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Biologically Guided Gamma Knife Dose Painting for Recurrent High-Grade Gliomas: A Retrospective Study Using Functional MRI Techniques

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Data Collection B
Statistical Analysis C
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Background: This study examines the efficacy of biologically guided dose painting in Gamma Knife stereotactic radiosurgery (GKSRS) to improve radiographic response in patients with recurrent high-grade gliomas by increasing radiation dosage in functionally active tumor subregions identified through magnetic resonance spectroscopy (MRS) and T1-weighted perfusion magnetic resonance imaging (T1-PMRI).

Material/Methods: In this single-arm cohort of patients (n=23) with recurrent high-grade glioma, all patients previously treated with surgery, chemotherapy, and fractionated radiotherapy underwent GKSRS. Functional imaging (MRS and T1-weighted PMRI) delineated metabolically active ("aggressive") and less active ("passive") tumor regions. A modified radiosurgery plan prescribed 18 Gy to aggressive and 15 Gy to passive zones. For intra-patient comparison, a uniform-dose plan (plan 1, 16 Gy) was generated but not delivered. All statistical analyses were performed in Python 3.11 (SciPy-v1.11, statsmodels-v0.14, lifelines-v0.28) executed in Visual Studio Code 1.88 (Microsoft).

Results: Across 23 patients, plan 2 vs plan 1 showed no significant change in whole-brain mean dose (*P*=0.716), integral dose (*P*=0.792), or V12 (*P*=0.583). Among 11 patients with follow-up imaging, K-trans decreased significantly (median, -18%; *P*=0.028; Wilcoxon) with a trend for initial area under the gadolinium concentration-time curve (IAUC; median, -22%; *P*=0.031 for table; overall *P*=0.08 for initial under curve analysis). Higher baseline K-trans correlated with greater K-trans reduction (r=-0.84, *P*=0.0012).

Conclusions: Using advanced MRI techniques (accounting for K-trans and IAUC on T1-PMRI, and MRS) to determine aggressive zones in salvage treatment for recurrent high-grade gliomas, and then focusing radiotherapy on these zones, can increase Gamma Knife efficiency without increasing the morbidity rate.

Keywords: **Perfusion Magnetic Resonance Imaging • Glioma • Radiosurgery**

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Introduction

High-grade gliomas, especially recurrent glioblastoma multiforme, continue to be invariably lethal despite comprehensive multimodal treatment [1]. A significant obstacle to advancement is the restricted capacity of traditional anatomical magnetic resonance imaging (MRI) to effectively differentiate between tumor recurrence and treatment-related alterations, as well as to identify physiologically aggressive sub-volumes suitable for focused dose escalation. Glioblastoma multiforme is a highly aggressive primary brain malignancy associated with poor prognosis despite multimodal treatment strategies [2-4]. The vast majority of gliomas are spread by local invasion, and recurrences usually arise from the tumor site and the adjacent zones of the tumor site [5]. Therefore, local tumor control is critical to extend the life span [6]. Advanced functional MRI biomarkers – specifically choline-to-N-acetyl-aspartate (Cho/NAA) ratios obtained from MR spectroscopy (MRS) and relative cerebral blood volume (rCBV) derived from dynamic susceptibility-contrast perfusion MRI (DSC-PWI) exhibit correlations with cellular proliferation and angiogenesis, respectively, thereby serving as significant targets for biologically guided radiosurgery [7]. Nevertheless, these strategies have not yet been consistently included into Gamma Knife (GK) dose planning. To avoid the complications that can develop after second re-radiation and to increase tumor control, stereotactic radiosurgery (SRS) can be considered as a salvage therapy for recurrent high-grade gliomas [8,9]. The efficacy of SRS using Gamma Knife radiosurgery (GKR) on gliomas is not clear. Several studies have shown that GKR has some effect on patient survival [8-10]. Because of the infiltrative nature of the tumor, the efficacy of post-GKR therapy is difficult to assess on MRI [11]. Increased contrast enhancement following GKR can be confused with contrast enhancement attributable to viable tumor or post-treatment enhancement (necrosis and pseudo-progression) [12]. In some cases, contrast enhancement does not provide sufficient information about the intensity of tumoral cells. In such cases, tumor markers, such as increased perfusion (rCBV), elevated microvascular permeability (K-trans), and increased Cho/NAA and Cho/Cre ratios, were used through advanced MRI techniques. These imaging biomarkers reflect tumor cell density and metabolic activity [13]. Recurrent high-grade glial tumors usually have a heterogeneous structure, and tumor density varies in different zones of the lesion. In this study, we aimed to assess if GK dose painting, guided by combined MRS and DSC-PWI biomarkers, enhances local radiographic response, compared with a conventional uniform-dose plan, in patients with recurrent high-grade gliomas.

This study pioneers the integration of 2 complementary functional MRI biomarkers – voxel-level metabolic maps from 3-dimensional proton MRS (Cho/NAA and Cho/Cr ratios) and perfusion metrics from T1-weighted dynamic-contrast perfusion MRI (PMRI) – K-trans and initial area under the gadolinium concentration-time curve (IAUC) – to direct sub-volume dose escalation during GKR for recurrent World Health Organization (WHO) grade III-IV gliomas. Earlier dose-painting investigations depended solely on anatomical contrast enhancement [14,15] or a single biologic imaging modality [16], overlooking the heterogeneous vascular and metabolic microenvironment that underlies radio-resistance. Concurrent exploitation of spectroscopy-defined cellular turnover and perfusion-defined hyper-vascularity yields a biologically grounded target that more precisely delineates aggressive tumor habitats. This dual-modality strategy bridges the divide between advanced imaging science and practical stereotactic radiosurgical planning, with the potential to improve local control while preserving uninvolved brain tissue.

Clinical Rationale for the Study

In this study, the area exhibiting elevated perfusion parameters (eg, K-trans ≥ threshold) and high spectral values on PMRI and MRS was identified as the active tumor zone, where glioblastoma multiforme tumor cells were clearly visualized. This area was treated as a separate tumor in the modified GK plan. Thus, the tumor area was divided into active and passive zones, and the GK plan was planned as 2 separate tumors, not as a single tumor (plan 2; **Figure 1**). Instead of a moderate dose to be used as a routine modality in the routine (simulated) plan (plan 1; **Figure 2**), our modified plan aimed to enhance GKR efficacy and reduce the morbidity by giving these tumors the maximum dose (18 Gy) to the active zone and the minimum dose (15 Gy) to other passive zones.

