concentration 6 hours after administration. A randomized control trial showed greater EOR was associated with higher rates of 6-month PFS for patients who had undergone surgery with 5-ALA versus those procedures completed under white light.[24] This randomized trial is also the strongest evidence that a higher rate of GTR led to improved PFS, despite no difference in overall survival.[24]

Given the importance of surgery in glioblastoma management, we sought to isolate the impact of IDH and the interaction between IDH and tumor volume while adjusting for known prognostic variables. Both overall survival and time to progression are analyzed. Exploring these relationships can ultimately aid in surgical planning and provide further insight into the distinct behavior of the IDH mutant molecular subgroup.

Materials and Methods

Cohort

A retrospective cohort study was performed using glioblastoma cases obtained from the Manitoba Cancer Registry. Patients diagnosed with glioblastoma from January 1st, 2012 to December 31st, 2015 were included if they had a surgical/biopsy procedure, received testing for

the presence of the IDH mutation and had MRI imaging available. Patients without a post-operative MRI who had undergone surgical de-bulking were eliminated. Variables such as age at diagnosis, sex, IDH status, tumor location, date at diagnosis, death, surgery/biopsy, radiation, concurrent chemotherapy and adjuvant chemotherapy were imported from the Maxon Database at CancerCare Manitoba. Chart review was done to determine ECOG performance status (PS) prior to surgery, and whether seizures, headaches, hemiplegia, gait disturbance and/or aphasia were a presenting symptom.

Progression after biopsy/surgery was defined with the use of the working group Response Assessment in Neuro-oncology (RANO) criteria.[26] The first post-operative MRI scan completed after the initial procedure (biopsy/surgery) was used as a baseline for each patient. Follow-up MRIs and accompanying clinical reports were then used to determine whether the patient had stable disease, progression, partial response, or complete response to treatment. In cases where numbers were needed for classification (ie. progression $\geq$25% increase in sum of products, partial response $\geq$50% decrease in sum of products), tumor dimensions were measured on IMPAX Client and the volume was calculated.

Volumetric Analysis

Pre-operative and post-operative tumor volumes were calculated using the quantitative semi-automated volumetric analysis tool Olea Sphere. The analysis was performed by a medical student blinded to the relevant molecular factors. Enhanced 3D MPRAGE or 2D T1 gadolinium scans were used depending on availability. For those in the cohort who underwent biopsy only, an MRI taken within one week of the procedure was used and the pre and post-operative volumes were assumed to be identical. For those who underwent some form of surgical resection, a pre-operative and post-operative MRI were used. The soonest post-operative MRI after surgery for each patient was used, with the majority obtained within 48 hours of the procedure. Since blood products may appear hyper-intense in the acute phase on T1-weighted or MPRAGE sequences and may inflate the postoperative enhancement volumes, T1 pre-contrast scans were analyzed simultaneously. For both pre-operative and post-operative scans, the general area of interest and a small volume of enhancing tumor was identified on the post-gadolinium scan by the observer, after which Olea Sphere automation was able to identify similar tissue and calculate the volume in cubic centimeters. Areas thought to represent blood products or other non-malignant enhancing tissue, such as blood vessels, could then be deselected and the volume recalculated. RTV was determined from the post-operative MRI for patients who had undergone surgical resection, and from the pre-operative MRI from those who had undergone biopsy only. EOR for those who had biopsy only was assumed to be 0%, while the formula used for those who had undergone resection was ((PTV-RTV)/PTV) x 100%) where PTV = pre-operative tumor volume.

IDH Status

In Manitoba, IDH1 R132H immunostaining is done on all gliomas including glioblastoma. Formalin-fixed, paraffin-embedded sections are immunostained using mouse monoclonal R132H IDH1 mutation–specific antibody (clone H09, Dianova); the antibody is detected using the DAKO Envision system. All IDH1/2 genomic studies are sent to Calgary. Calgary assesses the IDH mutation by PCR amplification/single base pair extension and capillary electrophoresis fragment analysis. This identifies mutations in IDH1 and/or IDH2. Genomic analysis is only done after a negative immunostain in circumstances where the glioblastoma may have arisen from a lower grade glioma, due to fiscal restrictions.

Analyses

Time-varying Cox regression models were used to predict overall survival and PFS from time of surgery/biopsy. Time-varying predictors included the variables of radiation therapy, concurrent chemotherapy, and adjuvant chemotherapy. The proportional hazard assumption was tested using Schoenfeld plots. Influence plots were used to detect influential outliers. Continuous predictors were converted using restricted cubic splines if they violated the assumption of linearity. Interaction terms were described with plotted hazard ratios adjusted for control variables held at their mean. Kaplan-Meier curves were produced and Cox regression models were run using the survival and rms packages in R (version 3.4.1).

Likelihood ratio testing was used for model building: at first excluding IDH and IDH/tumor volume interaction; then forcing IDH into the multivariable model; and finally, forcing an IDH/volume interaction term. Both EOR and RTV were analyzed as the "volume" variable of interest. AIC values were reported for each multivariable model. Differences in AIC of 2 or less indicate little difference between models and differences of 10 or more suggest that the more complex model is preferable.[27]

Results

Patient Characteristics

Table 1 summarizes the demographics of the 98 patients diagnosed with glioblastoma from January 1st, 2012 to December 31st, 2015, who met inclusion criteria. In this cohort, 92 (93.9%) were negative for the IDH mutation, 6 (6.2%) were positive for the mutation. The mean age at diagnosis was 60.8 years with a standard deviation of 12.6. Upon presentation, 74 (75.5%) of patients had an ECOG PS of 0-1, while 24 (24.49%) had an ECOG of 2-4. Death had occurred in 83 (84.69%) cases and progression or death was present in 92 (93.9%) cases. The median follow-up time of the cohort was 11.1 months. The median time to progression was 3.8 months while the median overall survival was 11.0 months.

