Original Article

Pattern of Recurrence of Glioblastoma Versus Grade 4 IDH-Mutant Astrocytoma Following Chemoradiation: A Retrospective Matched-Cohort Analysis

James Stewart, PhD[1] (ID), Arjun Sahgal, MD[1,2], Aimee K M Chan, MSc[3],
Hany Soliman, MD[1,2], Chia-Lin Tseng, MD[1,2] (ID), Jay Detsky, MD, PhD[1,2],
Sten Myrehaug, MD[1,2], Eshetu G Atenafu, MSc[4], Ali Helmi, MD[3],
James Perry, MD[5], Julia Keith, MD[6], Mary Jane Lim-Fat, MD, MSc[5],
David G Munoz, MD, MSc[7], Gelareh Zadeh, MD, PhD[8], David B Shultz, MD, PhD[2,9],
Sunit Das, MD, PhD[10], Catherine Coolens, PhD[2,9], Paula Alcaide-Leon, MD[11],
and Pejman Jabehdar Maralani, MD[3]

Abstract

Background and Purpose: To quantitatively compare the recurrence patterns of glioblastoma (isocitrate dehydrogenase-wild type) versus grade 4 isocitrate dehydrogenase-mutant astrocytoma (wild type isocitrate dehydrogenase and mutant isocitrate dehydrogenase, respectively) following primary chemoradiation. **Materials and Methods:** A retrospective matched cohort of 22 wild type isocitrate dehydrogenase and 22 mutant isocitrate dehydrogenase patients were matched by sex, extent of resection, and corpus callosum involvement. The recurrent gross tumor volume was compared to the original gross tumor volume and clinical target volume contours from radiotherapy planning. Failure patterns were quantified by the incidence and volume of the recurrent gross tumor volume outside the gross tumor volume and clinical target volume, and positional differences of the recurrent gross tumor volume centroid from the gross tumor volume and clinical target volume. **Results:** The gross tumor volume was smaller for wild type isocitrate dehydrogenase patients compared to the mutant isocitrate dehydrogenase cohort (mean ± SD: 46.5 ± 26.0 cm^3 vs 72.2 ± 45.4 cm^3, $P = .026$). The recurrent gross tumor volume was 10.7 ± 26.9 cm^3 and 46.9 ± 55.0 cm^3 smaller than the gross tumor volume for the same groups ($P = .018$). The recurrent gross tumor volume extended outside the gross tumor volume in 22 (100%) and 15 (68%) ($P = .009$) of wild type isocitrate dehydrogenase and mutant isocitrate dehydrogenase patients, respectively; however, the volume of recurrent gross tumor volume outside the gross tumor volume was not significantly different (12.4 ± 16.1 cm^3 vs 8.4 ± 14.2 cm^3, $P = .443$). The recurrent gross tumor volume centroid was within 5.7 mm of the closest gross tumor volume edge for 21 (95%) and 22 (100%) of wild type isocitrate dehydrogenase and mutant isocitrate dehydrogenase patients, respectively. **Conclusion:** The recurrent gross tumor volume extended beyond the gross tumor volume less often in mutant isocitrate dehydrogenase patients possibly implying a differential response to

[1] Department of Radiation Oncology, Sunnybrook Odette Cancer Centre, Toronto, Ontario, Canada
[2] Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada
[3] Department of Medical Imaging, University of Toronto, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[4] Department of Biostatistics, University of Toronto, University Health Network, Toronto, Ontario, Canada
[5] Division of Neurology, University of Toronto, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[6] Department of Laboratory Medicine & Pathobiology, University of Toronto, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[7] Department of Pathology, University of Toronto, St. Michael's Hospital, Toronto, Ontario, Canada
[8] Division of Neurosurgery, Department of Surgery, University of Toronto, University Health Network, Toronto, Ontario, Canada
[9] Department of Radiation Oncology, University Health Network, Toronto, Ontario, Canada
[10] Division of Neurosurgery, Department of Surgery, University of Toronto, St. Michael's Hospital, Toronto, Ontario, Canada
[11] Department of Medical Imaging, University of Toronto, University Health Network, Toronto, Ontario, Canada

Corresponding Author:
Pejman Jabehdar Maralani, Department of Medical Imaging, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, Ontario, Canada M4N 3M5.
Email: pejman.maralani@sunnybrook.ca

chemoradiotherapy and suggesting isocitrate dehydrogenase status might be used to personalize radiotherapy. The results require validation in prospective randomized trials.

Introduction

Isocitrate dehydrogenase (IDH) mutation status has established itself as a fundamental molecular biomarker in gliomas[1,2] and serves as a defining feature in the 2016 and 2021 World Health Organization classifications of brain tumors.[3,4] Patients with grade 4 IDH mutant astrocytoma[3] (previously referred to as IDH mutant glioblastoma[4]) have a demonstrated increase in progression-free and overall survival compared to patients with glioblastoma[3] (previously referred to as IDH wild-type glioblastoma[4]).[5–10] The current standard of care for both *de novo* glioblastoma and grade 4 IDH-mutant astrocytoma (these 2 groups are hereafter referred to as wtIDH and mutIDH, respectively) is maximal safe surgical resection, followed by radiotherapy to 54 to 60 Gy in 30 fractions with concurrent followed by adjuvant temozolomide chemotherapy.[11,12]

Within this treatment strategy, patterns of failure with respect to the high-dose radiation fields have been well characterized.[13–16] Such results have established the current radiation planning approach of targeting the surgical cavity and any residual enhancing tumor on T1-weighted MRI plus a 1.5 to 3 cm margin to include the region at the highest risk of recurrence.[11,17–19] Despite differences in the underlying biology,[20] the same radiotherapy guidelines are used for both wtIDH and mutIDH partially due to the lack of data on the relationship between IDH status and failure patterns.