Despite the increasing interest in stereotactic re-irradiation, most studies still employ a standardized margin-based dosage that neglects the varied biology of recurrent high-grade gliomas. Our research innovatively employs concurrent voxel-wise Cho/NAA and Cho/Cr ratios derived from 3-dimensional MRS, with rCBV/rCBF maps from DSC-PWI, to delineate biologically aggressive sub-volumes for GK dosage escalation. By merging these 2 functional MRI modalities, this study connects the advancements in metabolic imaging with practical GK planning [17,18].

Material and Methods

Study Design and Patient Selection

This retrospective study was approved by the institutional ethics committee (approval no. 2/12; date: Jan 27, 2016). Written informed consent was obtained from all participants. Patients included in the study had histologically confirmed WHO grade

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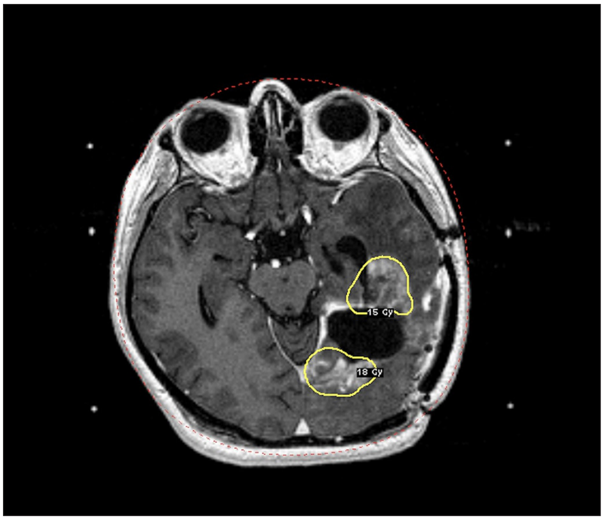

Figure 1. Delivered dose-painting plan (plan 2) with biologically defined sub-volumes. Treatment scheme of plan 2 (dose-painted 15-18 Gy; delivered). Images of representative patient with recurrent glioblastoma show biologically defined sub-volumes: aggressive voxels (18 Gy at 50% isodose) and passive tumor (15 Gy).

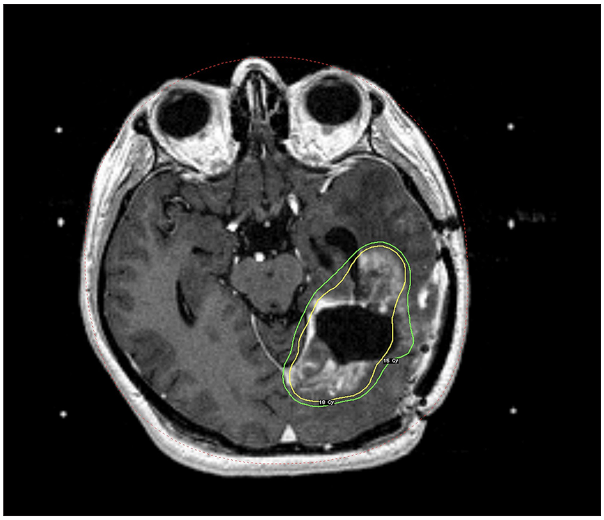

Figure 2. Simulated uniform-dose comparator (plan 1). Treatment scheme of simulated plan 1 (uniform 16 Gy; simulated, not delivered), in which the tumor was considered as a single mass of tumor, and 16 Gy (50% isodose) was simulated to be given. This plan was generated only for intra-patient (paired) dosimetric comparison with plan 2 (mean dose, integral dose, and volume of normal brain receiving ≥12 Gy [V12]).

III or IV gliomas, previously treated with maximal safe resection, 54 to 60 Gy of external beam radiotherapy in 2 Gy fractions, and adjuvant temozolomide chemotherapy in the Gamma Knife Unit of our hospital from December 2015 to December 2018. Patients were selected for GKSRS at recurrence based on radiographic progression, with tumor size less than 6 cm in maximal diameter.

The inclusion criteria were histologically confirmed WHO grade III-IV glioma; radiographic progression according to response assessment in neuro-oncology (RANO) criteria, with ≥25% increase in contrast-enhancing lesion volume or new nodular enhancement; maximal tumor diameter <6 cm; Karnofsky Performance Status ≥70; cumulative prior external-beam radiotherapy equivalent dose in 2 Gy fractions ≤60 Gy; and interval ≥6 months since completion of prior radiotherapy. Exclusion criteria were prior SRS to the index lesion, diffuse leptomeningeal disease, uncontrolled systemic illness, or MRI contraindications.

For each patient, 2 plans were created in Leksell GammaPlan version 5.34: a virtual uniform-dose plan (plan 1, uniform 16 Gy to the 50% isodose; simulated comparator, not delivered) and the administered biologically guided plan (plan 2, dose-painted 15-18 Gy; delivered), using a 3-zone discrete methodology (18 Gy to MRS/DSC-defined "active" voxels, 16.5 Gy to the penumbra, and 15 Gy to other areas). Zone borders were softened with a 2-mm gradient to prevent sharp dosage discontinuities. All clinical irradiations used plan 2. Dosimetric indices (whole-brain mean dose, integral dose, V12) were computed for both plans and compared within each patient.

Treatment Stages

All the patients were treated at our hospital (from the first surgery till receiving GK). All patients were treated surgically, and then they received fractional radiotherapy and adjunctive chemotherapy after histopathological diagnosis of high-grade glioma. Histopathologically, 20 patients received a diagnosis of glioblastoma multiforme, and 3 a diagnosis of anaplastic astrocytoma. A total of 54- to 60-Gy doses were given in conventional fractions of radiotherapy as 2-Gy daily doses. Radiotherapy was followed by temozolomide as adjunctive chemotherapy in all patients. The patients were followed up, and MRIs were performed in the first, third, and sixth months. This study covered tumors that showed progression on MRI and had a maximum diameter of less than 6 cm. These patients were considered to receive SRS.