The median post-residual volume of the tumor for this cohort was 7.87cm^3. The distribution of post-operative tumor values did not follow a normal distribution and the 25th percentile value was 2.99 cm^3 with a 75th percentile value of 20.78 cm^3. This indicates most post-operative tumor volumes were small but some cases had very high values. EOR values had a median of 65.5%, a 25th percentile value of 0 and a 75th percentile value of 88.6%. These distributions are due to the inclusion of biopsy cases with higher RTV and lower EORs.

Univariable Analysis

Univariable Cox regression was completed for all collected variables and the results for overall survival are summarized in Table 2. We analyzed three different volumetric variables, 2 as continuous variables (RTV and EOR), and a categorical EOR to determine if each influenced survival. Each of them were found to be significant predictors of both overall and PFS ($p<0.05$). We found each increase in 10cm^3 RTV, to be associated with decreased overall survival (HR=1.16). An increase in 10% EOR was associated with an increased overall survival (HR= 0.93) and an EOR <40% was also associated with a significant decrease in overall survival (HR=2.22). Similar results were found for PFS.

Beyond the tumor measurements themselves, significant predictors of overall survival included ECOG PS and adjuvant chemotherapy ($p<0.05$). Radiation therapy and adjuvant chemotherapy were significant predictors of overall survival and PFS. ($p<0.05$) A significant predictor for sooner progression was hemiplegia upon presentation ($p<0.05$) with a HR of 1.83. All other presenting symptoms, ie. headaches, aphasia, gait disturbance and seizures were not predictive of overall survival or PFS.

The Kaplan Meier curves for different RTVs for both overall and PFS are shown in Figures 1 and 2 respectively. Though our multivariable analysis suggested each $10cm^3$ increment of RTV has an impact on overall survival, Figure 1 and 2 demonstrate there is a significant increase in survival associated with a RTV $<5cm^3$. Similar curves for EOR are shown in figures 3 and 4. The Kaplan Meier curves demonstrating the survival benefit from the IDH mutation are found in Figures 5 and 6. In both overall and PFS, the benefit from IDH mutation became apparent at approximately 5 months.

Multivariable Analysis

To confirm each of their prognostic impact, both RTV and EOR were analyzed separately in multivariable Cox regression analyses. For each of the volumetric variables, EOR and RTV, multivariable Cox regression analyses were built with and without IDH as a covariate. We found RTV was a slightly better predictor of overall survival because the model with RTV had a slightly lower AIC value than the model with EOR, while having the same control variables. In both cases, the addition of IDH to the model provided a moderately better fit through AIC values. The multivariable analysis results for overall survival with RTV as a covariate can be found in Table 3. Similar results for EOR as a covariate are found in Table 4. The important predictors of survival for the multivariable models include RTV, ECOG PS, radiation therapy with concurrent chemotherapy and adjuvant chemotherapy ($p<0.05$).

The volumetric variables EOR and RTV underwent the same two multivariable analyses for PFS. In both cases, adding IDH as a covariate did not improve the models.

IDH Interaction

As part of our analysis, we determined how the IDH mutation impacts survival at different RTVs. Without accounting for this interaction, we assume the increased survival associated with decreased RTV is similar between IDH mutants and IDH wildtypes. The interaction variable was then added to the multivariable models for both overall survival and PFS. The insertion of this variable improved model fit, and the interaction term was statistically significant, indicating the effect of RTV is modified by the presence of an IDH mutation. The improved model fit was demonstrated by a significant decrease in AIC value, shown in Table 3. The relationship between RTV and IDH is shown in Figures 7 and 8 for both overall survival and PFS respectively. They suggest that the IDH mutation is more predictive of survival at lower RTV but becomes less predictive as RTV increases.

The interaction between IDH and EOR was also calculated and added to multivariable models for overall and PFS. The insertion of this variable was statistically significant and AIC values in Table 4 show there was little difference in model fit when the interaction was added. The interaction between IDH and EOR for overall and PFS are shown in Figures 9 and 10 respectively. The interaction suggests the IDH mutation has the greatest protective benefit at increased EOR, but becomes less of a factor as EOR decreases.

Discussion

Although many studies have shown the value of increased EOR in glioblastoma management, very few of these have included IDH mutation status in their analysis.[16,18,5] Of the studies that have accounted for molecular markers in EOR analyses[14,28], neither have characterized an interaction between IDH and RTV. In this study, we sought to determine whether EOR and/or RTV are still significant predictors of survival when IDH mutation status is incorporated into multivariable models. When analyzed separately, both increased EOR and decreased RTV are associated with increased survival. We found that RTV is the better predictor of overall survival. More importantly, we quantified the relationship between IDH status and RTV, and confirmed a similar relationship exists between IDH status and EOR. We found, the IDH mutation is more predictive of increased survival at lower RTVs, and appears to be less of a factor at higher volumes. Similarly, the IDH mutation is more predictive of increased survival for larger resections, and is less of a factor as EOR decreases. This implies that at a low RTV, an IDH mutation will be protective of overall survival, but at higher RTV, the prognosis of a patient with a tumor containing an IDH mutation may be similar to that of a patient whose tumor is IDH wildtype.

Our study supports maximal safe resection for all patients with glioblastoma, independent of IDH status. Many studies demonstrate the benefit associated with GTR, defined as removal of all contrast-enhancing tissue on T1-weighted MR images.[6,24] Enhancement represents defective integrity of the blood brain barrier, and likely correlates with the most aggressive area of tumor and hence the entire area is the target of resections. Though GTR is often a surgical goal, we show that when GTR is not possible, increasing EOR and minimizing RTV to what is feasibly safe will still be associated with longer overall survival and PFS. Previously, Lacroix et al[17] and Sanai et al[29] found resections of 98% and 78% respectively were thresholds needed for a significant impact on survival and recurrence. Later, Chaichana et al showed the threshold for a significant benefit was 70%.[7] They attributed this lower threshold to the addition of temozolomide as standard of care for glioblastoma in 2005,[5] reasoning it may be more effective at treating larger RTV, requiring less extensive resection to see a benefit in survival.[7] This demonstrates that with changing management and a growing understanding of this disease, resection must be continuously assessed to confirm its benefit. The way in which we address resection is also being questioned, due to the infiltrative nature and mobility of tumor cells that allow them to migrate to tissue outside that which is contrast-enhancing.[20] Li et al proposed in select cases, surgeons can do better than GTR of enhancing disease in glioblastoma management and removal of abnormal FLAIR tissue may be associated with increased survival.[20] Until recently, the 2007 CNS WHO classification system was used in clinical practice of gliomas and glioblastoma was defined as densely cellular; pleiomorphic neoplasms with mitotic activity, necrosis and microvascular proliferation.[30] The 2016 CNS WHO classification system still relies on these histological features as criteria but now categorizes glioblastoma according to IDH mutation status.[10] As such, effective practices for each subgroup remain to be determined. Here we conclude maximal safe resection will benefit both groups. This effect is particular seen at volumes $<5cm^3$, a finding in line with previously studies examining RTV thresholds and increased survival.[7]