The goal of this retrospective study was to quantitatively evaluate the pattern of recurrence between the wtIDH and mutIDH groups by assessing the volume of the recurrent tumor relative to the gross tumor and clinical target volumes from radiation planning. We also assessed the displacement of the centroid of the recurrent tumor relative to the radiotherapy planning volumes. Our hypothesis was that, given their underlying biologic differences, tumor recurrence pattern differs between the wtIDH and mutIDH groups following standard primary chemoradiotherapy.

Methods and Materials

Patient Characteristics, Treatment, and Imaging

This retrospective matched cohort study was approved by the Sunnybrook Health Sciences Centre Research Ethics Board with a waiver of the informed consent (under the auspices of project identification number 2724, "Magnetic Resonance Imaging as a Tool to Adapt Concurrent Chemoradiation Treatment for Malignant Gliomas"). The design and reporting of this study conforms to published STROBE guidelines.[21]

Using our local database from 2009 to 2019, 22 consecutive adult (18 years old or older) patients with *de novo* grade 4 IDH-mutant astrocytoma (mutIDH group) who completed primary radiation (54-60 Gy in 30 fractions) with concurrent temozolomide chemotherapy were identified. Patients who received any other chemotherapeutic agents such as bevacizumab were not included. Subsequently, 22 adult patients with *de novo* glioblastoma (wtIDH group) who also completed primary radiation (54-60 Gy in 30 fractions) with concurrent temozolomide chemotherapy were selected. To decrease biases between the groups and create a matched cohort, patients from the wtIDH group were selected such that the 2 groups were explicitly matched in the categories of sex, extent of surgical resection (gross total, subtotal, or biopsy), and involvement of corpus callosum. Matching by age was not feasible due to the generally younger age of mutIDH patients[22] and the resulting lack of age-matched wtIDH controls in our database. In addition, clinical characteristics such as performance status, individual radiotherapy details, and time from surgery to the radiotherapy planning were not available. Al patient details were de-identified for presentation in this manuscript. Patient characteristics are summarized in Table 1.

The gross tumor volume (GTV) and clinical target volume (CTV) contours from radiation planning along with the gadolinium-enhanced T1-weighted sequence from the MRI on which the recurrence was declared were downloaded to an offline workstation. The CTV and GTV were contoured in agreement with published guidelines,[23,24] and the declaration of recurrence was made in accordance with the Response Assessment in Neuro-Oncology (RANO) criteria.[25] To ensure that no cases of pseudoprogression were included in the dataset, the follow-up imaging performed after the declaration of recurrence by RANO criteria was also reviewed for every patient. The recurrent MRI was coregistered and resampled to the space of the planning CT or MRI using SPM12. The recurrent GTV (rGTV) was then manually contoured on the recurrent MRI as the T1 enhancing tumor by a neuroradiologist (PJM)

Table 1. Patient Characteristics.

	wtIDH (n = 22)	mutIDH (n = 22)	*P* value
Median age in years (range)	54.5 (39-68)	41.5 (24-82)	<.001
Sex			
Male	15 (68%)	16 (73%)	1.00
Female	7 (32%)	6 (27%)	
Resection type			
Gross total	4 (18%)	4 (18%)	1.00
Subtotal	17 (77%)	17 (77%)	
Biopsy	1 (5%)	1 (5%)	
Corpus callosum involvement			
Yes	12 (55%)	12 (55%)	1.00
No	10 (45%)	10 (45%)	

using ITK-SNAP[26] and was exported as binary mask. The rGTV contours were confirmed by a radiation oncologist specializing in the central nervous system (AS). The rGTV was said to extend outside the GTV if the volume of the rGTV outside the GTV was greater than 0.3 cm^3. This threshold was used to account for small spurious regions where the rGTV and GTV share a border (eg, along with the dura). All 3 contours analyzed in this work, along with the planned dose distribution, are illustrated for a single patient in Figure 1A.

Survival Analysis

Progression-free and overall survival were assessed from the date of the planning CT or MRI with the resulting progression-free survival (PFS) and overall survival (OS) rates calculated using the Kaplan-Meier product-limit method. The log-rank test was used to assess the statistical significance of IDH mutation status on PFS and OS.

Volumetric and Positional Analyses

The GTV, CTV, and rGTV contours were exported in Analyze 7.5 image format. Positional and volumetric analyses were performed after importing these masks into MATLAB (v. 9.7.0). The following metrics, illustrated in Figure 1B, were quantified for each patient using an in-house script:

1. Volume of the GTV, CTV, and rGTV.
2. Volume of the rGTV outside of the GTV (the region labeled "A" in Figure 1B) and CTV (region "B" in Figure 1B). This volume is presented as both an absolute volume and relative to the full rGTV volume.
3. Distance of the rGTV centroid from each of the GTV and CTV centroids, where the centroid is the geometric center of the 3-dimensional contour. These are denoted by the arrows connecting the rGTV centroid (orange) to the GTV (red) and CTV (blue) centroids, respectively, in Figure 1B.
4. Distance of the rGTV centroid to the closest edge of the GTV, illustrated by the arrow connecting the rGTV centroid (orange) to the GTV border (red) in Figure 1B. If

the rGTV centroid is within the GTV, this distance is zero.

