Peer Review File

Article information: https://dx.doi.org/10.21037/atm-21-3998

Reviewer A

Comment 1: The number was small. Moreover, multivariable assay was performed using only 11 cases. They should check the relation of growth rate and molecular biomarkers in each type of tumor using more cases. They showed the data in supplementary table 3, but the number of oligodendroglioma and diffuse astrocytoma, IDH mutant was 11 and 13, respectively according to table 1. The number was too small.

Response 1: I appreciate the reviewer comments. We re-reviewed our database to added number of patients in this study. And for new standard classification, patients with IDH wild type would be classified as WHO 4, so we only enrolled patient with IDH status and excluded patients with IDH wild-type.

Changes in the text: We re-did the data analysis and revised the sections of methods, results and discussions.

Comment 2: Introduction

L69: Although LGGs almost invariably progress to glioblastomas…..

As you know, oligodendroglioma dose not progress to glioblastoma.

Response 2: Thanks for point out this. We removed this sentence in the revised manuscript.

Changes in the text: We removed this sentence 'Although LGGs almost invariably progress to glioblastomas,' in L69.

Reviewer B

Comment 1. Specifically, I would suggest categorizing patients as:

IDH wild type

Astrocytoma IDH mutated, 1p19q intact/anueploid

Oligodendroglioma IDH mutated, 1p19q co-deleted

NOS

Response 1: Thanks a lot for your suggestion. As we know, in 2021 classification, patients with IDH wild type would be classified as WHO 4. Therefore, we excluded IDH wild-type patients in this study, and categorized patients as:

Diffuse astrocytoma, IDH wild-type

Oligodendroglioma, IDH mutant

Diffuse astrocytoma, NOS

Oligodendroglioma, NOS

Oligoastrocytoma, NOS

Changes in the text: We changed text in Table 1.

Characteristics	**No. / Value**	**eVDE (mm/year)**	**Estimated effects (mm/year ± SE)**	***p***

Sex				
Male	39	1.9	0.8 ± 0.3	0.02*
Female	17	2.6		
Age (Median, range, years)	36 (21-62)		-0.05 ± 0.01	< 0.01*
Histological classification				
Diffuse astrocytoma, IDH mutant	21	3.4	-2.1 ± 0.3	< 0.01*
Oligodendroglioma, IDH mutant	19	1.4		
Diffuse astrocytoma, NOS	4	3.3	—	—
Oligodendroglioma, NOS	4	1.7	—	—
Oligoastrocytoma, NOS	8	2.8	—	—
Initial mean tumor diameter (IMTD, median, range, cm)	2.7 (0.8-9.0)			
Initial tumor volume (Median, range, cm^3)	9.4 (0.2 - 368.0)	—	—	—
Preoperative tumor volume (Median, range, cm^3)	19.8 (1.9 - 404.1)	—	—	—
Interval time (Median, range, day)	472 (91 - 4799)	—	—	—
Number of Available MRI (Average, range, day)	2.6 (2 - 8)	—	—	—

Comment 2. I appreciate that limited diagnostic labels to those patients with known IDH and 1p19q status will leave a substantial number of the patient sample in the NOS subgroup, but histological diagnosis of glioma subgroup is no longer accepted in the WHO 2021 (or 2016) criteria.
Then, within each of these groups (perhaps excluding the IDH wt group due to size), present the effects of the remaining molecular biomarkers on tumor growth.

Response 2: Thank you for your comment. We re-reviewed institutional database and only enrolled patients with IDH status in the study. IDH wild-type patients, which are considered WHO 4 gliomas now, had been excluded. Effects of the remaining factors on tumor growth in diffuse astrocytoma, IDH mutant group and Oligodendroglioma, IDH mutant group were re-analyzed and shown in Table 4.

Changes in the text: We added Table 4 in the main text.

Table 4: The estimated effect of multiple-factor analysis using the multivariate linear mixed-effects model in diffuse astrocytoma, IDH mutant and oligodendroglioma, IDH mutant subgroups.

Molecular biomarkers	**Estimated effects**	***p*-value**

	(mm/year ± SE)	
Diffuse astrocytoma, IDH mutant		
Age	-0.09 ± 0.03	0.01*
Mutant p53 (High vs Low)	3.6 ± 1.7	0.04*
MGMT promoter (Methylation vs non-Methylation)	-2.6 ± 0.5	<0.01*
Oligodendroglioma, IDH mutant		
Age	-0.02 ± 0.006	<0.01*
Sex (Female vs male)	0.7 ± 0.2	<0.01*

Comment 3. Line 69: "almost invariably progress to glioblastomas": Under WHO 2021, only IDHwt gliomas can be classified as glioblastomas, so this is no longer true. Perhaps refer to the almost inevitable fatality.