Imaging Acquisition and Processing

MRI was acquired on a 1.5-Tesla scanner system (Avanto; Siemens Medical Systems, Erlangen, Germany) with the following parameters: 3-dimensional T1-weighted spoiled gradient echo (TR/TE=600/10 ms, flip angle=12°, slice thickness=1 mm); fluid attenuated inversion recovery (TR/TE/TI=9000/120/2500 ms, slice thickness=3 mm); DSC-PWI using gradient-echo EPI (TR/TE=2000/30 ms, 60

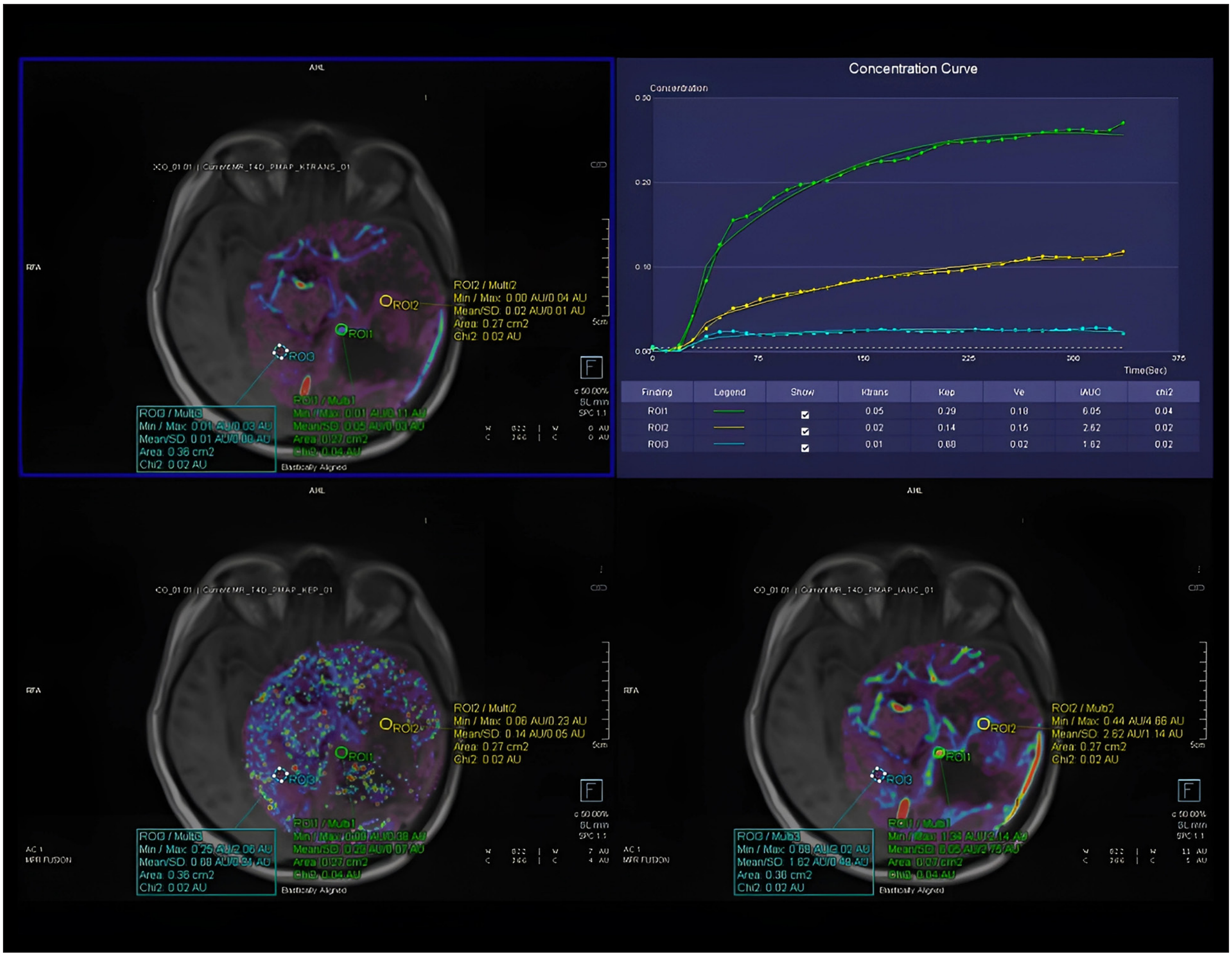

Figure 3. Baseline permeability map identifies the most aggressive sub-region. Pre-stereotactic radiosurgery dynamic contrast-enhanced MRI overlaid on post-contrast T1-weighted images in a recurrent glioblastoma shows region of interest 1 in the upper-medial tumor with volume transfer constant (K-trans)=0.05 min^{-1} (table and concentration-time curves shown). This voxel-wise permeability information was used, together with magnetic resonance spectroscopy and dynamic susceptibility contrast perfusion-weighted imaging, to define escalation sub-volumes for plan 2. IAUC – initial area under the gadolinium concentration-time curve.

dynamics, in-plane resolution 1.8×1.8 mm, slice thickness=4 mm) with a 0.1 mmol/kg gadobutrol bolus; and 3-dimensional multi-voxel MRS using PRESS (TR/TE=2000/135 ms, voxel size=10×10×10 mm). First, all patients underwent pre-GKSRS imaging with conventional non-contrast MRI. After the subsequent contrast injection, T1-weighted PMRI (K-trans, IAUC) sequences were obtained (**Figures 3, 4**). The K-trans value refers to the microcirculation perfusion in the tumor. It shows the entry of the contrast substance into the arteries. IAUC shows the contrast volume around the arteries. T2-weighted perfusion imaging (DSC) was performed with a second contrast injection approximately 5 to 8 min after the first contrast injection. Following the acquisition of perfusion-modulated radiological (PMRI) sequences, conventional contrast-enhanced MRI scans were obtained. Subsequently, MRS sequences were performed to assess metabolic profiles. Multivoxel short-echo and long-echo MRS sequences were taken from solid contrast sites (**Figure 5**). Permeability (T1-weighted perfusion) and PMRI (T2-weighted perfusion) sequences were performed together so that a single dose (0.1 mmol/kg) was divided into 2 equal injections, and a minimum of 10 mL of normal saline was given after each one. Automatic injection was used to set the gadolinium injection rate at 2 mL/s for 0.05 mmol/kg in dynamic contrast-enhanced (DCE) MRI and at 5 mL/s for 0.05 mmol/kg in DSC-MRI [19].

