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Clinical Outcomes of Moderately Hypofractionated Concurrent Chemoradiotherapy for Newly Diagnosed Glioblastoma

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Purpose: Hypofractionated radiotherapy (HypoRT) has recently been implemented in patients with glioblastoma (GBM) receiving concurrent temozolomide. Lymphopenia during treatment (LDT) is considered an important prognostic factor of clinical outcomes for GBM. We aimed to investigate the outcomes of HypoRT.

Materials and Methods: Among 223 patients with GBM, 145 and 78 were treated with conventionally fractionated RT (ConvRT, 60 Gy in 30 fractions) and HypoRT (58.5 Gy in 25 fractions), respectively. To balance characteristics between the two groups, propensity score matching (PSM) was performed.

Results: Patients in the HypoRT group were older and had smaller tumors than those in the ConvRT group (p<0.05). Furthermore, dose distributions to the brain were significantly lower in HypoRT than in ConvRT (p<0.001). Changes in absolute lymphocyte counts (ALC) during treatment were significantly lower after HypoRT than after ConvRT (p=0.018). With a median follow-up of 16.9 months, HypoRT showed comparable progression-free survival (9.9 months vs. 10.5 months) and overall survival (27.2 months vs. 26.6 months) to ConvRT (all p>0.05). Multivariable analysis before PSM revealed that ≥grade 2 LDT at 6 months was associated with inferior outcomes. Subsequent analysis demonstrated that HypoRT significantly reduced the rate of ≥grade 2 LDT at 6 months post-RT before and after PSM.

Conclusion: HypoRT with 58.5 Gy in 25 fractions could provide comparable oncologic outcomes and significantly reduce the ALC changes. In addition, HypoRT decreased the LDT. Further investigation should be warranted to suggest the significance of reduced LDT through HypoRT affecting survival outcomes.

Key Words: Glioblastoma, radiation therapy, hypofractionation, lymphopenia, temozolomide

INTRODUCTION

The mainstay of treatment for newly diagnosed glioblastoma (GBM) is maximal safe resection followed by radiotherapy (RT) of 60 Gy combined with temozolomide.[1-3] Short-course RT at a dose of 40 Gy in 15 fractions is recommended for frail patients with poor performance status or old age. With the advent of intensity-modulated RT (IMRT) with simultaneous integrated boost, moderately hypofractionated RT (HypoRT), rather than traditional conventionally fractionated RT (ConvRT, mostly 60 Gy in 30 fractions), has been explored as a treatment option for GBM in multiple series.[4-9]

Several tools using either risk partitioning analysis or nomograms have been developed to predict the outcomes of GBM patients.[10-12] Well-known clinicopathologic factors, such as extent of resection, age, and methylation status of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter, have been included in these tools. Recently, lymphopenia during treatment (LDT) has been advocated as a prognostic factor in

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patients with GBM.[13-17] A growing body of evidence has revealed a negative association between LDT and outcomes across cancer types, including esophageal, lung, pancreatic, and liver cancer.[18-22] Local RT delivered to organs with high blood flow (i.e., the brain, heart, or lung) could potentially lead to systematic lymphocyte depletion based on a mathematical model.[23,24] Although a number of studies suggest possible RT-related factors to be associated with LDT in patients with GBM, data on the effects of HypoRT are limited.[16,17,25,26]

This study aimed to examine the clinical outcomes (oncologic outcomes and LDT status) of HypoRT in comparison with those of ConvRT in patients with GBM treated with concurrent temozolomide.

MATERIALS AND METHODS

Patient population

Patients who were histologically diagnosed with GBM, IDH-wildtype between January 2013 and September 2021 were screened. Patients who were treated with concurrent chemoRT with temozolomide following surgery using the Stupp regimen were included.[27] Among these, patients treated with 15–20 fractions of short-course palliative (chemo)RT (n=33), patients with initial leptomeningeal seeding (n=10), or patients who did not complete the RT (n=3) were excluded. Finally, 223 patients, including 145 treated with conventional fractionated chemoRT (ConvRT group) and 78 treated with hypofractionated chemoRT (HypoRT group), were analyzed. This study was approved by our Institutional Review Board (no. SMC 2022-07-008), and the requirement for informed consent was waived due to the retrospective nature of this study.

Treatment and follow-up

The treatment strategy was determined on a case-by-case basis through discussions with a multidisciplinary neuro-oncology board comprising neurosurgeons, radiologists, radiation oncologists, and medical oncologists. The extent of resection was determined based on the results of magnetic resonance imaging (MRI) performed within 48 hours after tumor resection and intraoperative findings. Gross total resection (GTR, n=126, 56.5%), subtotal resection (n=65, 29.1%), partial resection (n=8, 3.6%), and biopsy (n=24, 10.8%) were defined as the absence of a visible contrast-enhanced portion, removal of at least 90% of the tumor, removal of less than 90% of the tumor, and performance of stereotactic biopsy, respectively. We also examined the methylation status of MGMT. All patients received concurrent chemoRT with the Stupp regimen (75 mg/m^2 of body surface area per day, 7 days per week, from the first to the last day of RT) followed by six cycles of adjuvant temozolomide (150–200 mg/m^2 for 5 days in every 28-day cycle).