A study by Kawaguchi et al, found WHO grade III gliomas with the IDH mutation and without 1p/19q deletion, had significant increased survival when they had undergone GTR versus non-GTR.[28] Though all molecular subgroups analyzed did better with GTR, they were able to conclude that patients with this specific molecular profile should be candidates for more radical resection. Though we did not consider 1p/19q mutation as it is not tested in glioblastoma, our findings that IDH mutants have better overall survival at lower tumor volumes

compared to IDH wildtype are in line with the results collected for grade III gliomas, which are known to be IDH mutant. Beyond this, we show that although GTR would theoretically be associated with the longest overall survival, when this is not attainable, pursuing the greatest resection possible to minimize the RTV will leave patients in the IDH mutation subgroup with an improved prognosis. Beiko et al showed that IDH mutant tumors in glioblastoma were associated with prolonged survival with maximal resections, defined as RTV <5cm^3.[14] Our results agree with this and suggest the relationship between RTV and IDH status is one of a continuous nature, where each incremental decrease in RTV has a greater protective benefit in IDH mutants. Though IDH mutants will have better prognosis than patients with IDH wildtype tumors with RTV <5cm^3, this relationship is even stronger with <2cm^3 and still present >5cm^3, but to a lesser extent.

It is well documented that patients with IDH mutant glioblastoma has a better prognosis than IDH wildtype[10], but whether this is due primarily to an improved intrinsic natural history or response to therapy (or both) is not fully understood. One study hypothesized that the prognostic significance of IDH1 may be partly due to its differential location, making the tumors themselves more amenable to GTR and placing them in less eloquent areas, decreasing morbidity.[30] However, in our analysis, we found that location was not predictive of overall or PFS. Unlike the documented improved response in methylated MGMT with treatment of temozolomide[13], examinations of response to radiation or chemotherapy in randomized trials have not found a therapeutic interaction between adjuvant treatment and IDH genotype.[31,32] One reason for this may be simply due to the fact that glioblastoma is a rare disease, and IDH mutant glioblastoma comprises only 10% of all cases.[10] Even when studies wish to determine a therapeutic interaction between therapeutic response and IDH status, they are limited by their population, as shown in a trial examining response to bevacizumab and temozolomide.[33] If low volumes of IDH mutant tumors are more susceptible to chemotherapy, this could in part influence why lower RTVs after surgery are associated with an enhanced survival rate in comparison with IDH wild type tumors. At the molecular level, it is believed the IDH mutation contributes to tumor pathogenesis early on in the disease. As the disease progresses however, the mutation may challenge survival of the tumor cell more than it aids it.[34] The mutation results in a gain of function which enhances the NADPH-dependent conversion of α-KG to 2HG.[11] Over time, this leaves cells with low NADPH levels and making them more susceptible to stress through free radicals and reactive oxygen species (ROS), such as those that may be induced by chemotherapy and concurrent radiation.[34] Though plausible, conclusive results regarding response to treatment cannot be drawn based on our results.

Understanding factors that influence survival and progression is important for treatment planning and individual prognostic counselling. We found RTV, ECOG PS, chemotherapy with concurrent radiotherapy, adjuvant chemotherapy and IDH status to all influence overall survival; findings in line with previous analyses.[35] Our study therefore helps support the importance of these variables as prognostic factors and they should continue to be of importance in treatment decisions and patient counselling. The same factors however were not significant for PFS. For PFS analyses, the only significant factor aside from resection was radiation therapy with chemotherapy. PFS is a less reliable as an outcome, as it is subjective in nature, and as such the implications of these findings to PFS are less clear. Graphs for the relationships between PFS and the IDH RTV interaction is shown in Figure 8, while that for the IDH EOR interaction is shown in Figure 9. Though a relationship exists, the AIC values for PFS models were significantly higher than those for overall survival. The interaction between IDH and RTV does impact PFS, but it does so in a complex way that requires better characterization.

Reports evaluating the benefits of increased EOR (reported as percentage volume of total tumor resected) may not fully capture the benefits of cytoreduction compared with studies evaluating benefits of minimizing RTV.[7,36] This is because EOR is not consistent among different tumor sizes. An 80% reduction of a 60cm^3 tumor would leave one with a RTV of 12cm^3 while a that same EOR in a 10cm^3 tumor would be left with a RTV of only 2cm^3. Discussion centering around EOR does not adequately depict residual disease burden that must be addressed with adjuvant therapies.[33] Chaichanna et al demonstrated increased EOR and decreased RTV independently influence overall survival, a finding we can confirm.[7.] In a study by Grabowski et al, RTV, pre-operative tumor volume, T2 FLAIR RTV and EOR were all volumetric measurements found to be significant predictors of survival when controlling for age and KPS score.[4.] Here, we support those findings, as both EOR and RTV were significant predictors of survival but RTV provided the best model fit for overall survival.