Biologic Biomarker Definition

Aggressive voxels were characterized by satisfying a minimum of 2 of the following criteria in comparison to contralateral normal-appearing white matter: Cho/NAA >2.0 or Cho/Cr ≥2.0 on MRS; increased K-trans or IAUC on DCEMRI; and hyperperfusion

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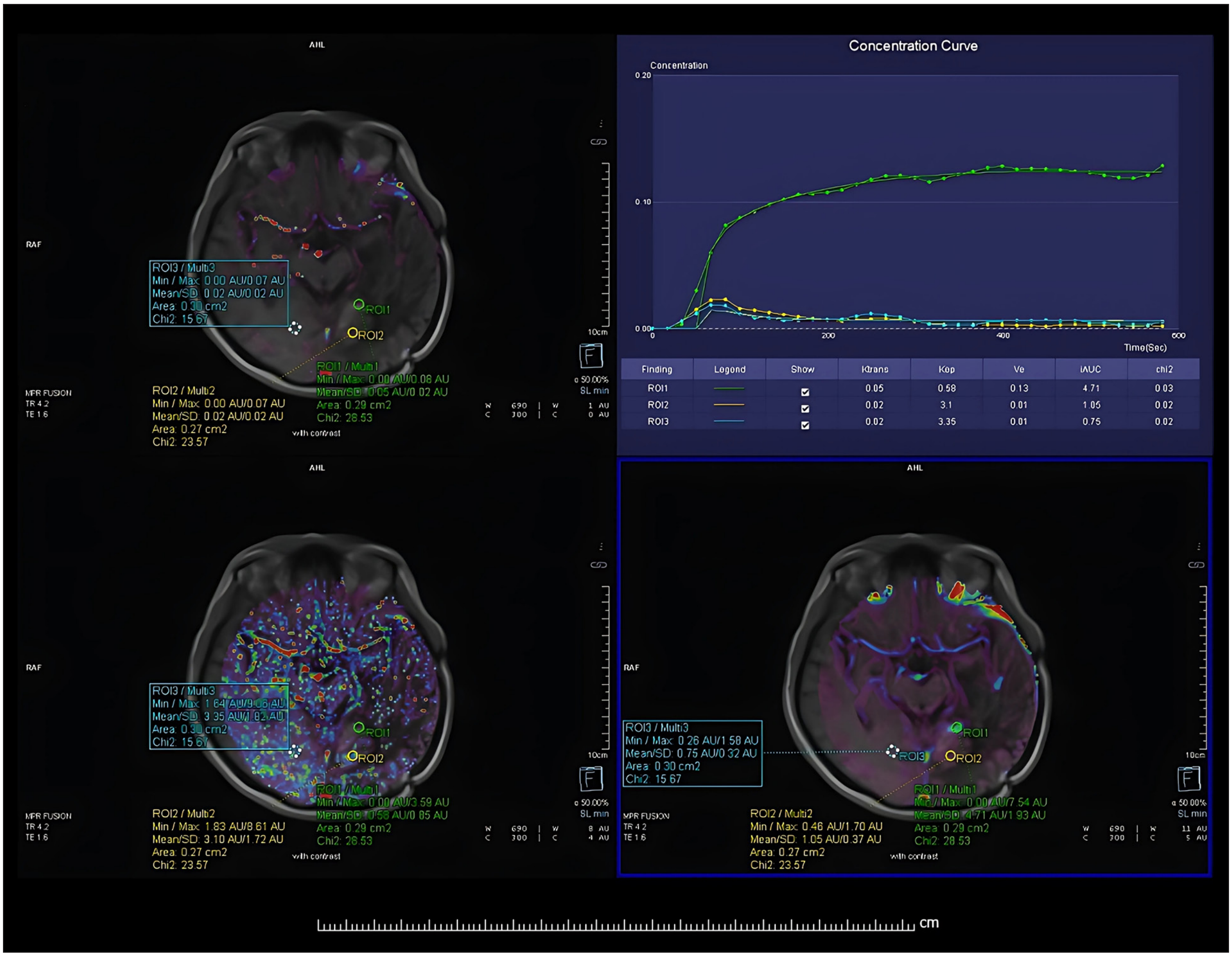

Figure 4. Post-stereotactic radiosurgery permeability reduction within escalation voxels. Follow-up dynamic contrast-enhanced MRI at approximately 3 months after Gamma Knife stereotactic radiosurgery demonstrates decreased volume transfer constant (K-trans) within the prior escalation sub-region (including region of interest 1), consistent with reduced vascular permeability after dose-painted treatment. Quantitative concentration-time curves and derived metrics are displayed for the same region of interests as in Figure 3. IAUC – initial area under the gadolinium concentration-time curve.

on DSC-PWI (elevation of rCBV). Penumbra was characterized as tissue that met 1 condition or was near to aggressive voxels; the remaining enhanced tumor was classified as passive.

MRI Data Analysis and Description of the 2 Plans

Aggressive tumor regions were defined by the presence of at least 2 of the following criteria: Cho/NAA or Cho/Cr ≥2 on MRS, elevated K-trans and IAUC compared with contralateral white matter, and hyperperfusion on T2-weighted DSC imaging. Non-aggressive regions lacked these features or demonstrated post-treatment normalization.

In the classic single-tumor site (control or simulated plan=plan 1, not delivered), the entire tumor was simulated to be given a dose of 16 Gy. A treatment plan was developed using 2 distinct tumor zones (modified dose-painting plan=plan 2). In plan 2, the maximum isodose administered to the aggressive location was equivalent to the dose allocated for recurrent glial tumors, specifically 18 Gy, whereas the minimum dose for the non-aggressive site was 15 Gy. To understand the radiation doses received by the surrounding brain tissues, the mean doses (ie, the average of the dose received by whole brain tissue), integral doses (ie, the average of the energy received by whole brain tissue), and V12 doses (ie, the volume of brain tissues that received a dose of 12 Gy) of both plans were calculated and statistically compared.

Statistical analyses were carried out in Python 3.11 (Anaconda distribution) using the SciPy (v1.11) and statsmodels (v0.14) packages for inferential statistics, lifelines (v0.28) for survival analysis, and pandas (v2.2) for data wrangling. All scripts were run in Visual Studio Code 1.88 under macOS Sequoia 15.5.