Statistical Analysis

Recurrence categorization outside the GTV and/or CTV was statistically assessed with Fisher exact test. Student paired *t* test was used to compare the contour volumes, volumetric data, and positional data between the wtIDH and mutIDH groups. In all analyses, *P* values were 2-sided and *P* < .05 was considered statistically significant. All statistical analyses were performed using the SAS software suite (v 9.4).

Results

Patient characteristics are summarized in Table 1 demonstrating matching in terms of sex, extent of resection, and involvement of corpus callosum between the wtIDH and mutIDH groups.

Survival Analysis

Kaplan-Meier estimates of PFS and OS are outlined in Figure 2. Median PFS was 9.0 (95% confidence interval (CI): 4.9-12.9) and 15.1 months (95% CI: 6.0-28.6) for the wtIDH and mutIDH groups (*P* = .016), respectively. Similarly, median OS was 14.2 (95% CI: 10.1-19.2) and 42.2 months (95% CI, 23.8-50.2) for the same respective groups (*P* < .001).

Volumetric Analysis of Recurrence

Volumes of the GTV, CTV, and rGTV are summarized in Figure 3A to C. The mean GTV was smaller in the wtIDH versus the mutIDH group (mean ± SD: 46.5 ± 26.0 cm^3 vs 72.2 ± 45.4 cm^3, *P* = .026). The CTV and rGTV volumes were not statistically different between wtIDH and mutIDH groups (*P* = .545 and *P* = .308, respectively). The difference between GTV and rGTV volumes in individual patients is illustrated in Figure 3D to E; the rGTV was on average 10.7 ± 26.9 cm^3 smaller than the original GTV for the wtIDH group, and 46.9 ± 55.0 cm^3 smaller for mutIDH group (*P* = .018).

The volume of the rGTV outside the planning contours is summarized in Figure 4A. For the wtIDH and mutIDH groups, the mean volume of the rGTV outside the GTV was 12.4 ± 16.1 cm^3 (26.8 ± 22.4% of the rGTV) and 8.4 ± 14.2 cm^3 (28.4 ± 29.5%) (*P* = .443). The rGTV extended outside the GTV in 22 (100%) and 15 (68%) of wtIDH and mutIDH groups, respectively (*P* = .009). In other words, the rGTV was completely contained within the GTV for 0 (0%) and 7 (32%) of wtIDH and mutIDH patients. The rGTV extended outside the CTV in 6 (27%) and 7 (32%) patients from the same groups (*P* = 1.00). Finally, the mean rGTV volume outside the CTV was 2.7 ± 11.0 cm^3 (5.2 ± 20.5% of the rGTV) and 0.6 ± 1.2 cm^3 (4.5 ± 7.2%) (*P* = .387) for wtIDH and mutIDH patients, respectively.