With regard to fractionation, ConvRT or HypoRT was adopted based on the physician's preference. Briefly, HypoRT is considered for patients with 1) a small contrast-enhancing tumor (4 cm) defined by preoperative MRI, 2) small peritumoral edema defined by postoperative T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI (less than two lobes), or 3) old age (>65 years) but tolerable performance status [≥Karnofsky performance status (KPS) 80]. Gross tumor volumes (GTVs) in both groups included the resection cavity and residual contrast-enhancing lesions observed on postoperative MRI scans. The clinical target volume (CTV) included abnormalities on T2-FLAIR MRI and GTV plus a 1–1.5 cm margin. Reduced-field CTV (RF-CTV) was delineated by adding a 0.5-cm margin to the GTV. The planning target volume (PTV or RF-PTV) was defined as the CTV or RF-CTV plus a 3-mm margin. In the ConvRT group, a radiation dose of 50 Gy in 25 fractions to the PTV and a sequential boost of 10 Gy in 5 fractions to the RF-PTV were prescribed. For patients in the HypoRT group, a radiation dose of 50 Gy in 25 fractions to the PTV and a radiation dose of 58.5 Gy in 25 fractions to the RF-PTV were prescribed using a simultaneous integrated boost technique. All CTVs received 95% of the prescribed dose. Detailed information on the dose constraints for organs-at-risk is summarized in Supplementary Table 1 (only online). Patients in the ConvRT group were treated with either three-dimensional conformal RT (3D-CRT, n=61, 42.1%) or IMRT (n=84, 57.9%), while those in the HypoRT group received only IMRT (100.0%).

All patients were followed until death or the time of analysis. After treatment, follow-up MRI was performed 1 month after the planned chemoRT, every 3 months for the first 2 years, and every 6 to 12 months thereafter.

Lymphocyte counts

Since all patients were treated under the Stupp regimen with six cycles of adjuvant temozolomide after chemoRT, peripheral blood counts were assessed at five time points: preoperative, pre-RT, 1 month after RT, 3 months after RT, and 6 months after RT. LDT was graded according to the Common Terminology Criteria for Adverse Events version 5.00 based on the following absolute lymphocyte count (ALC): grade 1 (800≤ ALC <1000/μL), grade 2 (500≤ ALC <800/μL), grade 3 (200≤ ALC <500/μL), or grade 4 (ALC <200/μL).

Statistical analyses

Pearson's chi-square or Fisher's exact tests were used to analyze categorical variables, while the Mann-Whitney U test (non-normally distributed) were employed to compare continuous variables among patient and treatment characteristics between the two groups. The R package "MaxStat," which iteratively tests all possible cutoff points to determine ones that achieve the maximum rank statistic, was used to dichotomize PTV and RF-PTV volumes.[28] Progression-free survival (PFS) and overall survival (OS) rates were calculated from the date of surgery or biopsy to the date of the event or death from any cause. The Kaplan-Meier method was employed to estimate PFS and OS rates,

while the log-rank test was used to assess the prognostic significance. Multivariable analysis was performed according to the Cox regression model using significant factors in univariable analysis. A mixed model was used to ascertain associations between ALC and absolute neutrophil count (ANC) changes and fractionation schedules (ConvRT or HypoRT). Logistic regression analysis was performed to evaluate predictive factors for LDT. The factors were selected in stepwise regression after 10-fold cross-validation and were included in the multivariate analysis for LDT. In addition, propensity score matching (PSM) was performed to minimize selection bias and the effects of potential confounders. Using the "MatchIt" package, propensity scores were calculated among age (continuous), extent of resection (biopsy, partial resection, subtotal resection, and GTR), PTV (continuous), RF-PTV (continuous), and RT modality (3D-CRT vs. IMRT). Patients were matched using 1:1 nearest matching with a caliper 0.05 standard deviations of the logit of the calculated propensity score. McNemar's test or the Wilcoxon signed-rank test was used to compare variables after PSM. A two-tailed p-value of <0.05 was considered significant. All statistical analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

Overall, the median age of the patients was 59 years [interquartile range (IQR), 53–67]. The median KPS scores at the time of surgery and RT were 80 (IQR, 70–90) and 80 (IQR, 80–90), respectively. Patients in the HypoRT group were older than those in the ConvRT group (median age, 62 years vs. 58 years, p=0.015), and the proportion of patients aged ≥70 years was higher in the HypoRT group than in the ConvRT group (23.1% vs. 12.4%,