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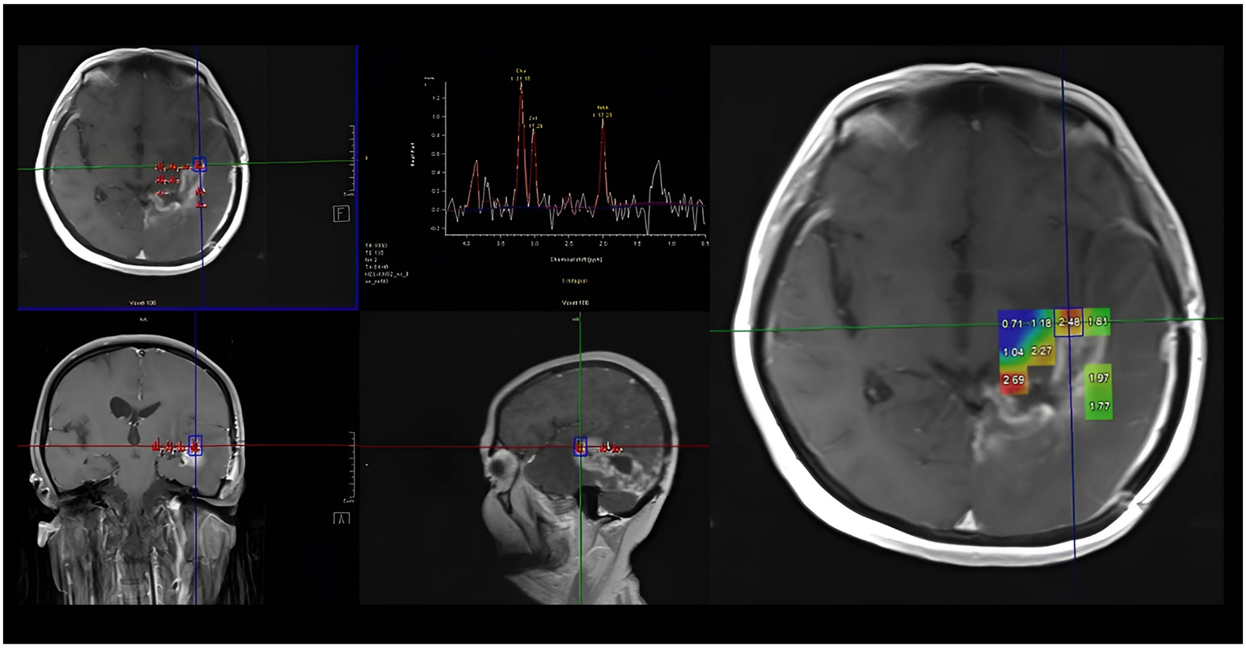

Figure 5. Pre-stereotactic radiosurgery multivoxel MR spectroscopy supports biologic target definition. Multiplanar views show MR spectroscopy voxel placement and spectra in the recurrent glioblastoma. The medial tumor voxel demonstrates elevated choline (Cho) and reduced N-acetylaspartate (NAA) with Cho/NAA approximately equal to 2.69 (values displayed), corresponding spatially to the high-volume transfer constant (K-trans) region on dynamic contrast-enhanced MRI. These metabolite abnormalities contributed to labeling the sub-volume as aggressive for dose escalation in plan 2.

To minimize the potential confusion on MR images caused by contrast enhancement following GKR, K-trans on T1-weighted PMRI and MRS was used. For residual and recurrent tumor analysis, hyperintense zones on T2-weighted FLAIR sequences and high-contrast zones were considered in line with the RANO criteria [20]. The increased signal of the mass effect on the T2-weighted FLAIR sequences or the restriction of diffusion around the resection cavity has been accepted as tumor progression [21]. After 6 months of tumor resection, the patients who were treated with radiotherapy and temozolomide may have had increased contrast, post-treatment effect, or tumor progression. PMRI and MRS were employed to differentiate these changes. The Cho/NAA, Cho/Cre ratios, rCBV, and K-trans parameters were investigated using long echo MRS sequences. If the ratios of Cho/NAA and Cho/Cre were less than 2, the zone was regarded as a normoperfusion area. The hypoperfusion area observed on T2-weighted PMRI, which demonstrated a similar or decreased K-trans level compared to the contralateral normal side on T1-weighted PMRI, was accepted as indicative of posttreatment recovery. If Cho/NAA and Cho/Cre ratios were greater than or equal to 2, the hyperperfusion area on T2-weighted PMRI and increased K-trans level compared with the contralateral normal side on T1-weighted PMRI were accepted as the development of recurrent or residual tumor. Subsequently, a decrease in contrast enhancement with ongoing diffusion restriction was accepted as a pseudoresponse, which refers to the presence of the tumor with less edema and compression. This can be associated with low rCBV or low/stable signal changes, compared with the contralateral normal side on T1-weighted PMRI, or low/stable signal changes on the T2-weighted FLAIR sequences [22,23]. Aggressive (active) tumor zones are considered to have very high hyperperfusion, very high K-trans or Cho/NAA, and Cho/Cre values higher than the general tumor area.

SRS Treatment

A stereotactic frame was implanted under local anesthesia. Radiosurgical planning was performed with the Leksell GammaPlan version 5.34 (Elekta, Sweden). Radiosurgery was performed using the Gamma Knife model C (Elekta, Sweden). The treatment was done in 1 session. Before the radiotherapy, MRS and T1-weighted PMRI (K-trans) were performed using 1.5-Tesla Siemens magnets, and 2-mm thin sequences of contrast-enhanced MRI images were obtained. When the gamma plan was made (plan 2, **Figure 1**), the zone that was hyperperfused, based on DCE-MRI metrics and having high spectral values on PMRI and MRS, was the active tumor zone where the tumoral cells of the glioblastoma multiforme mass were confirmed to be hyperintense; this zone was accepted as a separate tumor in our modified GK plan. Thus, the tumor area was divided into active and passive zones, and the GK plan was planned as 2 separate tumors, not as a single tumor (plan 2, **Figure 1**). Instead of a moderate dose to be used as a

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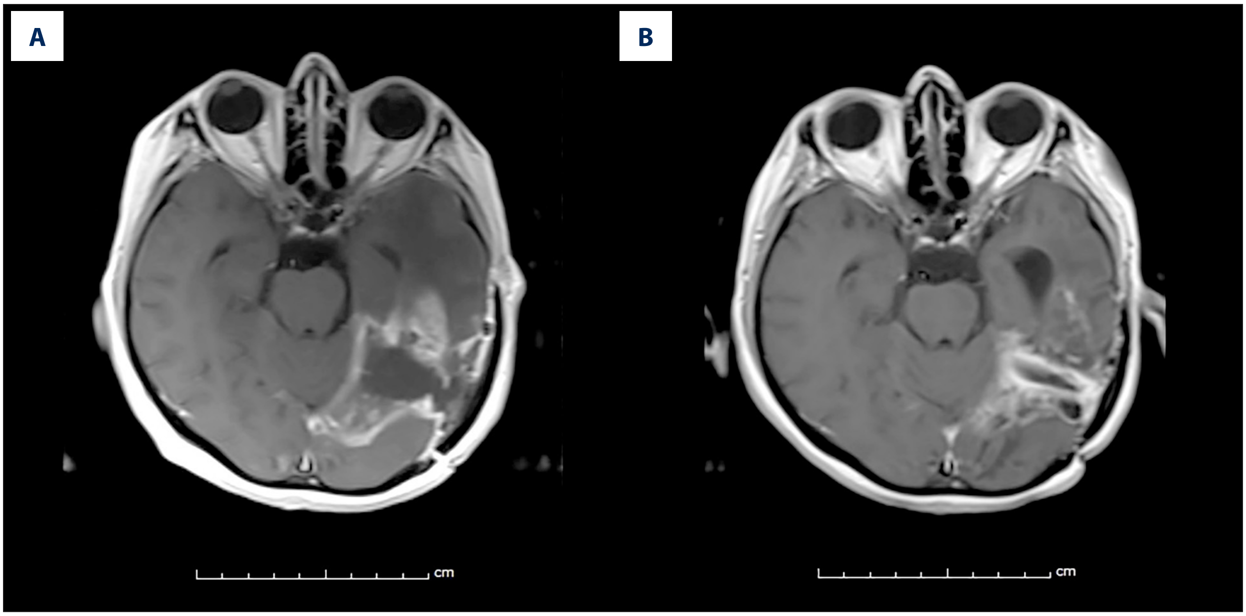