Positional Analysis of Recurrence

The displacement of the rGTV centroid from the GTV and CTV centroids is outlined in Figure 4B. The displacement of rGTV
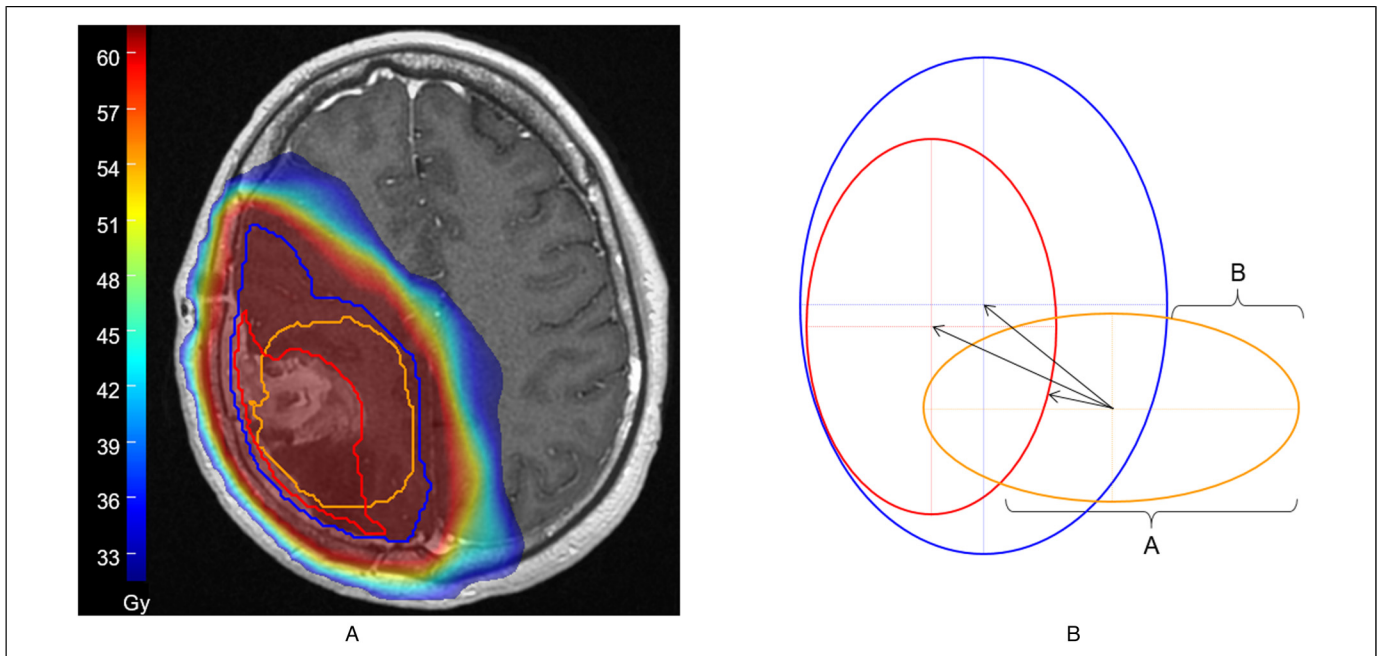
Figure 1. (A) Axial slice of patient with grade 4 IDH-mutant astrocytoma illustrating analysis volumes. Overlaid on the T1 with gadolinium contrast planning MRI are the gross tumor volume (GTV; red contour), clinical target volume (CTV; blue contour), and recurrent gross tumor volume (rGTV; orange contour). Also shown for illustrative purposes is the 60 Gy prescribed radiotherapy dose plan (colorwash). (B) Schematic 2-dimensional illustration of metrics quantified in this study. The GTV, CTV, and rGTV are denoted by the red, blue, and orange contours, respectively; the centroid of each contour is indicated. The 3 arrows demonstrate the distance of the rGTV centroid from the GTV centroid, CTV centroid, and closest edge of the GTV. The regions of the rGTV outside the GTV (labeled "A") and CTV ("B") are also indicated.
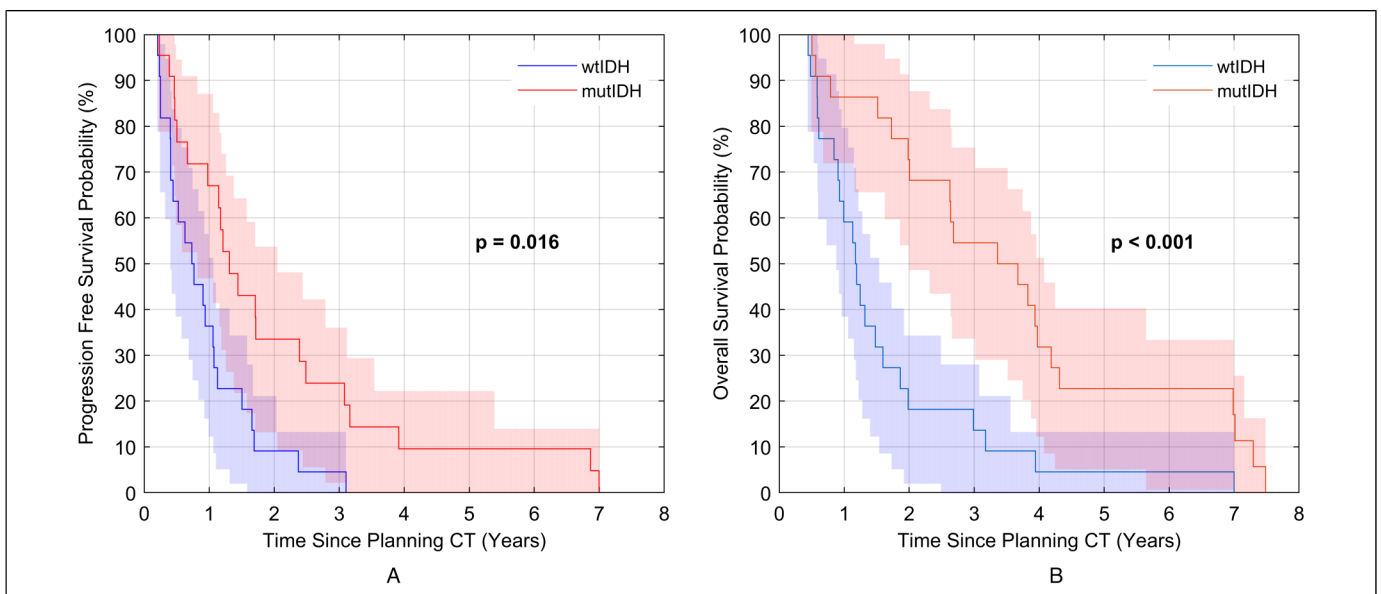

Figure 2. Kaplan-Meier estimates of progression-free survival (A) and overall survival (B) for the wtIDH (blue) and mutIDH (red) groups with 95% confidence interval delineated by the shaded region.

centroid for the wtIDH and mutIDH groups was 9.5 ± 9.8 mm and 12.2 ± 8.4 mm ($P = .446$) from the GTV, and 14.6 ± 8.8 mm and 15.9 ± 7.9 mm ($P = .718$) from the CTV, respectively.