Table 1. Patient and Treatment Characteristics Before and After PSM

	Before PSM			After PSM		
	ConvRT (n=145)	HypoRT (n=78)	*p* value	ConvRT (n=27)	HypoRT (n=27)	*p* value
Sex			0.984			0.097
Male	82 (56.6)	44 (56.4)		13 (48.1)	19 (70.4)	
Female	63 (43.4)	34 (43.6)		14 (51.9)	8 (29.6)	
Age, yr	58 [52–64]	62 [54–69]	0.015	59 [54–66]	59 [53–67]	0.993
≥70 years	18 (12.4)	18 (23.1)	0.039	4 (14.8)	5 (18.5)	0.715
Preoperative KPS	80 [70–90]	80 [70–90]	0.354	80 [70–90]	80 [70–90]	0.102
KPS≤70	56 (38.6)	24 (30.8)	0.244	12 (44.4)	7 (25.9)	0.154
Pre-RT KPS	80 [80–90]	80 [80–90]	0.586	80 [80–90]	80 [80–90]	0.778
KPS≤70	33 (22.8)	12 (15.4)	0.191	7 (25.9)	2 (7.4)	0.068
Extent of resection			<0.001			0.663
Biopsy	4 (2.8)	20 (25.6)		2 (7.4)	1 (3.7)	
PR	5 (3.4)	3 (3.8)		1 (3.7)	0 (0.0)	
STR	52 (35.9)	13 (16.7)		7 (25.9)	9 (33.3)	
GTR	84 (57.9)	42 (53.8)		17 (63.0)	17 (63.0)	
MGMT promoter			0.861			0.580
Methylated	67 (46.2)	37 (47.4)		12 (44.4)	10 (37.0)	
Unmethylated	78 (53.8)	41 (52.6)		15 (55.6)	17 (63.0)	
Adjuvant temozolomide	6 [2–6]	6 [2–6]	0.971	6 [2–6]	6 [2–6]	0.981
Total dose			<0.001			<0.001
58.5 Gy	0 (0.0)	78 (100.0)		0 (0.0)	27 (100.0)	
60 Gy	145 (100.0)	0 (0.0)		27 (100.0)	0 (0.0)	
PTV volume	241.6 [195.9–298.9]	152.0 [111.3–221.5]	<0.001	212.5 [164.8–248.3]	205.4 [154.5–245.1]	0.559
≥300 cm^3	36 (24.8)	8 (10.3)	0.009	4 (14.8)	4 (14.8)	>0.999
RF-PTV volume	93.0 [69.1–127.7]	49.2 [33.2–74.7]	<0.001	74.0 [60.2–94.5]	74.3 [54.1–88.2]	0.511
≥120 cm^3	43 (29.7)	1 (1.3)	0.005	2 (7.4)	1 (3.7)	0.552
RT modality						
3D-CRT	61 (42.1)	0 (0.0)	<0.001	0 (0.0)	0 (0.0)	>0.999
IMRT	84 (57.9)	78 (100.0)		27 (100.0)	27 (100.0)	

PSM, propensity score matching; ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy; KPS, Karnofsky performance status; PR, partial resection; STR, subtotal resection; GTR, gross total resection; MGMT, O^6-methylguanine-DNA methyltransferase; PTV, planning target volume; RF, reduced field; 3D-CRT, three-dimensional conformal radiotherapy; IMRT, intensity-modulated radiotherapy.
Values are presented as the number of patients (%) or medians [interquartile range].

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p=0.039). In addition, the rate of biopsy was higher in the HypoRT group than in the ConvRT group (25.6% vs. 2.8%). Regarding RT planning, smaller PTV and RF-PTV were observed in the HypoRT group than in the ConvRT group. Patients in the HypoRT were more frequently treated with IMRT than those in the ConvRT group. After PSM, there were no differences in patient, tumor, and RT volume characteristics between two groups. The patient's baseline characteristics and treatment details before and after PSM are provided in Table 1.

Meanwhile, significant differences were found in the dose-volume parameters of the brain between the two groups (Supplementary Table 2, only online). Although the differences in dose distribution to the brain decreased after PSM, patients in the HypoRT group showed tended to involve lower dose distributions to the brain than those in the ConvRT group both before and after PSM.

Clinical outcomes

With a median follow-up of 16.9 months (IQR, 10.2–26.7), the median PFS and OS of the entire cohort were 10.1 and 27.2 months, respectively. Of note, HypoRT showed comparable PFS and OS outcomes to those of ConvRT (median PFS: 9.9 months vs. 10.5 months, *p*=0.560; median OS: 27.2 months vs. 26.6 months, *p*=0.490) (Fig. 1). In subgroup analysis of OS, no major differences or interactions were found for the effects of fractionation according to the clinical and treatment factors (Fig. 2). In every subgroup, HypoRT exhibited OS outcomes similar to those of ConvRT.

Regarding toxicities, the rates of ≥grade 2 acute toxicities during chemoRT and symptomatic radionecrosis after chemoRT were also comparable between the two groups (Table 2). Overall, 17 (7.6%), 39 (17.5%), 52 (23.3%), and 63 (28.3%) patients experienced ≥grade 2 LDT at pre-RT, 1 month post-RT, 3 months post-RT, and 6 months post-RT, respectively. Among them, 3