Response 3: Thanks for point out this. We removed this sentence in the revised manuscript.

Changes in the text: We removed this sentence "Although LGGs almost invariably progress to glioblastomas," in L69.

Comment 4. Line 110: It's unclear if only 2 MRIs were included for each patient, or if all available MRIs prior to surgery were included. If more were included, indicate the average number and range in the text or one of the tables.

Response 4: I appreciate the reviewer comment. All available MRIs prior to surgery were included in this study and we added the average number and range in table 1.

Changes in the text: We added the average number and range of available MRI in Table 1

Characteristics	**No. / Value**	**eVDE (mm/year)**	**Estimated effects (mm/year ± SE)**	***p***
Sex				
Male	39	1.9	0.8 ± 0.3	0.02*
Female	17	2.6		
Age (Median, range, years)	36 (21-62)		-0.05 ± 0.01	< 0.01*

Histological classification				
Diffuse astrocytoma, IDH mutant	21	3.4	-2.1 ± 0.3	< 0.01*
Oligodendroglioma, IDH mutant	19	1.4		
Diffuse astrocytoma, NOS	4	3.3	—	—
Oligodendroglioma, NOS	4	1.7	—	—
Oligoastrocytoma, NOS	8	2.8	—	—
Initial mean tumor diameter (IMTD, median, range, cm)	2.7 (0.8-9.0)			
Initial tumor volume (Median, range, cm^3)	9.4 (0.2 - 368.0)	—	—	—
Preoperative tumor volume (Median, range, cm^3)	19.8 (1.9 - 404.1)	—	—	—
Interval time (Median, range, day)	472 (91 - 4799)	—	—	—
Number of Available MRI (Average, range, day)	2.6 (2 - 8)	—	—	—

Comment 5: Line 142: I suggest moving the choice of linear or non-linear growth model from the discussion to the methods. Also, clearly state that the mean tumor diameter is an estimate based on the measured tumor volume, and not a directly measured single-slice diameter measurement, as used in the Macdonald and RANO criteria for measuring tumor size and treatment response. Perhaps discuss the relationship between RANO and MTD measurements, as RANO are required in most clinical trials.

Response 5: Thanks for your suggestion, we revised methods to more clearly showed the reason why we chose the linear growth model and the method we calculated mean tumor diameter. Voxel-based calculation of area of interest (ROI) volume is a widely accepted method for quantitative neuroimaging research at present, so we do not need to discuss the comparison with other volume calculation methods.

Changes in the text: Line 142 were revised as

'Most studies that quantitatively measured tumor growth rate used the linear growth model[1, 8-10, 12, 13, 15, 24, 25], and since it is not affected by the initial volume, the linear model can intuitively observe the differences of growth rates between different subgroups. Therefore, we chose the linear growth model to analysis influence of different factors on growth rate. The changes in tumor size are represented by the change in mean tumor diameter (MTD, $MTD = (2 \times V)^{1/3}$) [16] over time, and the tumor volume (V) was calculated using MATLAB (version 2014a, The MathWorks Inc.) based on voxels of the segmented tumor region on T2-weighted images (Fig 1).'

Comment 6: Line 138: "A dichotomy was utilized to obtain the subgroups": How were these variables dichotomized into high and low? It appears some criteria was used, rather than a median split, as the numbers are unequal. Are there references for the cut-off values used for high and low groups for each biomarker? Also, why are continuous variables like age and IMTD dichotomized?

Response 6: Thanks for your comments. We set We revised the Supplementary Table 1to show how were these variables dichotomized into high and low more clearly. And according your suggestion, 'age' was used as a continuous variable in the subsequent analysis.

Changes in the text: Supplementary Table 1. The final standard of the high expression level of each biomarker. When we calculated the effect of a biomarker, we analyzed the effects of different high-expression cutoffs (> 0, > 1, > 2, and > 3). When the effects of the biomarker expression levels were most significant, the cutoff of the expression level was determined to be the final cutoff.