Limitations

Our study has a number of limitations. We separated our cohort based on IDH status, but it is possible that not all IDH mutants were captured. There may be some IDH mutants in our cohort that were R132H wildtype but that would have been found to be mutant if they had undergone genomic analysis. The retrospective nature of this study suffers similar shortcomings present in previous literature on this topic including selection bias, lack of randomization and difficulty in controlling for confounders and establishing cause and effect. However, it would be unethical and impractical to perform a prospective study to determine RTV as a prognostic factor in the presence of IDH mutation. This would involve the creation of a study group receiving intended subtotal resection when a number of retrospective studies have found increased survival with increased resection.

Another limitation, also present in previous volumetric studies, is the uncertainty that exists with tumor volume measurements. Since the study was limited by the availability of scans completed for past patients, some of the 3-dimensional volumetric measurements were based on 2-dimensional imaging, and therefore limited by slice thickness and imaging resolution. Beyond this, only a single observer had reviewed the scans at the time of data analysis. Once a second independent observer has reviewed these same scans and inter-observer variability is accounted for, the results can be conveyed more confidently. Finally, this study is limited by its size. As there were only 6 IDH mutant cases in our population, power would be limited for interaction effects and the generalizability of the results would be limited. To support the relationship between IDH and RTV larger studies at a multi-center level must be done.

Conclusion

Our study found maximal safe surgical resection, measured by decreasing RTV and increasing EOR, are significant prognostic factors for all patients with glioblastoma. When IDH was incorporated into multivariable models, RTV provided a slightly better fit than the model with EOR. Beyond this, we found that IDH interacts with RTV, such that the lowest RTVs are associated with the greatest protective benefit from IDH. This interaction suggests that although the benefit of lower RTV is seen independent of other factors, IDH mutants should be the target of aggressive surgical resections in order to minimize RTV.

References

1. Progress M, Wen PY, Kesari S. Malignant Gliomas in Adults. N Engl J Med. 2008;359(5):492-507.

2. Grabowski M, Recinos P, Nowacki A. Residual tumor volume versus extent of resection: predictors of survival after surgery for glioblastoma. *J Neurosurg*. 2014;121(5):1115-1123.

3. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study : 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 10(5):459-466. doi:10.1016/S1470-2045(09)70025-7.

4. Deangelis L. Brain Tumors. *Med Prog N Engl J Med*. 2001;344(2):114-123. doi:10.1227/01.NEU.0000311254.63848.72.

5. Amico RSD, Englander ZK, Canoll P, Bruce JN. Literature Review Extent of Resection in Glioma e A Review of the Cutting Edge. *World Neurosurg*. 2017;103:538-549. doi:10.1016/j.wneu.2017.04.041.

6. Brown TJ, Brennan MC, Li M, et al. Association of the Extent of Resection With Survival in Glioblastoma. *JAMA Oncol*. 2016;352(10):987-996. doi:10.1001/jamaoncol.2016.1373.

7. Chaichana KL, Jusue-torres I, Navarro-ramirez R, et al. Establishing percent resection and residual volume thresholds affecting survival and recurrence for patients with newly diagnosed intracranial glioblastoma. *Neuro Oncol*. 2014;16(1):113-122. doi:10.1093/neuonc/not137.

8. Staedtke V, Holdhoff M, Program C. Actionable molecular biomarkers in primary brain tumors. *Trends Cancer*. 2016;2(7):338-349. doi:10.1016/j.trecan.2016.06.003.Actionable.

9. Kos I, Ph D, Batinic-haberle I, et al. Mutations in Gliomas. *N Engl J Med*. 2009;360(8):765-773.

10. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131(6):803-820. doi:10.1007/s00401-016-1545-1.

11. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462(10):739-744. doi:10.1038/nature08617.

12. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 Mutations are Early Events in the Development of Astrocytomas and Oligodendrogliomas. *Am J Pathol*. 2009;174(4):1149-1153. doi:10.2353/ajpath.2009.080958.

13. Hegi ME, Diserens A-C, Gorlia T, et al. Gene Silencing and Benefit from Temozolomide in Glioblastoma. *N Engl J Med*. 2005;352(10):997-1003.

14. Beiko J, Suki D, Hess KR, et al. IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. *Neuro Oncol*. 2014;16(1):81-91. doi:10.1093/neuonc/not159.

15. Vecht J, Avezaat J, Putten WLJ Van, Eijkenboom WMH, Stefanko SZ. The influence of the extent of surgery on the neurological function and survival in malignant glioma . A retrospective analysis in 243 patients. 1990:466-471.

16. Oszvald A, Güresir E, Setzer M, et al. Glioblastoma therapy in the elderly and the importance of the extent of resection regardless of age. *J Neurosurg*. 2012;116(2):357-364. doi:10.3171/2011.8.JNS102114.

17. Lacroix M, Abi-said D, Fourney D. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg*. 2001;95:190-198.

18. Marko NF, Weil RJ, Schroeder JL, Lang FF, Suki D, Sawaya RE. Extent of resection of glioblastoma revisited: Personalized survival modeling facilitates more accurate survival prediction and supports a maximum-safe-resection approach to surgery. *J Clin Oncol*. 2014;32(8):774-782. doi:10.1200/JCO.2013.51.8886.

19. Kelly PJ, Daumas-Duport C, Kispert DB, Kall BA, Scheithauer BW, Illig JJ. H Imaging extent: Imaging-based stereotaxic serial biopsies in untreated intracranial glial neoplasms. *J Neurosurg*. 1987;66(6):865-874. doi:10.3171/jns.1987.66.6.0865.

20. Li YM, Suki D, Hess K, Sawaya R. The influence of maximum safe resection of glioblastoma on survival in 1229 patients: Can we do better than gross-total resection? *J Neurosurg*. 2015;124:1-12. doi:10.3171/2015.5.JNS142087.

21. Hervey-Jumper SL, Berger MS. Maximizing safe resection of low- and high-grade glioma. *J Neurooncol*. 2016;130(2):269-282. doi:10.1007/s11060-016-2110-4.

22. Bogomolny DL, Petrovich NM, Hou BL, Peck KK, Kim MJJ, Holodny AI. Functional MRI in the Brain Tumor Patient. *Top Magn Reson Imaging*. 2004;15(5):325-335.