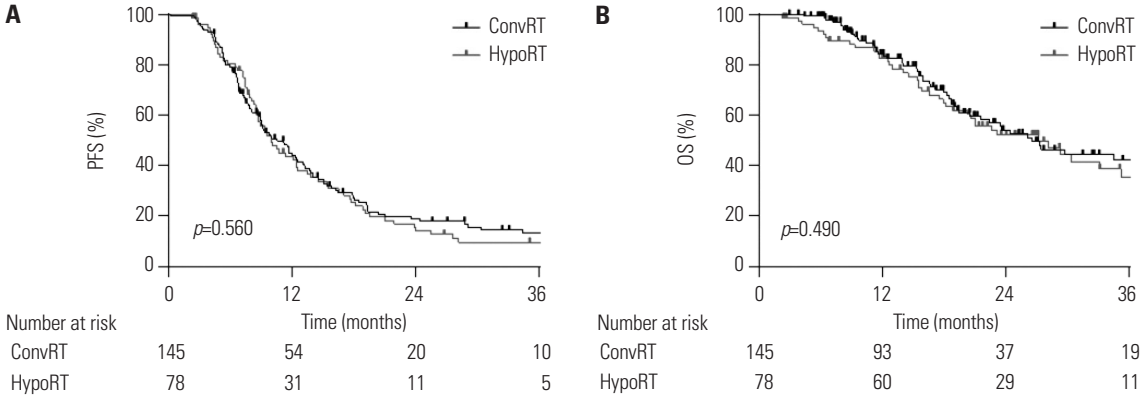

Fig. 1. Kaplan–Meier curves for progression-free survival (PFS) (A) and overall survival (OS) (B) stratified by fractionation schedules. ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy.

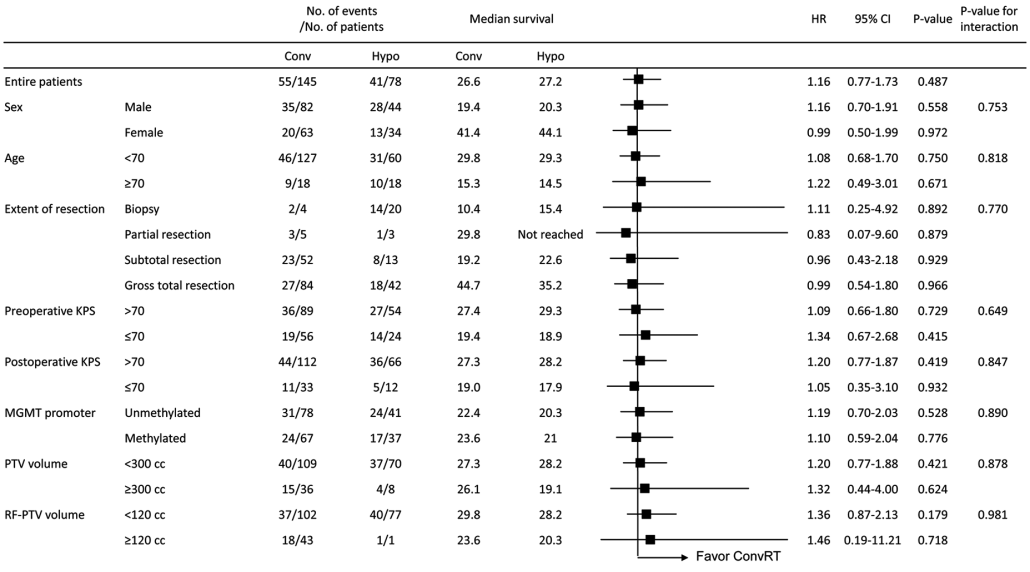

Fig. 2. OS according to subgrouping. HRs and rates of OS among patients stratified by fractionation schedules are shown. The dashed vertical line at 1.2 indicates the overall HR estimate. The HRs are shown on a logarithmic scale. ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy; HR, hazard ratio; CI, confidence interval; KPS, Karnofsky performance status; MGMT, O^6-methylguanine-DNA methyltransferase; PTV, planning target volume; RF, reduced field; OS, overall survival.

(1.4%), 9 (4.0%), 15 (6.7%), and 26 (11.7%) patients were categorized as having ≥grade 3 LDT at pre-RT, 1 month post-RT, 3 months post-RT, and 6 months post-RT, respectively. Although no differences were observed in the rates of ≥grade 2 or ≥grade 3 LDT at pre-RT and 1 month post-RT, HypoRT was related with lower rates of ≥grade 2 LDT at 3 months (15.4% vs. 26.2%, p=0.045) and 6 months post-RT (14.1% vs. 35.9%, p=0.001). After mixed-model analysis, the changes in ALC during treatment courses were significantly different between the HypoRT and ConvRT groups (p=0.018) (Fig. 3A). However, the changes in ANC during the treatment course were comparable between the HypoRT and ConvRT groups (p=0.158) (Fig. 3B). Detailed information on changes in ALC and ANC are provided in Supplementary Table 3 (only online).

Table 2. Treatment-Related Adverse Events Stratified by Radiotherapy Schedule

	ConvRT (n=145)	HypoRT (n=78)	p value
Grade 2 or more acute toxicity			
Fatigue	37 (25.5)	23 (29.5)	0.524
Nausea	60 (41.4)	28 (35.9)	0.424
Headache	33 (22.8)	20 (25.6)	0.630
Grade 2 or more radionecrosis or pseudoprogression	47 (32.4)	26 (33.3)	0.889
Grade 2 or more lymphopenia (<800/uL)			
Pre-RT	11 (7.6)	6 (7.7)	0.977
Post-RT 1 month	29 (20.0)	10 (12.8)	0.178
Post-RT 3 month	40 (27.6)	12 (15.4)	0.040
Post-RT 6 month	52 (35.9)	11 (14.1)	0.001
Grade 3 or more lymphopenia (<500/uL)			
Pre-RT	3 (2.1)	0 (0.0)	0.201
Post-RT 1 month	7 (4.8)	2 (2.6)	0.413
Post-RT 3 month	12 (8.3)	3 (3.9)	0.208
Post-RT 6 month	21 (14.5)	5 (6.4)	0.073

ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy.
Values are presented as the number of patients (%).