The rGTV centroid was on average 1.4 ± 6.7 mm and 0.7 ± 1.5 mm ($P = .615$) from the closest edge of the GTV for the wtIDH and mutIDH groups, respectively. As summarized in Figure 4C, this distance was 0 mm (ie, the rGTV centroid
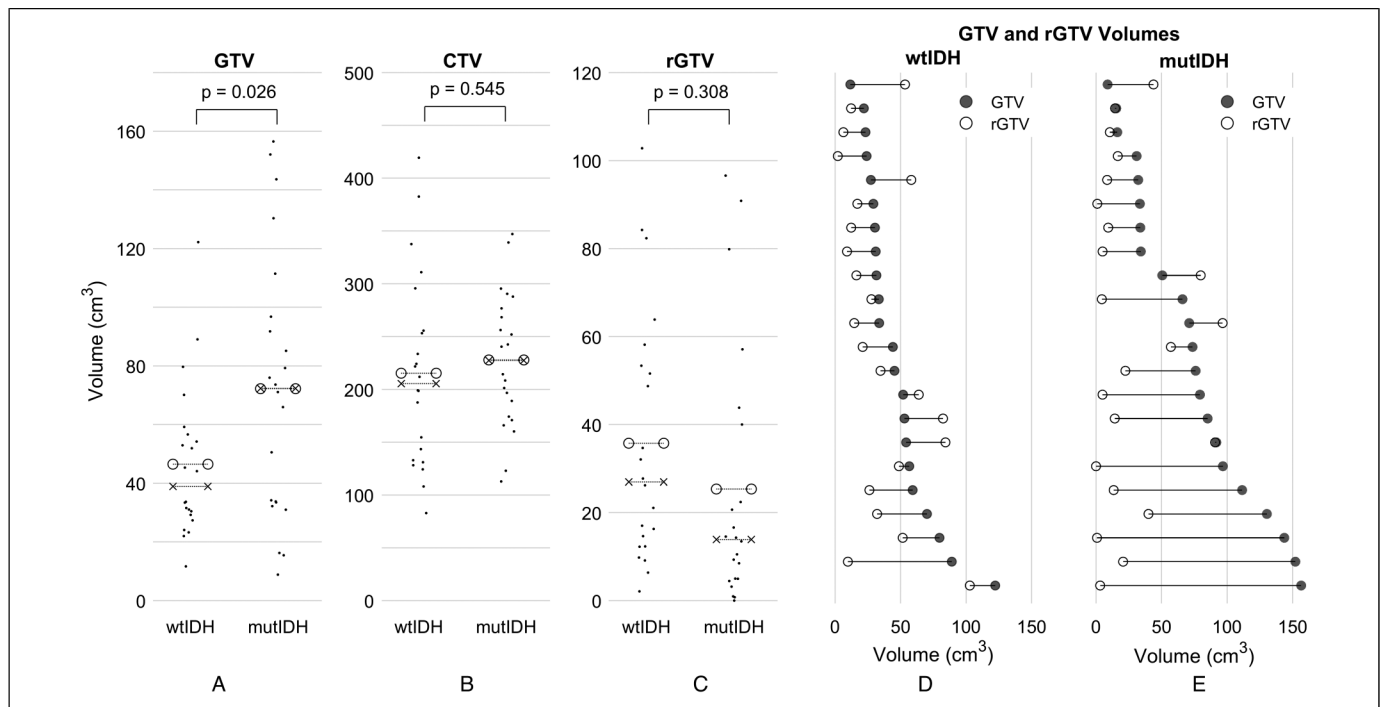
Figure 3. (A-C) Gross tumor (GTV), clinical targeting (CTV), and recurrent gross tumor (rGTV) volumes for the wtIDH and mutIDH groups. In all 3 panels, the scatter points denote individual patient volumes; points are randomly offset along with the abscissa to improve visualization. The horizontal dashed lines with "o" and "x" endpoints denote the mean and median, respectively, of the group. The GTV and rGTV volume data are delineated in a different forms in panels (D-E) to highlight individual patient differences between these 2 volumes. In both panels, the $n = 22$ patients in each of the wtIDH (D) and mutIDH (E) groups are shown along with the ordinal with the filled and open circles denoting the GTV and rGTV volumes, respectively. Patients are sorted by their GTV volume. The rGTV was 10.7 ± 26.9 cm^3 (mean $\pm$ SD) and 46.9 ± 55.0 cm^3 smaller than the GTV for wtIDH and mutIDH groups, respectively ($P = .018$).

was within the GTV) for 21 (95%) of the wtIDH patients; the sole patient with a nonzero distance had an rGTV centroid 31.4 mm from the closest portion of the GTV. This distance was also zero in 18 (82%) mutIDH patients and ranged from 2.0 to 5.7 mm in the 4 (18%) remaining mutIDH patients.

Discussion

Radiotherapy in glioblastoma and grade 4 IDH-mutant astrocytoma relies critically on the geometric expansion from the GTV to the CTV. An opportunity to refine these relatively large CTV margins relies on identifying patient subgroups for which this expansion could potentially be decreased. In this retrospective matched cohort, we demonstrated a differential recurrence pattern between these 2 groups with the rGTV extending outside the planning GTV less often in the mutIDH group as compared to wtIDH group. The volume of the recurrence extending beyond the GTV and CTV, however, was not statistically different between the 2 groups nor was the displacement of the recurrent tumor centroid relative to the planning GTV or CTV.

IDH mutation is an increasingly important molecular biomarker of in terms of tumor behavior and therapy response in patients with high-grade gliomas. In our patient cohort, the GTV was larger in mutIDH patients. Unlike the wtIDH