Biomarkers	**Number of patients with different scores**					**Standard of high expression level**	**Estimated effects (High vs low, mm/year ± SE)**	***p***
	0	**1**	**2**	**3**	**4**			
ATRX	9	9	3	1	3	> 0	-2.5 ± 0.4	0.16×10^{-8}*
						> 1	-2.3 ± 0.4	0.68×10^{-7}
						> 2	-1.2 ± 1.2	0.34
						> 3	-1.9 ± 1.3	0.16
EGFR	0	0	4	3	9	> 0	-	
						> 1	-	
						> 2	1.0 ± 0.9	0.27
						> 3	1.8 ± 0.6	0.003*
Mutant p53	9	7	6	3	5	> 0	-0.7 ± 0.7	0.28
						> 1	2.4 ± 0.4	0.14×10^{-8}
						> 2	3.0 ± 0.4	0.12×10^{-10}*
						> 3	2.6 ± 0.5	0.4×10^{-6}

						> 0	-	-
Ki-67	0	18	15	1	2	> 1	-0.7 ± 0.4	0.05*
						> 2	1.7 ± 0.9	0.07
						> 3	1.6 ± 1.0	0.10

Comment 7: Line 165: "histological classification". Again, WHO 2021 diagnostic groups should be used, rather than histology-based groups. Also, with this classification, it is unnecessary to include TERT status, as it was 100% covariant with 1p19q status.

Response 7: Thanks for your comments. TERT promoter status was still included in the revised manuscript because Some patients tested for TERT promoter status but not for 1p/19q status.

Comment 8: Line 172: Were any corrections for multiple comparisons performed?

Response 8: No. In revised manuscript, we didn't use multiple comparison.

Comment 9: Line 205: The results within each diagnostic subgroup (or at least Astrocytoma IDH mutated and Oligodendroglioma IDH mutated. 1p19q co-deleted) should be reported in the main text.

Response 9: Thanks for your suggestion, we added results of multifactor mLME analysis for diffuse astrocytoma, IDH mutant and oligodendroglioma, IDH mutant in the main text, Table 4 and Table 5.

Changes in the text: Line 210-219 were revised as 'The interaction effects of status of data missing in different biomarkers were shown in Supplementary Table 2. Status of EGFR and TERT promoter distributed inconsistency in missing data group and non-missing group were excluded. Histological classification which was determined by biomarkers was also excluded. Status of Ki-67, mutant p53, 1p/19q, ATRX, MGMT promoter, age and sex were both added in the mLME as the interaction terms, to assess whether they were independent influencing factors. In the multiple-factor analysis, it was observed that age ($p < 0.01$) and status of mutant p53 ($p < 0.01$), ATRX ($p < 0.01$) and MGMT promoter ($p < 0.01$) still had a significant impact on eVDE (Table 3).

The estimated effect of factors in the diffuse astrocytoma, IDH mutant subgroup and oligodendroglioma, IDH mutant subgroup were also analyzed by Multiple-factor analysis (Table 4). The status of age, mutant p53, MGMT promoter were independent factor influenced growth rate of the diffuse astrocytoma, IDH mutant subgroup, while age and sex were independent factor that influenced growth rate of oligodendroglioma, IDH mutant subgroup.

Table 4: The estimated effect of multiple-factor analysis using the multivariate linear mixed-effects model in diffuse astrocytoma, IDH mutant and oligodendroglioma, IDH mutant subgroups.

Molecular biomarkers	Estimated effects (mm/year ± SE)	*p*-value
Diffuse astrocytoma, IDH mutant		
Age	-0.09 ± 0.03	0.01*
Mutant p53 (High vs Low)	3.6 ± 1.7	0.04*
MGMT promoter (Methylation vs non-Methylation)	-2.6 ± 0.5	<0.01*
Oligodendroglioma, IDH mutant		
Age	-0.02 ± 0.006	<0.01*
Sex (Female vs male)	0.7 ± 0.2	<0.01*

Comment 10: Line 210: Again, the WHO 2021 diagnostic groups should be used in the multi-variate analysis, and the molecular factors that make up those diagnoses should then be left out (IDH, 1p19q, TERT).

Response 10: Thanks for your comments, status of Ki-67, mutant p53, 1p/19q, ATRX, MGMT promoter, age and sex were finally used in the multi-variate analysis.