Prognostic factors for PFS and OS

HypoRT was not associated with inferior PFS [hazard ratio (HR): 1.09, 95% confidence interval (CI): 0.81–1.47, p=0.566] or OS (HR: 1.16, 95% CI: 0.77–1.73, p=0.487) in univariable analysis. After PSM, HypoRT consistently did not affect PFS and OS outcomes (Supplementary Table 4, only online). In multivariable analysis before PSM, male sex, non-GTR, unmethylated MGMT promoter status, and ≥grade 2 LDT at 6 months post-RT were related to inferior PFS outcomes (Table 3). Specifically, ≥grade 2 LDT at 6 months was associated with inferior PFS, compared with grade 0–1 LDT (HR: 1.63, 95% CI: 1.16–2.29, p=0.005). With regard to OS, ≥grade 2 LDT consistently showed a significant association with inferior OS outcomes, compared with grade 0–1 LDT (HR: 1.94, 95% CI: 1.20–3.14, p=0.007), after the multivariable analysis. In addition, female sex, age <70 years, and GTR were statistically significant factors affecting improved OS outcomes (all p<0.05). After PSM, ≥grade 2 LDT at 6 months (HR: 2.52, 95% CI 1.11-5.70, p=0.027) was related with inferior PFS (Supplementary Table 4, only online). However, ≥grade 2 LDT at 6 months showed insignificance in OS outcomes after PSM. Sixty-three patients (28.3%) with ≥grade 2 LDT at 6 months showed inferior median PFS (8.1 months vs. 12.0 months, p=0.006) (Fig. 4A) and OS (18.7 months vs. 29.8 months, p=0.026) (Fig. 4B), compared to 160 patients (71.7%) without ≥grade 2 LDT, at 6 months.

Predictive factors for LDT at 6 months

Since ≥grade 2 LDT at 6 months was a significant prognostic factor for both PFS and OS, we analyzed the predictive factors that influence the incidence of ≥grade 2 LDT at 6 months before and after PSM (Table 4).

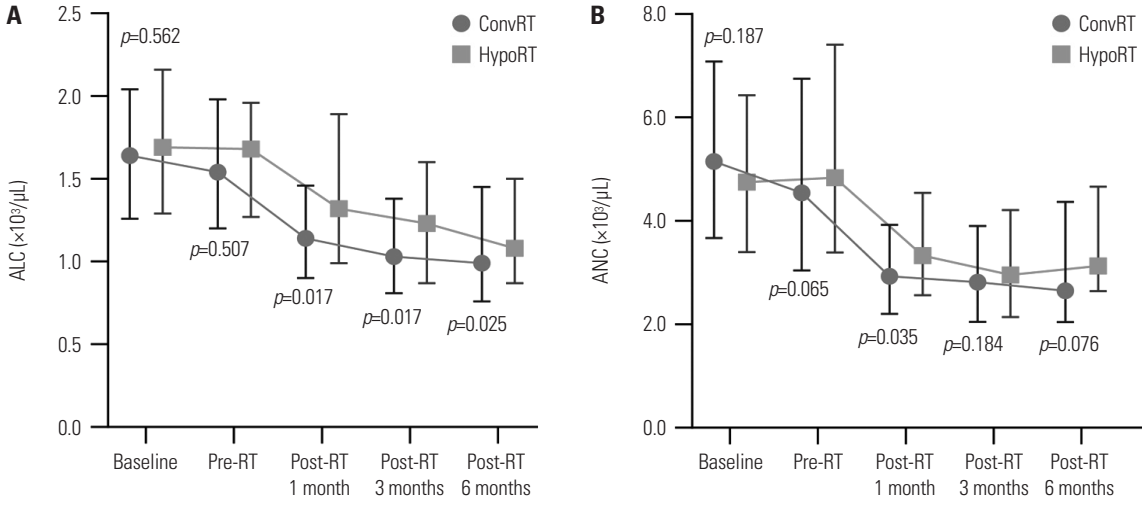

Fig. 3. Changes in ALC (A) and ANC (B) during treatment stratified by fractionation schedules. ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy; ALC, absolute lymphocyte count; ANC, absolute neutrophil count.

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HypoRT showed a significant association with ≥grade 2 LDT at 6 months in multivariate analysis [odds ratio (OR): 0.23, 95% CI: 0.10–0.48, p<0.001]. In addition, female sex (OR: 2.39, 95% CI: 1.26–4.58), pre-RT ALC (OR: 0.45, 95% CI: 0.25–0.79), and higher brain volume receiving more than 30 Gy (brain V30Gy, OR: 1.03, 95% CI: 1.02–1.10) were identified as independent predictors of ≥grade 2 LDT at 6 months. After PSM, HypoRT was consistently related to ≥grade 2 LDT at 6 months in multivariate analysis (OR: 0.09, 95% CI: 0.01–0.48, p=0.012).