Figure 6. **Axial contrast-enhanced T1-weighted MRI before and approximately 3 months after Gamma Knife treatment.** (**A**) Baseline (pre-treatment) scan shows an irregularly enhancing temporal-lobe mass consistent with recurrent high-grade glioma. (**B**) Approximately 3-month post-treatment scan demonstrates decreased size and intensity of enhancement, compatible with radiographic response after dose-painted Gamma Knife stereotactic radiosurgery.

routine modality in the simulated plan, our new plan tried to increase GKR efficacy and reduce morbidity by giving these tumors the maximum dose (18 Gy) to the active zone and the minimum dose (15 Gy) to other passive zones. The maximum dose (18 Gy) was used as the standard in the active site (50% isodose). In the simulated control plan (plan 1), the tumor was considered as a single mass of tumor, and 16 Gy (50% isodose) was planned to be given (**Figure 2**). Mean doses, integral doses, and V12 values were calculated on both plans, to compare the radiation dose received by surrounding brain tissues.

Follow-Up

Patients were followed up clinically and radiologically at 3-month intervals after SRS. Radiologically, K-trans and IAUC values were accounted for on T1-weighted PMRI (**Figures 3, 4**). T1- and T2-weighted conventional MRI, contrast-enhanced MRI (**Figure 6A, 6B**), and MRS were performed together in the follow-up visits. A significant decrease in the K-trans and IAUC values was regarded as indicative of the therapeutic effect of GKR. The primary outcome was the percentage change in K-trans within the dose-escalation zone at 3 months.

Statistical Analysis of Imaging Biomarkers

The primary endpoint was the percent change in K-trans within dose-escalation (aggressive) voxels at approximately 3 months after SRS. Secondary endpoints were percent change in IAUC in the same voxels and paired dosimetric differences between plan 2 (dose-painted; delivered) and plan 1 (uniform 16 Gy; simulated) for whole-brain mean dose, integral dose, and V12. Data distributions were screened with the Shapiro-Wilk test; given the small sample and non-normality, paired comparisons used the Wilcoxon signed-rank test with Hodge-Lehmann median difference and 95% CIs; and effect sizes are reported as matched-pairs rank-biserial r. Associations between baseline biomarkers (eg, baseline K-trans) and subsequent change used Pearson r on log-transformed values when assumptions held, otherwise Spearman ρ. All *P* values are 2-sided (α=0.05). Exploratory linear models adjusted for age, Karnofsky performance status, and prior external beam radiotherapy equivalent dose in 2-Gy fractions were considered hypothesis-generating. Analyses were performed in Python 3.10 (scipy.stats).

The Mann-Whitney U test was used to compare the average dose of the whole brain, energy, and 12 Gy space volume (V12) of the whole brain between the same 23 patients in both plan 1 (simulation, not delivered plan), which was made by accepting the tumor as the same area, and plan 2 (modified dose-painting, treatment plan), which was made by dividing the tumor areas into active and passive zones. The Shapiro-Wilk test was used to evaluate the normality of continuous variables. Due to the predominance of non-normal distributions and the restricted sample size, non-parametric tests (Mann-Whitney U and Wilcoxon signed-rank) were employed. The Wilcoxon signed-rank test was used to evaluate the efficacy of the control treatment in only 11 patients who came to follow-up visits for various reasons (most of these patients came from outside the city or from other countries). Pearson correlation analysis was performed to evaluate the linear association between baseline imaging biomarkers and treatment response to GKR, as defined by the change in K-trans (ΔK-trans) on post-treatment DCE-MRI. The Pearson correlation examined linear relationships following the log transformation of unbalanced variables. Complete-case analysis was augmented with a sensitivity analysis that presumed constant biomarker levels for missing data. Exploratory multivariable linear regression, accounting for age, Karnofsky performance status, and preceding radiotherapy dose, assessed potential confounding variables.

Two pre-specified predictors were assessed: (1) baseline K-trans, reflecting tumor microvascular permeability, and (2) Cho/NAA ratio, reflecting metabolic activity. Correlation strength was categorized as strong ($|r| \geq 0.70$), moderate ($0.30 \leq |r| < 0.70$), or weak ($|r| < 0.30$). Statistical significance was defined as $P<0.05$. Analyses were conducted using the scipy.stats package in Python 3.10.

Results

Patient Cohort

A total of 23 patients (12 women, 11 men; mean age: 45.7 years, range 28-76) with recurrent high-grade gliomas were included. The average tumor volume was 13 mL (range 9.4-43.7 mL). Third-month post-treatment MRI was available in only 11 patients (most of these patients came from outside the city or from other countries).

Dosimetric Comparison

The mean doses, integral doses, and V12 values of both plans are given and compared in **Tables 1 and 2**. The average of the mean dose in plan 1, the standard plan, was 2.23 Gy, versus 2.16 Gy in plan 2, the modified dose-painting plan. The average integral dose in plan 1 was 7.17 J, versus 7.03 joules in plan 2. The average of the V12 in plan 1 was 116.71 cc, versus 94.69 cc in plan 2. The difference was not significantly different between the 2 plans in the doses of radiation that were received by brain tissues (for mean doses, P=0.716; for integral doses, P=0.792; and for V12, P=0.583; all P values were >0.05). The radiotherapy efficiency was increased in plan 2 by giving 18 Gy to the active tumor zone, versus the mean of 16 Gy dose that was planned to be given in plan 1. Although our modified plan was applied to 23 patients and all their pretreatment MRI scans were available, for different reasons, 3-month post-treatment MRI scans were taken from 11 patients. The comparison was performed between pre- and post-GKR MRIs of these 11 patients.