group, the tumor in mutIDH patients is thought to predominantly progress from a clinically silent lower grade glioma[27] over a long period of time and, therefore, more time to slowly grow to larger volumes prior to symptom development. We also observed a larger decrease between the volume of the planning GTV and the recurrent tumor in the mutIDH group, which may suggest a better response to chemoradiation in mutIDH patients. In turn, this may imply that interfraction variability is greater in the mutIDH group, and patients with grade 4 IDH-mutant astrocytoma might therefore benefit from more frequent interfraction treatment assessment and adaptation.[28–32] An important caveat is that subtle imbalances in how CTVs were created between the 2 groups cannot be definitively ruled out. Final GTV/CTV contours were available to us but the underlying geometric expansions and amount of FLAIR hyperintensity subsequently included in CTV for each individual case were not available for all cases. That said, a smaller recurrence volume in the mutIDH group with a centroid of the rGTV within 5.7 mm of the initial GTV for all patients in the mutIDH group suggests further clinical trials with adaptive limited margin CTVs are warranted. This may be of particular interest for mutIDH patients, as they are generally younger with prolonged survival and may preferentially benefit from improved neurocognitive outcomes with smaller RT margins.[33] In addition, the temporal and spatial heterogeneity
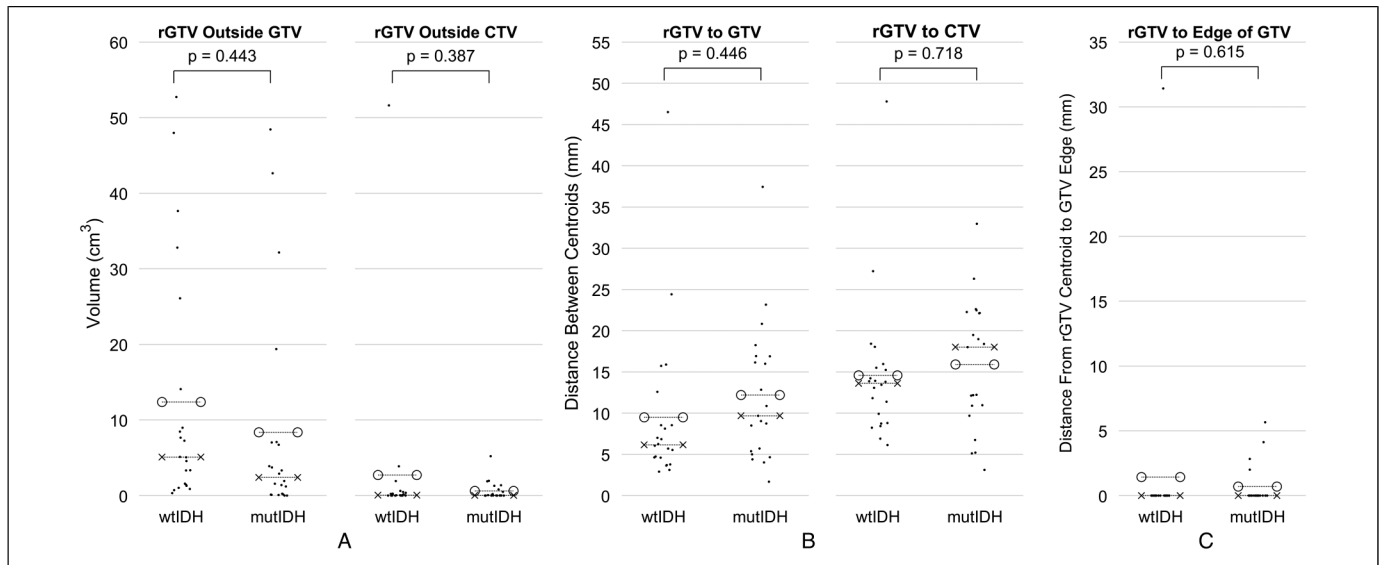
Figure 4. Volumetric (A) and positional (B-C) analyses of recurrent gross tumor volume (rGTV) for the wtIDH and mutIDH groups. (A) rGTV volume outside the gross tumor volume (GTV; left panel) and clinical target volume (CTV; right panel). (B) Distance of the rGTV centroid from the GTV (left panel) and CTV (right panel) centroids. (C) Distance of the rGTV centroid from the closest edge of the GTV. In all panel groups in the scatter points denote individual patient measurements; these points are randomly offset along with the abscissa to improve visualization. The horizontal dashed lines with "o" and "x" endpoints denote the mean and median, respectively, of the group.

of IDH status in high-grade gliomas is currently an open question. Although such heterogeneity has been identified for other glioblastoma molecular markers,[34–36] it is unclear if similar heterogeneity exists for IDH status. Should such heterogeneity in IDH status be discovered, it may be a contributing factor to the interfraction variability observed in the present study.

The macroscopic changes in the tumor and target volumes identified in this work occur in the context of both radiotherapy and chemotherapy treatments. Although the addition of temozolomide chemotherapy to radiotherapy is the current standard of care due to its demonstrated survival benefit,[11] approximately 50% of patients demonstrate a poor response to this agent.[37] The exact mechanism for this resistance is thought to involve MGMT expression[38] and likely involves other aspects of the glioblastoma microenvironment.[39] As the targeted agent bevacizumab has not demonstrated a survival benefit in the context of glioblastoma,[40] recent efforts have focused on targeting different pathways such as IDH.[41] With the present study demonstrating a difference between mutIDH and wtIDH patients in terms of radiotherapeutic response, we look forward to revisiting and expanding these results in the context of IDH targeted chemotherapy should this agent enter widespread clinical use.

We have demonstrated in this small cohort that recurrent tumor extends less often beyond the planning GTV in the mutIDH group. Knowing that the main reason for GTV-to-CTV expansion is to account for infiltrative microscopic disease, our results suggest that the current guidelines for the expansion of GTV to CTV for radiation planning in glioblastoma patients[11,12] could be revisited for the mutIDH group in the setting of a clinical trial. However, caution is required as

the recurrent tumor still extended outside the planning GTV in 68% of mutIDH patients, and a traditional CTV was treated in all patients in this study. There have been preliminary studies supporting limited CTV expansions (down to 0.5 cm) and this is an active area of research.[42,43] In the future, molecular analysis[44,45] may contribute to identify a subgroup where margin reduction is warranted.