23. Sanai N, Berger MS. Operative Techniques for Gliomas and the Value of Extent of Resection. *Neurotherapeutics*. 2009;6:478-486.

24. Stummer W, Pichlmeier U, Meinel T, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma : a randomised controlled multicentre phase III trial. *Lancet Oncol*. 2006;7:392-401. doi:10.1016/S1470-2045(06)70665-9.

25. Coburger J, Hagel V, Wirtz CR, König R. Surgery for Glioblastoma : Impact of the Combined Use of 5-Aminolevulinic Acid and Intraoperative MRI on Extent of Resection and Survival. *PLoS One*. 2015;10(6):1-13. doi:10.1371/journal.pone.0131872.

26. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: Response assessment in neuro-oncology working group. *J Clin Oncol*. 2010;28(11):1963-1972. doi:10.1200/JCO.2009.26.3541.

27. Burnham, KP, Anderson, DR. (2002). Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer, New York.

28. Kawaguchi T. Impact of gross total resection in patients with WHO grade III glioma harboring the IDH 1 / 2 mutation without the 1p / 19q. *J Neurooncol*. 2016;129(3):505-515. doi:10.1007/s11060-016-2201-2.

29. Sanai N, Polley M-Y, McDermott MW, Parsa AT, Berger MS. An extent of resection threshold for newly diagnosed glioblastomas. *J Neurosurg*. 2011;115(1):3-8. doi:10.3171/2011.2.JNS10998.

30. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*. 2007;114(2):97-109. doi:10.1007/s00401-007-0243-4.

30. Paldor I, Drummond KJ, Kaye AH. IDH1 mutation may not be prognostically favorable in glioblastoma when controlled for tumor location : A case-control study. *J Clin Neurosci*. 2016;34:117-120. doi:10.1016/j.jocn.2016.05.016.

31. Hegi ME, Wanyu RJ, Thierry LL, et al. Presence of an oligodendroglioma-like component in newly diagnosed glioblastoma identifies a pathogenetically heterogeneous subgroup and lacks prognostic value: central pathology review of the EORTC_26981 /NCIC_CE.3 trial. *Acta Neuropathol*. 2012;123:841-852. doi:10.1007/s00401-011-0938-4.

32. Dubbink HJ, Taal W, van Marion R, et al. IDH1 mutations in low-grade astrocytomas predict survival but not response to temozolomide. Neurology. 2009;73(21):1792–1795.

33. Omuro A, Beal K, Gutin P, et al. Phase II Study of Bevacizumab , Temozolomide , and Hypofractionated Stereotactic Radiotherapy for Newly Diagnosed Glioblastoma. *Clin Cancer Res*. 2014;20(19):5023-5032. doi:10.1158/1078-0432.CCR-14-0822.

34. Maus A, Peters GJ. Glutamate and α-ketoglutarate : key players in glioma metabolism. *Amino Acids*. 2017;49(1):21-32. doi:10.1007/s00726-016-2342-9.

35. Almeida JP, Chaichana KL. The Value of Extent of Resection of Glioblastomas : Clinical Evidence and Current Approach. *Curr Neurol Neurosci Rep*. 2015;15(2):517. doi:10.1007/s11910-014-051

Tables and Figures

Table 1. Patient Demographics and Clinical Characteristics of 98 Patients with Glioblastoma

Characteristic	N	Percent
Residual Tumor Volume Median (Q1-Q3)	7.87 cm^3	2.99-20.78
Resection Percentage Median (Q1-Q3)	65.5	0.00-88.57
IDH Mutation		
Yes	6	6.1
No	92	93.9
Age Mean (SD)	60.8	12.6
Gender		
Female	43	43,9
Male	55	56.1
Location		
Frontal	33	33.7
Other	65	66.3
ECOG		
0-1	74	75.5
2-4	24	24.5
Presenting Symptoms		
Headache	41	41.8
Aphasia	27	27.6
Gait Disturbance	18	18.4
Hemiplegia	28	28.6
Seizures	10	10.2
Radiation Therapy		
With Chemotherapy (Co)	72	73.5
RT Only	8	8.2
No RT	18	18.4
Adjuvant Chemotherapy		
Yes	37	37.8
No	61	62.2
Events		
Death	83	84.7
Progression or death	92	93.9
Follow-up (months)	Median	11.1

Table 2. Univariable Analysis for Predictors of Overall Survival

Characteristic	HR	95% CI	P
Residual Tumor Volume / $10cm^3$	1.16	1.03-1.30	**0.013**
Resection Percentage / 10%	0.93	0.88-0.98	**0.009**
Resection as a Category			
<40	2.22	1.22-4.06	**0.025**
40-89	1.48	0.79-2.77	
90+	1		
IDH mutation			
Yes	0.41	0.13-1.31	0.133
No	1		
Age / 10 years	1.04	0.86-1.26	0.682
Sex			
Female	1.44	0.93-2.22	0.102
Male	1		
Location			
Frontal	0.70	0.43-1.14	0.153
Other	1		
ECOG			
2-5	1.89	1.15-3.11	**0.011**
0-1	1		
Presenting Symptoms			
Headache	1.02	0.66-1.58	0.916
Aphasia	1.22	0.76-1.95	0.406
Gait Disturbance	1.57	0.90-2.72	0.111
Hemiplegia	1.15	0.71-1.85	0.571
Seizures	1.24	0.62-2.49	0.544
Radiation Therapy			
With Chemotherapy (co)	0.28	0.19-0.63	**<0.001**
RT Only	0.97	0.26-1.49	
No RT	1		
Adjuvant Chemotherapy			
Yes	0.48	0.30-0.79	**0.004**
No	1		