We performed subsequent analysis for the cutoff point for

Table 3. Prognostic Factors for PFS and OS Using Cox Regression Analysis

	(ref. vs.)	Univariable analysis			Multivariable analysis		
		HR	95% CI	*p* value	HR	95% CI	*p* value
PFS							
Fractionation	(ConvRT vs. HypoRT)	1.09	0.81–1.47	0.566	1.23	0.87–1.72	0.238
Sex	(Male vs. female)	0.66	0.49–0.88	0.006	0.66	0.48–0.91	0.012
Age	(<70 years vs. ≥70 years)	1.13	0.74–1.71	0.571			
Preoperative KPS	(>70 vs. ≤70)	1.42	1.04–1.95	0.027	1.26	0.91–1.73	0.160
Postoperative KPS	(>70 vs. ≤70)	1.23	0.84–1.81	0.291			
Extent of resection	(GTR vs. non-GTR)	2.06	1.35–3.14	0.001	1.69	1.25–2.30	0.001
RT modality	(3D-CRT vs. IMRT)	1.05	0.76–1.44	0.773			
MGMT promoter	(Unmethylated vs. Methylated)	0.63	0.47–0.84	0.002	0.69	0.51–0.93	0.014
PTV volume	(<300 cm^3 vs. ≥300 cm^3)	1.24	0.86–1.79	0.260			
RF-PTV volume	(<120 cm^3 vs. ≥120 cm^3)	1.67	1.16–2.42	0.006	1.33	0.89–1.99	0.157
Post RT acute LDT	(Grade 0–1 vs. Grade 2–3)	0.73	0.43–1.24	0.243			
Post RT 6-month LDT	(Grade 0–1 vs. Grade 2–3)	1.56	1.13–2.13	0.006	1.63	1.16–2.29	0.005
OS							
Fractionation	(ConvRT vs. HypoRT)	1.16	0.77–1.73	0.487	2.12	0.91–4.49	0.648
Sex	(Male vs. female)	0.50	0.33–0.77	0.001	0.45	0.28–0.71	0.001
Age	(<70 years vs. ≥70 years)	2.40	1.44–4.00	0.001	2.15	1.26–3.68	0.005
Preoperative KPS	(>70 vs. ≤70)	1.45	0.95–2.22	0.087			
Postoperative KPS	(>70 vs. ≤70)	1.40	0.82–2.42	0.222			
Extent of resection	(GTR vs. non-GTR)	3.42	2.00–5.87	<0.001	2.01	1.31–3.07	0.001
RT modality	(3D-CRT vs. IMRT)	0.66	0.44–0.99	0.043	0.71	0.47–1.07	0.102
MGMT promoter	(Unmethylated vs. Methylated)	0.63	0.42–0.94	0.024	0.59	0.39–1.02	0.054
PTV volume	(<300 cm^3 vs. ≥300 cm^3)	1.27	0.77–2.11	0.353			
RF-PTV volume	(<120 cm^3 vs. ≥120 cm^3)	1.71	1.03–2.84	0.040	1.60	0.93–2.72	0.090
Post RT acute LDT	(Grade 0–1 vs. Grade 2–3)	0.73	0.43–1.24	0.243			
Post RT 6-month LDT	(Grade 0–1 vs. Grade 2–3)	1.64	1.06–2.56	0.027	1.94	1.20–3.14	0.007

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy; KPS, Karnofsky performance status; MGMT, O6-methylguanine-DNA methyltransferase; PTV, planning target volume; RF, reduced field; LDT, lymphopenia during treatment; GTR, gross total resection; 3D-CRT, three-dimensional conformal radiotherapy; IMRT, intensity-modulated radiotherapy.
The foreparts of the parentheses indicate the reference groups.

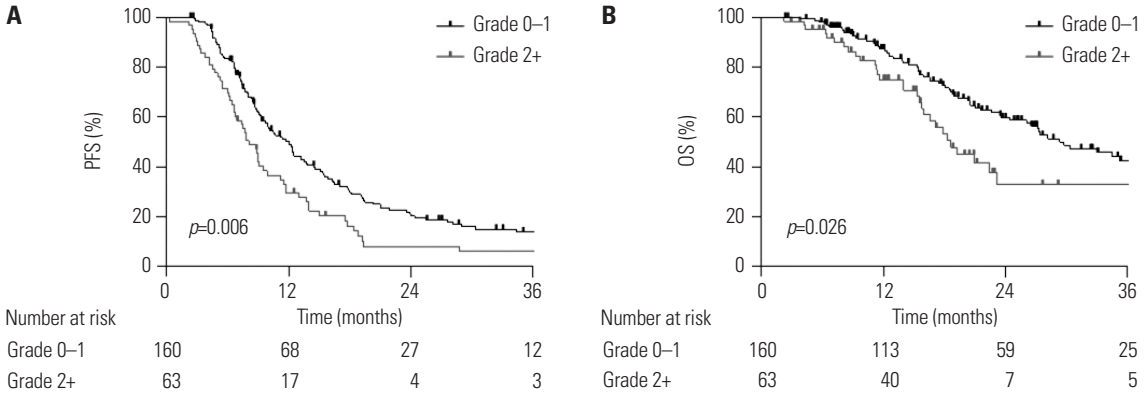

Fig. 4. Kaplan–Meier curves for progression-free survival (PFS) (A) and overall survival (OS) (B) according to lymphopenia at 6 months after radiotherapy.