This study had several limitations. The retrospective nature of the study is a source of bias and the sample size in our study was small. With the recent 2021 WHO classification of central nervous system tumors and consistently improving molecular characterization, we look forward to having these results revisited in future studies with larger patient numbers. Also, clinical characteristics including performance status, time from surgery to the planning CT, and per-patient radiotherapy details were not available. We cannot rule out, for example, that the prescribed radiotherapy dose was unbalanced between the mutIDH and wtIDH groups. Finally, data regarding MGMT methylation status and other molecular biomarkers of high-grade gliomas[46] were not available for this study. As a result of these limitations, this study should be viewed as exploratory and hypothesis generating. Larger prospective studies and ultimately randomized trials evaluating margin reduction are required before the current radiotherapy margin paradigm can be changed.

Conclusions

Although failure patterns in high-grade gliomas have been well-characterized across unstratified patient populations, it is unknown whether these patterns are influenced by isocitrate dehydrogenase mutation (IDH) status. We quantitatively

evaluated recurrence tumor volumes in a matched cohort of adult patients with de novo IDH-wild type glioblastoma (wtIDH) and grade 4 IDH-mutant astrocytoma (mutIDH) and, to the best of our knowledge, this study is the first to demonstrate 3 key results. First, we demonstrated that the tumor recurrence may less often extended beyond the radiotherapy gross tumor volume (GTV) in mutIDH patients. Second, we quantified that the GTV in mutIDH patients can be larger than wtIDH patients at the time of radiation planning. Third, in patients with mutIDH, the tumor size may decrease to a much larger extent compared to wtIDH patients. Finally, we note that the centroid of the recurrence was within 6 mm of the GTV border for 43 of 44 (98%) patients in the combined cohort, potentially supporting the use of small margin radiotherapy strategies.

Authors' Note

Acknowledgments

Declaration of Conflicting Interests

Funding

ORCID iDs

James Stewart ![ORCID] https://orcid.org/0000-0002-3134-3415
Chia-Lin Tseng ![ORCID] https://orcid.org/0000-0002-3700-0886

References

1. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol*. 2010;120(6):707-718. doi:10.1007/s00401-010-0781-z

2. Songtao Q, Lei Y, Si G, et al. IDH Mutations predict longer survival and response to temozolomide in secondary glioblastoma. *Cancer Sci*. 2012;103(2):269-273. doi:10.1111/j.1349-7006.2011.02134.x

3. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23(8):1231-1251. doi:10.1093/neuonc/noab106

4. 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

5. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. *N Engl J Med*. 2015;372(26):2499-2508. doi:10.1056/nejmoa1407279

6. Verhaak RGW, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98-110. doi:10.1016/J.CCR.2009.12.020

7. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009;360(8):765-773. doi:10.1056/nejmoa0808710

8. Combs SE, Rieken S, Wick W, et al. Prognostic significance of IDH-1 and MGMT in patients with glioblastoma: one step forward, and one step back? *Radiat Oncol*. 2011;6(1). doi:10.1186/1748-717x-6-115

9. Gravendeel LAM, Kouwenhoven MCM, Gevaert O, et al. Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. *Cancer Res*. 2009;69(23):9065-9072. doi:10.1158/0008-5472.CAN-09-2307

10. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*. 2006;9(3):157-173. doi:10.1016/j.ccr.2006.02.019

11. Stupp R, Mason WP, Van Den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987-996.

12. Becker KP, Yu J. Status quo-standard-of-care medical and radiation therapy for glioblastoma. *Cancer J*. 2012;18(1):12-19.

13. Burger PC, Dubois PJ, Schold SC, et al. Computerized tomographic and pathologic studies of the untreated, quiescent, and recurrent glioblastoma multiforme. *J Neurosurg*. 1983;58(2):159-169. doi:10.3171/jns.1983.58.2.0159

14. Gaspar LE, Fisher BJ, Macdonald DR, et al. Supratentorial malignant glioma: patterns of recurrence and implications for external beam local treatment. *Int J Radiat Oncol Biol Phys*. 1992;24(1):55-57. doi:10.1016/0360-3016(92)91021-E

15. Halperin EC, Bentel G, Heinz ER, Burger PC. Radiation therapy treatment planning in supratentorial glioblastoma multiforme: an analysis based on post mortem topographic anatomy with ct correlations. *Int J Radiat Oncol Biol Phys*. 1989;17(6):1347-1350. doi:10.1016/0360-3016(89)90548-8

16. Chan JL, Lee SW, Fraass BA, et al. Three-dimensional conformal radiotherapy. *J Clin Oncol*. 2002;20(6):1635-1642. doi:10.1016/s0169-5002(97)90162-8

17. Chang EL, Akyurek S, Avalos T, et al. Evaluation of peritumoral edema in the delineation of radiotherapy clinical target volumes

for glioblastoma. *Int J Radiat Oncol*. 2007;68(1):144-150. doi:10.1016/J.IJROBP.2006.12.009

18. Mason WP, Del Maestro R, Eisenstat D, et al. Canadian recommendations for the treatment of glioblastoma multiforme. *Curr Oncol*. 2007;14(3):110-117.

19. Panet-Raymond V, Souhami L, Roberge D, et al. Accelerated hypofractionated intensity-modulated radiotherapy with concurrent and adjuvant temozolomide for patients with glioblastoma multiforme: a safety and efficacy analysis. *Int J Radiat Oncol Biol Phys*. 2009;73(2):473-478. doi:10.1016/j.ijrobp.2008.04.030

20. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462(7274):739-744. doi:10.1038/nature08617

21. Von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies*. *Bull World Health Organ*. 2007;85(11):867-872. doi:10.2471/BLT.07.045120

22. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. *Clin Cancer Res*. 2013;19(4):764-772.