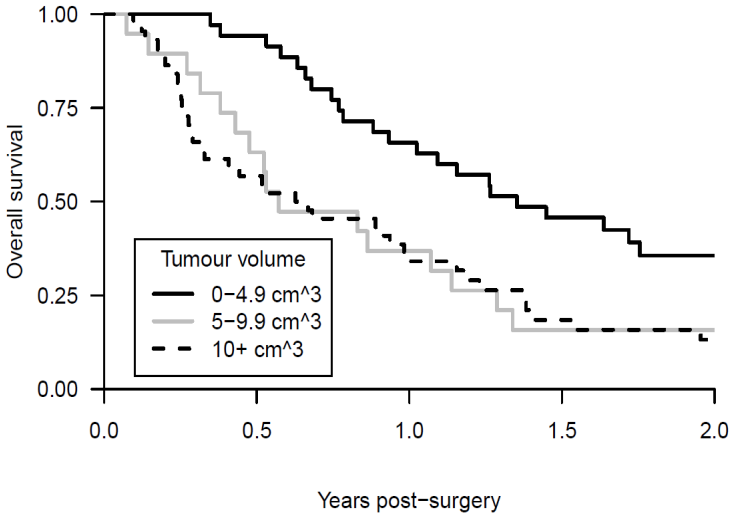

Figure 1. Kaplan Meier curve for overall survival by RTV (residual tumor volume)

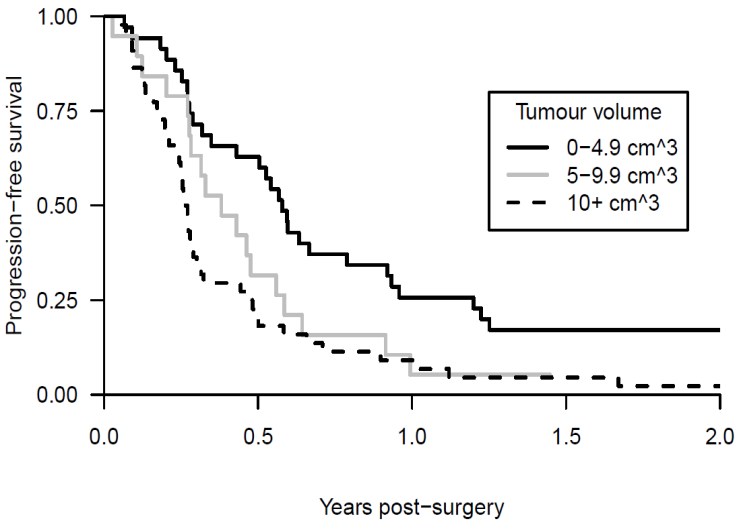

Figure 2. Kaplan Meier curve for progression-free survival by RTV (residual tumor volume)

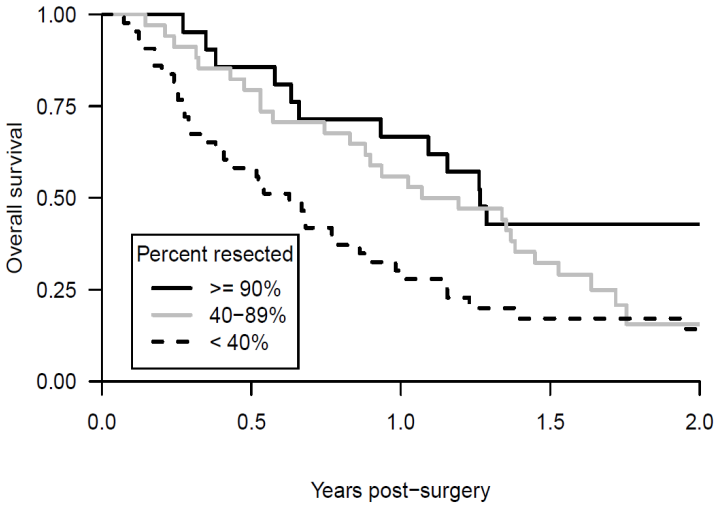

Figure 3. Kaplan Meier curve for overall survival by EOR (extent of resection)

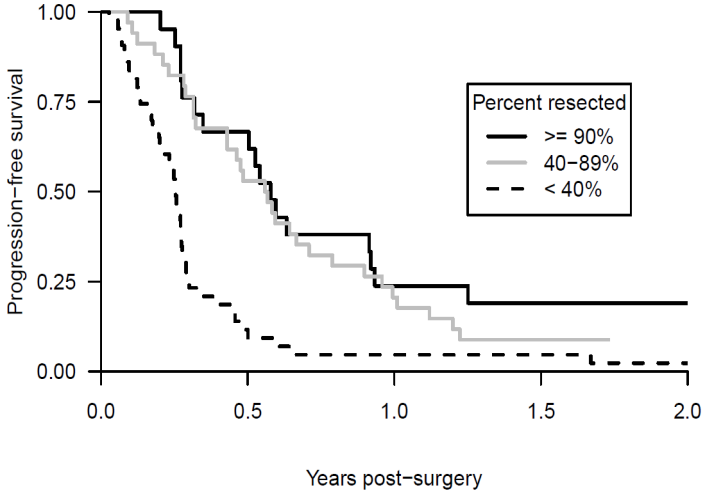

Figure 4. Kaplan Meier curve for progression-free survival by EOR (extent of resection)

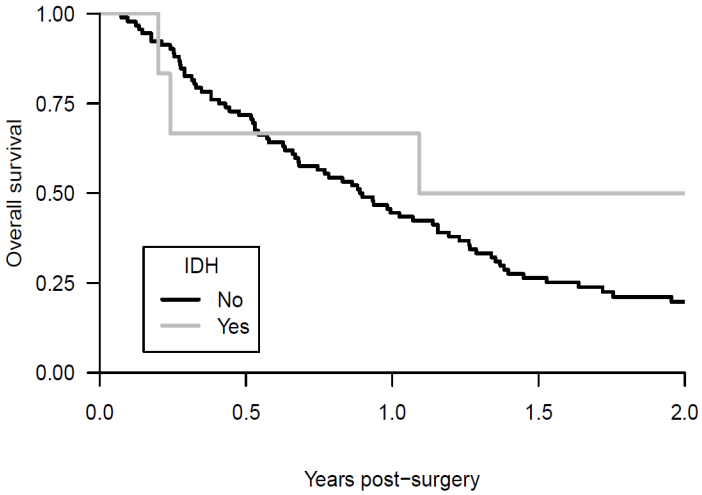

Figure 5. Kaplan Meier curve for overall survival comparing IDH mutant and IDH wild-type