Table 4. Predictive Factors for Grade 2 or More Lymphopenia at 6 Months after Chemoradiotherapy

		Univariate analysis			Multivariate analysis		
		OR	95% CI	*p* value	OR	95% CI	*p* value
Post RT 6-month lymphopenia before PSM							
Fractionation	(ConvRT vs. HypoRT)	0.29	0.14–0.59	0.001	0.23	0.10–0.48	<0.001
Sex	(Male vs. female)	2.37	1.31–4.34	0.004	2.39	1.26–4.58	0.008
Age	(<70 years vs. ≥70 years)	0.97	0.42–2.10	0.945			
Preoperative KPS	(>70 vs. ≤70)	1.26	0.68–2.28	0.457			
Postoperative KPS	(>70 vs. ≤70)	1.35	0.66–2.70	0.398			
Extent of resection	(GTR vs. Biopsy/PR)	1.35	0.69–2.58	0.375			
	(GTR vs. STR)	1.10	0.44–2.56	0.825			
MGMT promoter	(Unmethylated vs. Methylated)	0.67	0.37–1.21	0.193			
Baseline ALC	Continuous	0.57	0.34–0.89	0.020	0.68	0.38–1.15	0.173
Pre-RT ALC	Continuous	0.39	0.22–0.66	0.001	0.45	0.25–0.79	0.007
Adjuvant temozolomide	Continuous	0.87	0.67–1.15	0.657			
RT modality	(3D-CRT vs. IMRT)	0.54	0.29–1.02	0.056	0.68	0.38–1.13	0.197
PTV volume	(<300 cm^3 vs. ≥300 cm^3)	0.81	0.37–1.69	0.593			
RF-PTV volume	(<120 cm^3 vs. ≥120 cm^3)	1.60	0.79–3.20	0.185			
PTV volume	Continuous	1.00	1.00–1.00	0.831			
RF-PTV volume	Continuous	1.00	0.99–1.01	0.417			
Mean brain dose	Continuous	1.01	0.97–1.06	0.495			
Brain V5Gy	Continuous	1.00	0.98–1.02	0.862			
Brain V10Gy	Continuous	1.00	0.98–1.02	0.962			
Brain V15Gy	Continuous	1.00	0.98–1.02	0.957			
Brain V20Gy	Continuous	1.00	0.98–1.02	0.749			
Brain V30Gy	Continuous	1.01	0.98–1.03	0.648	1.03	1.02-1.10	0.046
Post RT 6-month lymphopenia after PSM							
Fractionation	(ConvRT vs. HypoRT)	0.14	0.02–0.60	0.017	0.09	0.01–0.48	0.012
Sex	(Male vs. female)	1.63	0.44–6.06	0.461			
Age	(<70 years vs. ≥70 years)	1.00	0.13–4.98	0.100			
Preoperative KPS	(>70 vs. ≤70)	0.90	0.21–3.38	0.879			
Postoperative KPS	(>70 vs. ≤70)	1.00	0.13–4.98	0.100			
Extent of resection	(GTR vs. Biopsy/PR)	0.64	0.13–2.59	0.553			
	(GTR vs. STR)	0.10	0.12–7.19	0.993			
MGMT promoter	(Unmethylated vs. Methylated)	0.95	0.26–3.68	0.941			
Baseline ALC	Continuous	0.48	0.14–1.45	0.215			
Pre-RT ALC	Continuous	0.30	0.08–0.98	0.061	0.17	0.02–0.71	0.032
Adjuvant temozolomide	Continuous	0.68	0.77–1.11	0.687			
PTV volume	(<300 cm^3 vs. ≥300 cm^3)	0.21	0.15–3.33	0.994			
RF-PTV volume	(<120 cm^3 vs. ≥120 cm^3)	0.15	0.31–1.06	0.994	0.98	0.87–1.06	0.620
PTV volume	Continuous	1.00	0.99–1.00	0.412			
RF-PTV volume	Continuous	0.99	0.97–1.01	0.445			
Mean brain dose	Continuous	0.93	0.81–1.03	0.186			
Brain V5Gy	Continuous	0.96	0.91–1.01	0.120			
Brain V10Gy	Continuous	0.97	0.92–1.01	0.179			
Brain V15Gy	Continuous	0.96	0.91–1.00	0.104			
Brain V20Gy	Continuous	0.96	0.91–1.01	0.168			
Brain V30Gy	Continuous	0.96	0.89–1.01	0.188			

RT, radiotherapy; PSM, propensity score matching; OR, odds ratio; CI, confidence interval; ConvRT, conventional fractionated radiotherapy; HypoRT, hypofractionated radiotherapy; KPS, Karnofsky performance status; MGMT, O6-methylguanine-DNA methyltransferase; ALC, absolute lymphocyte count; PTV, planning target volume; RF, reduced field; GTR, gross total resection; PR, partial resection; STR, subtotal resection; 3D-CRT, three-dimensional conformal radiotherapy; IMRT, intensity-modulated radiotherapy; VXXGy, volume receiving more than XX Gy; NA, not available.
The foreparts of parentheses indicate the reference groups.