23. Niyazi M, Brada M, Chalmers AJ, et al. ESTRO-ACROP guideline "target delineation of glioblastomas". *Radiother Oncol*. 2016;118(1):35-42. doi:10.1016/j.radonc.2015.12.003

24. Tseng CL, Stewart J, Whitfield G, et al. Glioma consensus contouring recommendations from a MR-Linac International Consortium Research Group and evaluation of a CT-MRI and MRI-only workflow. *J Neurooncol*. 2020;149(2):305-314. doi:10.1007/s11060-020-03605-6

25. 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.

26. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006;31(3):1116-1128. doi:10.1016/j.neuroimage.2006.01.015

27. Ichimura K, Pearson DM, Kocialkowski S, et al. IDH1 Mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neuro Oncol*. 2009;11(4):341-347. doi:10.1215/15228517-2009-025

28. Lee S, Stewart J, Lee Y, et al. Improved dosimetric accuracy with semi-automatic contour propagation of organs-at-risk in glioblastoma patients undergoing chemoradiation. *J Appl Clin Med Phys*. 2019;20(12):45-53. doi:10.1002/acm2.12758

29. Manon R, Hui S, Chinnaiyan P, et al. The impact of mid-treatment MRI on defining boost volumes in the radiation treatment of glioblastoma multiforme. *Technol Cancer Res Treat*. 2004;3(3):303-307. doi:10.1177/153303460400300308

30. Tsien C, Gomez-Hassan D, Ten Haken RK, et al. Evaluating changes in tumor volume using magnetic resonance imaging during the course of radiotherapy treatment of high-grade gliomas: implications for conformal dose-escalation studies. *Int J Radiat Oncol Biol Phys*. 2005;62(2):328-332. doi:10.1016/j.ijrobp.2004.10.010

31. Kim TG, Lim DH. Interfractional variation of radiation target and adaptive radiotherapy for totally resected glioblastoma. *J Korean Med Sci*. 2013;28(8):1233-1237. doi:10.3346/jkms.2013.28.8.1233.

32. Stewart J, Sahgal A, Lee Y, et al. Quantitating interfraction target dynamics during concurrent chemoradiation for glioblastoma: a prospective serial imaging study. *Int J Radiat Oncol Biol Phys*. 2021;109(3):736-746. doi:10.1016/j.ijrobp.2020.10.002

33. Kumar N, Kumar R, Sharma SC, et al. Impact of volume of irradiation on survival and quality of life in glioblastoma: a prospective, phase 2, randomized comparison of RTOG and MDACC protocols. *Neuro-Oncol Pract*. 2020;7(1):86-93. doi:10.1093/nop/npz024

34. Montemurro N, Fanelli GN, Scatena C, et al. Surgical outcome and molecular pattern characterization of recurrent glioblastoma multiforme: a single-center retrospective series. *Clin Neurol Neurosurg*. 2021;207:106735. doi:10.1016/j.clineuro.2021.106735

35. Feldheim J, Kessler AF, Monoranu CM, et al. Changes of O^6-methylguanine DNA methyltransferase (MGMT) promoter methylation in glioblastoma relapse—a meta-analysis type literature review. *Cancers (Basel)*. 2019;11(12):1837. doi:10.3390/cancers11121837

36. Parker NR, Khong P, Parkinson JF, et al. Molecular heterogeneity in glioblastoma: potential clinical implications. *Front Oncol*. 2015;5. doi:10.3389/fonc.2015.00055

37. Lee SY. Temozolomide resistance in glioblastoma multiforme. *Genes Dis*. 2016;3(3):198-210.

38. Chen X, Zhang M, Gan H, et al. A novel enhancer regulates MGMT expression and promotes temozolomide resistance in glioblastoma. *Nat Commun*. 2018;9(1):1-14. doi:10.1038/s41467-018-05373-4

39. Fanelli GN, Grassini D, Ortenzi V, et al. Decipher the glioblastoma microenvironment: the first milestone for new groundbreaking therapeutic strategies. *Genes (Basel)*. 2021;12(3):445. doi:10.3390/genes12030445

40. Kim MM, Umemura Y, Leung D. Bevacizumab and glioblastoma past, present, and future directions. *Cancer J (United States)*. 2018;24(4):180-186.

41. Golub D, Iyengar N, Dogra S, et al. Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics. *Front Oncol*. 2019;9:417. doi:10.3389/FONC.2019.00417

42. Paulsson AK, McMullen KP, Peiffer AM, et al. Limited margins using modern radiotherapy techniques does not increase marginal failure rate of glioblastoma. *Am J Clin Oncol Cancer Clin Trials*. 2014;37(2):177-181. doi:10.1097/COC.0b013e318271ae03

43. McDonald MW, Shu HKG, Curran WJ, Crocker IR. Pattern of failure after limited margin radiotherapy and temozolomide for glioblastoma. *Int J Radiat Oncol Biol Phys*. 2011;79(1):130-136. doi:10.1016/j.ijrobp.2009.10.048

44. Wen PY, Weller M, Lee EQ, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. *Neuro Oncol*. 2020;22(8):1073-1113. doi:10.1093/NEUONC/NOAA106

45. Lim-Fat MJ, Youssef GC, Touat M, et al. Clinical utility of targeted next generation sequencing assay in IDH-wildtype glioblastoma for therapy decision-making. *Neuro Oncol*. 2021. doi:10.1093/NEUONC/NOAB282

46. Zhang P, Xia Q, Liu L, et al. Current opinion on molecular characterization for GBM classification in guiding clinical diagnosis, prognosis, and therapy. *Front Mol Biosci*. 2020;7(562798):1–13.