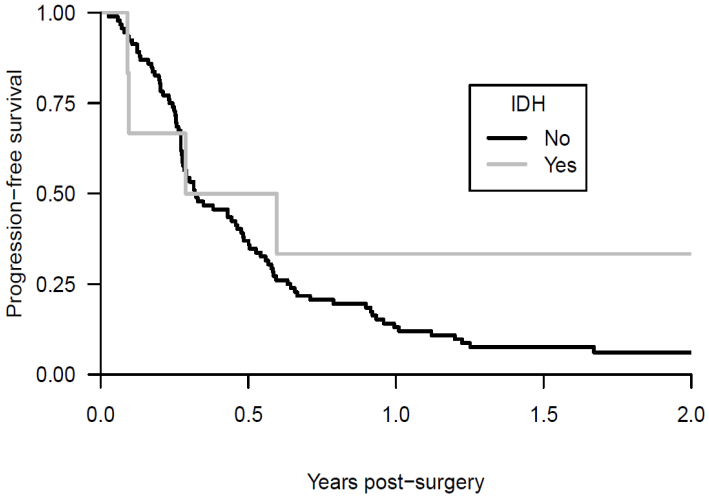

Figure 6. Kaplan Meier curve for progression-free survival comparing IDH mutant and IDH wild-type

Table 3. Multivariable Cox PH Regression for Overall Survival in 3 Different Models using Residual Tumor Volume (RTV): one without IDH as a covariate, one adding IDH as a covariate and one adding the IDH-RTV interaction as a covariate

Characteristic	Multivariable			Multivariable + IDH			Multivariable + IDH Int		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
RTV / $10cm^3$	1.20	1.06-1.36	**0.004**	1.17	1.03-1.32	**0.015**	1.16	1.02-1.32	**0.022**
ECOG 2-4 0-1	 2.27 1	 1.35-3.82	 **0.002**	 2.39 1	 1.42-4.03	 **0.001**	 2.51 1	 1.48-4.26	 **<0.001**
Radiation Therapy With Chemotherapy (co) RT Only No RT	 0.21 0.55 1	 0.11-0.39 0.22-1.37	 **<0.00 1**	 0.17 0.71 1	 0.09-0.32 0.29-1.73	 **<0.001**	 0.17 0.82 1	 0.09-0.32 0.33-2.02	 **<0.001**
Adjuvant Chemotherapy Yes No	 0.46 1	 0.27-0.77	 **0.003**	 0.48 1	 0.28-0.80	 **0.005**	 0.49 1	 0.29-0.82	 **0.007**
IDH mutation Yes No				 0.23 1	 0.07-0.78	 **0.018**	 0.03 1	 0.00-0.38	 **0.007**
IDH * RTV Interaction							20.97	3.73-117.89	**0.001**
AIC	**600.0**			**594.0**			**584.2**		

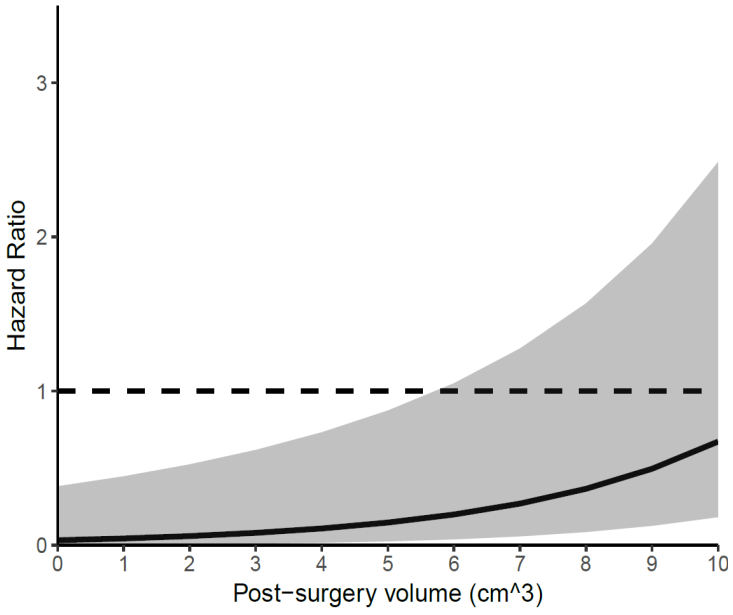

Figure 7. Estimates of Hazard Ratios for Overall Survival in IDH-mutants over a range of post-surgical volumes

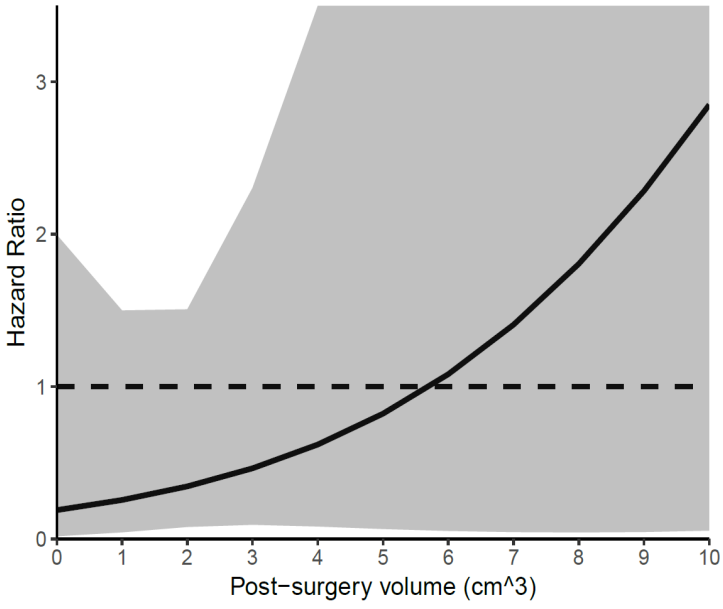

Figure 8. Estimates of Hazard Ratios for Progression-Free Survival in IDH-mutants over a range of post-surgical volumes