Imaging Biomarker Response Within Escalation Voxels

According to the Wilcoxon signed-rank test, significant reductions of K-trans and IAUC values were found in the control MRIs (for K-trans, P=0.01 and for IAUC, P=0.08; **Table 3**). In the same way, the masses were diminished on conventional MRIs. In these 11 patients, the median K-trans within the escalation zone decreased by 18% (IQR 12-25%, P=0.028), and the median IAUC decreased by 22% (IQR 15-29%, P=0.031) compared with baseline, while no significant alteration was observed in non-escalated voxels (P>0.10). These data collectively corroborate our hypothesis that physiologically driven dosage painting selectively influences vascular permeability in metabolically active tumor areas.

Correlation Analyses

Correlation analyses are summarized in **Table 4**. A strong and statistically significant inverse correlation was observed between baseline K-trans and ΔK-trans (r=-0.841, P=0.0012), indicating that tumors with elevated baseline vascular permeability exhibited greater reductions in K-trans following GKR. This supports the role of K-trans as a robust imaging biomarker of vascular response to SRS.

In contrast, the Cho/NAA ratio demonstrated a weak inverse correlation with ΔK-trans (r=0.091, P=0.791), which did not reach statistical significance. Although directionally consistent with reduced treatment responsiveness in metabolically active tumors, this association may require further validation in a larger cohort.

Summary of Key Findings

Collectively, dose-painting GKSRS delivered biologically escalated doses to aggressive sub-volumes without worsening whole-brain dosimetry, and produced permeability reductions on DCE-MRI within targeted regions. These data support integrating vascular and metabolic imaging biomarkers to guide and monitor SRS in recurrent high-grade glioma.

Discussion

In 23 patients with recurrent high-grade gliomas, we delivered GK dose painting guided by MRS/PMRI and compared it with a simulated uniform 16 Gy plan. Dose painting did not increase whole-brain dose metrics (mean dose, integral dose, V12) but, in 11 patients with about 3-month follow-up imaging, reduced permeability biomarkers in escalated voxels (K-trans -18%, IAUC -22%) and higher baseline K-trans predicted greater reduction (r=-0.841). Overall, functional-imaging-guided escalation produced a measurable biologic response without extra normal-brain dose, extending prior dose-painting evidence using MRS/

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Table 1. Patient-level dosimetry comparing uniform 16 Gy (plan 1) vs dose-painted 15-18 Gy (plan 2). Comparison of mean dose, integral dose, and volume of normal brain receiving ≥12 Gy (V12) between standard treatment (plan 1: uniform 16 Gy, simulated, not delivered) and dose-painting plans (plan 2: dose-painted 15-18 Gy; delivered).

Sex	Plan 1 (16 Gy)			Plan 2 (15-18 Gy)		
	Mean (Gy)	Integral dose (J)	V12 (cc)	Mean (Gy)	Integral dose (J)	V12 (cc)
M	2.2	5.8	71.4	2.1	5.8	70.25
F	2.7	8.9	116.58	2.6	8.6	110.61
F	2.9	8.9	126.03	2.8	8.7	124.57
M	2.1	7.9	85.35	2.1	7.9	85.41
M	3	9.9	163	3	9.8	162.97
M	3	9.9	134	3	10	136.4
M	1.5	5	550.9	1.5	4.9	55.04
F	2.4	8.4	104.13	2.4	8.5	105.46
F	1.4	4.6	14.92	1.3	4.3	13.92
F	1.5	4.7	58.55	1.5	4.7	57.36
M	2.5	7.5	130.39	2.5	7.4	130.11
F	0.9	2.9	24.26	0.8	2.8	23.92
F	1.2	3.4	32.11	1.1	3.3	32.09
F	1.5	4.7	60.33	1.4	4.6	60.28
F	3.1	9.3	91.47	2.9	8.7	91.32
F	1.1	2.5	14.87	1	2.4	14.48
F	2.2	7	91.39	2.1	7	91.3
M	2.4	8.2	93.28	2.3	8.1	93.15
M	3.8	13.9	239.75	3.7	13.7	238.25
M	1.3	5	45.71	1.2	5	45.68
M	3.6	12	250.21	3.5	11.9	250.18
M	1.9	6.1	63.28	1.8	6	63.2
F	3	9.4	126.49	3	9.2	126.38

The mean dose – the average of the dose received by whole brain tissue; integral dose – average of the energy received by whole brain tissue; V12 dose – volume of brain tissues that received a dose of 12 Gy. F – female; M – male.

perfusion guidance. It has been well reported that SRS has no significant impact on the life span of patients with operated glioblastoma multiforme when it was used as an adjunctive treatment. SRS is more beneficial for recurrent and progressive intracranial lesions [24]. For a long time, increased enhancement of lesions on contrast-enhanced MRI sequences has been evaluated as progression in operated glioblastoma multiforme and in follow-up post-SRS visits. However, intense contrast enhancement can happen in radiation therapy- and SRS-induced necrosis and pseudo-progression. Therefore, evaluating such patients according to contrast enhancement is insufficient, and this explains the demand for different diagnostic tools for accurate diagnosis. T1- and T2-weighted PMRI images provide more accurate details about the prognosis of gliomas than do contrast-enhanced MRIs. Increased blood flow in the tumor area, increased perfusion, and increased microvascular permeability are indicative of high grading and poor prognosis [22,23,25]. However, there is still debate whether the blood flow in the tumor site demonstrates the prognosis or not [26]. Post-SRS progression can be understood by detecting tumor blood flow on T1-weighted PMRI [25]. MRS provides us with the Cho/NAA and Cho/Cre ratios. These ratios can be used to determine the active

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Table 2. Summary dosimetric metrics and paired comparisons. The average, minimum, and maximum of the mean dose, integral dose (ID), and volume of normal brain receiving ≥12 Gy (V12) doses for both plans were calculated and compared using the Mann-Whitney U test.

Dose/plan	n	Min	Max	Mean	Std deviation	*P*
Mean						0.716
Plan 1	23	1	4	2.23	0.827	
Plan 2	23	1	4	2.16	0.831	
ID						0.792
Plan 1	23	2	13	7.17	2.832	
Plan 2	23	2	13	7.03	2.764	
V12						0.583
Plan 1	23	15	550	116.71	112.661	
Plan 2	23	14	250	94.69	61.98	

The mean dose – the average of the dose received by whole brain tissue; ID integral dose – average of the energy received by whole brain tissue; V12 dose – volume of brain tissues that received a dose of 12 Gy.

Table 3. Volume transfer constant (K-trans) and initial area under the gadolinium concentration-time curve (IAUC) metrics before and approximately 3 months after stereotactic radiosurgery in escalation voxels (n=11).