Changes in the text: Line 210-219 were revised as 'The interaction effects of status of data missing in different biomarkers were shown in Supplementary Table 2. Status of EGFR and TERT promoter distributed inconsistency in missing data group and non-missing group were excluded. Histological classification which was determined by biomarkers was also excluded. Status of Ki-67, mutant p53, 1p/19q, ATRX, MGMT promoter, age and sex were both added in the mLME as the interaction terms, to assess whether they were independent influencing factors. In the multiple-factor analysis, it was observed that age ($p < 0.01$) and status of mutant p53 ($p < 0.01$), ATRX ($p < 0.01$) and MGMT promoter ($p < 0.01$) still had a significant impact on eVDE (Table 3).'

Comment 11: Line 232: "the first study" – It's not clear which is the first study, and how it differs from the others, or why that matters, as the results seem the same.

Response 11: It's the first study use linear mixed model to evaluate eVDE of a group of LGG. In many other studies, reported VDE was not calculated by LME and was just mean VDE or median VDE. To make it clearly, we revised Line 232.

Changes in the text: Line 232:

'While the eVDE in the first study calculated by linear mixed model was 4.1 mm/year….'

Comment 12: Line 260: Survival time results mentioned here are not described in the methods or reported in the results.

Response 12: Prognostic data were not included in this study. Diffuse astrocytomas have been reported to have a shorter survival period than oligodendrogliomas. We revised the manuscript to make it clearer.

Changes in the text: Line 260:

'Diffused astrocytoma, IDH mutant-type, grew the faster and was reported had a shorter survival time, while oligodendroglioma, IDH mutant, grew the slower and was reported had a longer survival time.'

Comment 13: Line 282: What is the new method provided by this study? All the methods used here have been previously reported, although the application to this data sample is new. Furthermore, the methods and results here are not clinically useful for "preoperative" assessment of patients, exactly, because the biomarkers examined here are only available from tissue samples collected during surgery. It seems that the results here suggest that these biomarkers, once acquired, will be useful post-operatively to predict growth rates of residual tumor, and use that information in treatment planning.

Response 13: Thanks for your comments. This sentence was not appropriate and we have removed it in the revised manuscript.

Changes in the text: Table 2: Estimated effects of the expression (high or low) of molecular biomarkers on the growth rate of low-grade gliomas.

Molecular biomarkers	**Subgroup**	**N**	**eVDE (mm/year)**	**Estimated effects (mm/year ± SE)**	***p***
Ki-67	Low	18	2.6	-0.7 ± 0.4	0.05*
	High	18	1.9		
	Missing data	20	1.9		
Mutant p53	Low	22	1.5	3.0 ± 0.4	<0.01*
	High	8	4.5		
	Missing data	26	2.0		
TERT promoter	Wild-type	25	3.4	-1.8 ± 0.3	<0.01*
	Mutant	20	1.6		
	Missing data	11	1.5		
1p/19q	Non-codeletion	21	3.4	-1.9 ± 0.3	<0.01*
	Codeletion	19	1.4		
	Missing data	16	2.2		
EGFR	Low	7	2.2	1.8 ± 0.6	<0.01*
	High	9	4.0		
	Missing data	40	1.8		

ATRX	Low	9	3.6	-2.5 ± 0.4	<0.01*
	High	16	1.1		
	Missing data	31	1.9		
MGMT promoter	Non-methylation	8	5.0	-3.0 ± 0.6	< 0.01*
	Methylation	31	2.0		
	Missing data	17	1.7		

Comment 14: Table 2: Give column labels for all columns, and perhaps separate molecular biomarker rows with a line, as it is difficult to follow biomarkers horizontally.

Response 14: Thanks for your suggestion. We revised Table 2 to be more clearly.

Changes in the text: Line 282: we removed 'Our study provides a new method for the preoperative assessment of patients with LGGs by evaluating the status of biomarkers and determining prognostics according to the tumor growth rate.'

Reviewer C

Comment 1: This work was planned with the WHO 2016 diagnostic criteria ongoing. Unfortunately, this year will be published new criteria that it will takes into account molecular information at the same level of the histological findings, as it was recommended by the cIMPACT group during the past years. The advance of the new novelties has been published this June in Neuro-Oncology by Drs Wen and Packer. Therefore, this work includes tumors that it will not be considered LGG (6 IDHwt, and 20 NOS, almost the half of the sample), or even it would not be considered LGG following the cIMPACT recommendations in the past years. I'm afraid that this work will born dead or expired due to these changes.