Table 4. Multivariable Cox PH Regression for Overall Survival in 3 Different Models using Percent Resected: one without IDH as a covariate, one adding IDH as a covariate and one adding the IDH-EOR (Extent of Resection) interaction as a covariate

Characteristic	Multivariable			Multivariable + IDH			Multivariable + IDH Int		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Percent Resected / 10%	0.94	0.88-1.00	**0.041**	0.96	0.90-1.01	0.152	0.96	1.02-1.32	0.181
ECOG 2-4 0-1	2.40 1	1.41-4.09	**0.001**	2.47 1	1.45-4.20	**0.001**	2.57 1	1.48-4.26	**0.001**
Radiation Therapy With Chemotherapy (co) RT Only No RT	0.29 0.78 1	0.15-0.53 0.30-2.05	**<0.001**	0.22 0.91 1	0.11-0.42 0.35-2.36 1	**<0.001**	0.21 1.03 1	0.11-0.42 0.40-2.65	**<0.001**
Adjuvant Chemotherapy Yes No	0.48 1	0.28-0.81	**0.006**	0.48 1	0.28-0.80	**0.005**	0.49 1	0.28-0.79	**0.005**
IDH mutation Yes No				0.23 1	0.07-0.78	**0.018**	0.47 1	0.28-0.79	**0.005**
IDH * EOR Interaction							0.66	0.44-0.99	**0.047**
AIC	**600.0**			**597.2**			**596.1**		

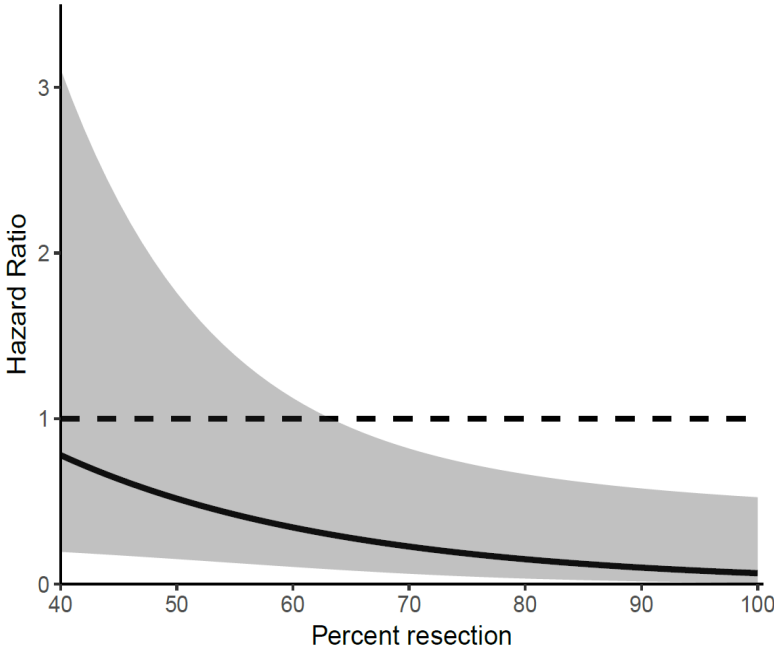

Figure 9. Estimates of Hazard Ratios for Overall Survival in IDH-mutants over a Range of Resection Values

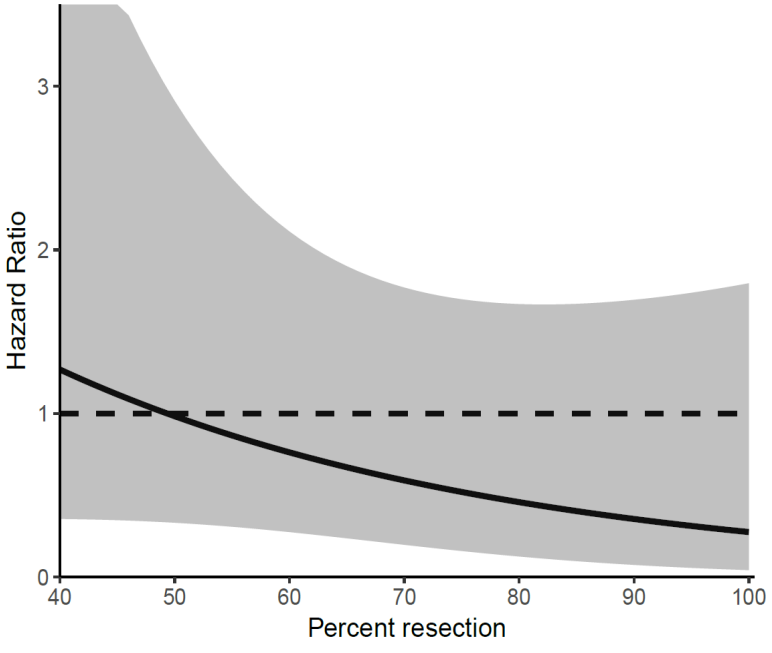

Figure 10. Estimates of Hazard Ratios for Progression-Free Survival in IDH-mutants over a Range of Resection Values

Appendix

ECOG Performance Status	
Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

From ECOG website - http://www.ecog.org/general/perf_stat.html

RANO Criteria				
Criterion	CR	PR	SD	PD*
T1 gadolinium enhancing disease	None	≥50% or ↓	<50% ↓ but <25% ↑	≥25%↑
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑
New lesion	None	None	None	Present
Corticosteroids	None	Stable or ↓	Stable or ↓	NA
Clinical Status	Stable or ↑	Stable or ↑	Stable or ↑	↓
Requirement for response	All	All	All	Any
RANO – response assessment in neuro-oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid attenuated inversion recovery; NA, not applicable *progression occurs when any one of the criteria are present				

From the RANO working group[26]