N	K-trans		IAUC	
	Before	After	Before	After
1	0.02	0.01	4.17	0.67
2	0.02	0.01	1.94	1.45
3	0.01	0.01	1.55	2.16
4	0.01	0.02	1.39	0.58
5	0.02	0.01	2.16	0.90
6	0.03	0.01	3.01	0.96
7	0.04	0.01	3.83	0.39
8	0.05	0.01	4.07	2.4
9	0.19	0.13	3.04	2.88
10	0.17	0.02	5.57	3.30
11	0.16	0.09	3.68	1.22

Table 4. Pearson correlation analysis revealing associations between baseline imaging biomarkers and post-treatment changes in volume transfer constant (ΔK-trans).

Comparison	Pearson r	*P* value	Interpretation
Baseline K-trans vs ΔK-trans	-0.841	0.0012	Strong inverse correlation (statistically significant)
Baseline Cho/NAA vs ΔK-trans	0.091	0.791	Very weak positive correlation (not statistically significant)

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tumor zones and sites of intense tumor cells, where the PMRI is insufficient. Because of the inhomogeneous property of the tumor, in some postoperative cases, T2-weighted PMRI and MRS can be relatively limited, and these images are still insufficient to show the progression. In such cases, accounting for K-trans on T1-weighted PMRI sequences imaging (which refers to permeability maps) successfully demonstrates tumor enhancement [22,23]. Our modified GK treatment plan depends on the properties of tumor sites obtained using these advanced techniques. The tumor was divided into 2 zones: aggressive (active) and non-aggressive (passive). Then, the plan was prepared for these 2 targets. Routine SRS planning is based on contrast-enhanced cranial MRI and computerized tomography [28]. However, the limitations of these imaging modalities limit optimal SRS treatment. To compensate for these limitations, in our study, GK plans were additionally planned using T1-weighted PMRI and MRS. In our modified plans, the sites showed hyperperfusion, high K-trans, and high ratios of Cho/NAA and Cho/Cre, which were accepted as aggressive tumor sites. In the modified GK treatment plan, the tumoral area was divided into 2 distinct regions: an active (more aggressive) area and a passive (less aggressive) area. At this point, the maximum dose that can be given to the recurrent gliomas (18 Gy) has been applied to active zones, and the minimum dose (15 Gy) to other passive zones. Thus, instead of a moderate dose being applied to the whole tumor area, the GKR efficacy in actively aggressive sites was increased, and the morbidity rate was reduced by giving the peripheral sensitive and eloquent areas the minimum dose. In both plans (treatment and simulated plans), the radiation doses that were received by the peripheral cerebral tissues were calculated. Our results show that active tumor sites received the highest dose, while the peripheral brain tissues received the lowest dose. As a result, the suggested modified GK plan, by using advanced MRI techniques such as MRS and accounting for K-trans on T1-weighted PMRI, improved the efficiency of GKR in the treatment of recurrent glial tumors without raising the morbidity rate. K-trans value refers to the microcirculation perfusion in the tumor. It shows the entry of the contrast substance into the arteries. IAUC refers to showing the contrast volume around the arteries, so a significant decrease in the 2 values can be regarded as indicative of the therapeutic effect of GKR.

Limitations

This study has several important limitations. First, it was a retrospective, single-center analysis, which introduces the potential for selection bias and limits generalizability. Second, the control arm (plan 1) was simulated and not applied to actual patients, which limits the ability to directly compare clinical outcomes between the 2 treatment strategies. Third, post-treatment follow-up imaging was only available for a subset of patients (11 out of 23), thereby reducing the statistical power of radiographic outcome analyses. Fourth, the follow-up period was relatively short, precluding the assessment of long-term tumor control, progression-free survival, or overall survival. Fifth, although biologically guided dose painting was implemented based on advanced imaging (MRS and PMRI), no histopathological or molecular validation of tumor heterogeneity was available. Thus, the correlation between imaging-defined "aggressive" subregions and actual cellular or molecular tumor characteristics (eg, IDH mutation status, MGMT promoter methylation, or cellular proliferation indices) could not be confirmed. Finally, no neurocognitive or quality-of-life data were collected, which would be essential to assess the potential morbidity reduction claimed by limiting dose exposure to passive zones. Future prospective studies with a larger patient cohort, histological correlation, molecular profiling, and long-term outcome assessment are warranted to validate these findings.

Clinical Implications and Future Directions

Using advanced MRI techniques (K-trans and IAUC on PMRI with MRS) and conventional MRI to determine aggressive areas in salvage treatment for recurrent glial tumors, and then dividing tumor areas into aggressive and non-aggressive areas in a modified GK plan leads to increased treatment efficiency without raising the morbidity rate. The morbidity rate was not changed, because the dose of radiation in the peripheral zones did not change.

Future prospective multicenter trials using serial DSC-PWI and MRS, in conjunction with AI-based voxel-level outcome modeling, are necessary to confirm the clinical advantages of this biologically guided approach.

The present data directly confront the limitations of structural MRI-guided SRS by evidencing biomarker-confirmed normalization of vascular permeability within escalating voxels, an effect that was previously suggested but not quantified in recurrent high-grade gliomas.

Conclusions

The study illustrates that biologically guided GK dose painting, using advanced functional imaging, such as MRS and T1-weighted PMRI, is a viable approach for enhancing locoregional treatment accuracy in recurrent high-grade gliomas. The modified SRS approach involved delineating metabolically and vascularly active tumor subregions and assigning increased radiation doses to these areas. This method preserved the dosimetry of surrounding normal brain tissue while producing radiographic responses, as indicated by reductions in K-trans and IAUC values.

The findings, despite being constrained by their retrospective design and a small follow-up cohort, endorse the clinical value of

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incorporating quantitative imaging biomarkers, such as K-trans and Cho/NAA, into radiosurgical planning. This personalized radiotherapy method shows potential for improving local tumor control, reducing treatment-related toxicity, and progressing precision neuro-oncology. Future studies should involve larger patient cohorts, extended follow-up periods, and the inclusion of molecular pathology to validate and enhance these findings.

Biologically guided GK dosage painting, informed by combined MRS and DSC-PWI biomarkers, is both practical and safe for recurrent high-grade glioma. Preliminary evidence of reduced K-trans and IAUC in escalating voxels indicates a potential to improve focal control while preserving normal brain tissue; nevertheless, validation through prospective multicenter trials is necessary prior to general application.

Institution Where Work Was Done

Bezmialem Vakif University, Istanbul, Türkiye.

Patient Permission

Informed consent was obtained from all individual participants included in the study.

Ethic Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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