Response 1: I appreciate the reviewer comments. We re-reviewed our database to added number of patients in this study. And for new standard classification, patients with IDH wild type would be classified as WHO 4, so we only enrolled patient with IDH status and excluded patients with IDH wild-type and NOS.

Comment 2: In addition to have a better homogeneous sample of LGG, the authors should have to include the CDNK1A7B deletion, the status of histone G34 and the chromosomes 7 and 10, and avoid the NOS in their determinations and statistical model.

Response2: Thanks for your advice, for a better homogeneous sample of LGG, we excluded IDH wild-type patients. As a retrospective study, we haven't tested for these molecular markers in most of the last 13 years, and We hope to include these indicators in future studies.

Comment 3: The other important concern is the assumption of linear growth in gliomas or specifically in LGG. Without this assumption, the statistical approach done by the authors should be wrong, and the findings irrelevant. And at this point, it has controversy; LGG has linear or Gompertzian growth? Moreover, some of the

references provided for the authors to support this assumption, highlight the doubts of this approach ('the linear radial growth results in a cubic growth of its visible volume. While all of the cells can potentially be proliferating exponentially, the radius of the visible bulk is increasing linearly and its volume is growing cubically'). I'm not mathematic, but the experience shows me that some LGG can have a very small growth or even growth stabilization during many years, and at some point, it can start to growth or accelerate its growth. Therefore, depending on the temporal range of your observation window, you can extrapolate different growing patterns or models. Your own graphics (Fig 2) exemplify this point, if you compare the growth behavior of patients with the below or above median interval time of following. In any way and putting aside this controversy, it should be worthy to consider this kind of statistical approach that the patients included would have a homogeneous interval of MRI observations.

Response 3: Thanks for your comments. Gompertzian growth were mainly reported in research of GBM, due to the extremely fast growth rate, limited by the cranial cavity, the growth rate decreases after reaching a certain size, and there is still little evidence on whether it is applicable to LGGs. The linear growth model in past studies and this study means to the linear growth of tumor diameter over time, while the change of tumor volume is exponential growth. These two are not contradictory. Linear growth models are widely used in LGG, and can easily and intuitively quantify the effects of different factors on growth rate. Therefore, we choose the linear diameter growth model for analysis in this study. I appreciate with your suggestion that regular follow-up time points can better observe changes in tumor growth rate, and we hope to include this method in future studies.

Comment 4: It should have to be improved the justification and the explanation of the cut-offs employed for the molecular markers and its use for statistical analysis.

Response 4: Thanks for your suggestion. We revised the Supplementary Table 1to show the origin of cutoff selection.

Changes in the text: Supplementary Table 1. The final standard of the high expression level of each biomarker. When we calculated the effect of a biomarker, we analyzed the effects of different high-expression cutoffs (> 0, > 1, > 2, and > 3). When the effects of the biomarker expression levels were most significant, the cutoff of the expression level was determined to be the final cutoff.

Biomarkers	**Number of patients with different scores**					**Standard of high expression level**	**Estimated effects (High vs low, mm/year ± SE)**	***p***
	0	**1**	**2**	**3**	**4**			
ATRX	9	9	3	1	3	> 0	-2.5 ± 0.4	0.16×10^{-8}*
						> 1	-2.3 ± 0.4	0.68×10^{-7}

						> 2	-1.2 ± 1.2	0.34
						> 3	-1.9 ± 1.3	0.16
EGFR	0	0	4	3	9	> 0	-	
						> 1	-	
						> 2	1.0 ± 0.9	0.27
						> 3	1.8 ± 0.6	0.003*
Mutant p53	9	7	6	3	5	> 0	-0.7 ± 0.7	0.28
						> 1	2.4 ± 0.4	0.14×10^{-8}
						> 2	3.0 ± 0.4	0.12×10^{-10}*
						> 3	2.6 ± 0.5	0.4×10^{-6}
Ki-67	0	18	15	1	2	> 0	-	-
						> 1	-0.7 ± 0.4	0.05*
						> 2	1.7 ± 0.9	0.07
						> 3	1.6 ± 1.0	0.10

Comment 5: If the justification of the study is the heterogeneous behavior of LGG, why do not exclude the histology in the model, and add the IDH status and the NOS? for me seems more logical, with the additional advantage to assess the impact of the IDH status.

Response 5: Thanks for your comments, in revised manuscript, we only enrolled patients with mutant status of IDH.