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brain V30Gy because V30Gy showed an association with ≥grade 2 LDT at 6 months in multivariate analysis before PSM. With a cutoff value of V30Gy>30% for predicting ≥grade 2 LDT at 6 months, 44/112 patients (39.3%) with V30Gy>30% experienced ≥grade 2 LDT at 6 months, whereas only 19/111 patients (17.1%) with V30Gy≤30% showed ≥grade 2 LDT at 6 months (p<0.001).

DISCUSSION

Our study demonstrated that HypoRT for newly diagnosed GBM patients receiving concurrent temozolomide shows comparable oncologic outcomes and reduced LDT to those of ConvRT. Moreover, late LDT (6 months post-RT) was associated with inferior PFS and OS outcomes. Notably, HypoRT reduced the incidence of grade 2 or higher LDT at 6 months post-RT.

Apart from ConvRT explored in the Stupp regimen,[27] HypoRT with simultaneous integrated boost offers tolerable outcomes in patients with GBM receiving concomitant temozolomide.[4-9] A recent phase II study of 89 patients treated with 60 Gy in 20 fractions (50 Gy to low-risk areas) demonstrated tolerable toxicity.[4] They also showed comparable outcomes to those of ConvRT with median PFS and OS of 13.1 and 15.2 months, respectively. Cho, et al.[5] reported the outcomes of 40 patients with high-grade gliomas treated with 60 Gy in 25 fractions (50 Gy to low-risk areas). The median PFS and OS for GBM were 8.2 and 12.4 months, respectively. Another retrospective study with dose escalation of 64 Gy in 27 fractions (60 and 54 Gy to intermediate- and low-risk areas, respectively) showed comparable outcomes (median PFS and OS: 15 and 21 months, respectively) without any severe toxicities.[6] Although the radiation dose of 58.5 Gy used in the current study is not a dose-escalated regimen, as reported in aforementioned series, a dose scheme of 58.5 Gy in 25 fractions did not lead to inferior outcomes, compared with those reported in previous studies (median PFS and OS: 9.9 and 27.2 months, respectively). A recent meta-analysis of 399 patients across 12 studies including dose-escalated regimens also showed that dose escalation in the chemoRT had no beneficial effects in patients with GBM.[7] Also, a prospective randomized trial of NRG Oncology BN-001 demonstrated that dose escalation did not confer any survival benefit, compared with standard RT.[8] Therefore, the current HypoRT regimen without dose escalation could be considered as a non-inferior alternative treatment option with shortened overall treatment time for physicians and patients. In addition, there are several shorter HypoRT regimens for patients with GBM receiving concomitant temozolomide. Several retrospective studies with 40–45 Gy in 15 fractions showed a median OS of 5.6–11.0 months, inferior to the current OS outcomes. However, these studies mostly included older frail patients.[29-31]

A mathematical computational model has demonstrated that typical ConvRT at doses of 0.5 Gy and 2 Gy might induce radiation exposure to circulating blood cells and lymphocytes.[24] Given the radiosensitivity of lymphocytes among the hematopoietic cells, exposure to this radiation dose could induce LDT. Previous studies have suggested that brain V25Gy <40% and <56% could be optimal cutoff values for predicting LDT within 3 months after chemoRT.[17,25] In a comparative analysis of X-ray and proton beam therapy, brain V20Gy was considered as a significant independent variable.[26] Despite determining LDT at different timepoints, brain V30Gy <30% was consistently associated with a decreased incidence of LDT at 6 months after chemoRT in this study. This suggests that reduced radiation exposure from HypoRT significantly reduced the ALC changes during the treatment course, compared with ConvRT. The protective effect of HypoRT on ALC recovery remained significant during the 6-month Stupp treatment period. One of the potential benefits of HypoRT is minimization of LDT due to the reduced overall treatment time compared with ConvRT. Additionally, female patients were more prone to developing LDT than male patients, which is consistent with the findings of previous studies.[16,17,25,26] Mohan, et al. suggested that high cerebral blood flow and glucose metabolic rates observed in women might increase exposure levels of circulating lymphocytes to RT,[26,32] thus resulting in frequent LDT. Based on the results of the current analysis, HypoRT might be the preferred modality to prevent severe LDT in women with GBM.

The prognostic value of LDT in GBM has been explored in several studies.[13-17] Rudra, et al.[17] found that any LDT event within 3 months after the completion of RT was independently associated with inferior OS outcomes (HR: 1.83, 95% CI: 1.20–2.80). Byun, et al.[16] also found that 118 patients with LDT (35.1% of the entire cohort) had significantly worse clinical outcomes than those without LDT (median OS: 18.2 months vs. 22.0 months, p=0.028). They also pointed out the importance of assessing LDT in patients treated with modern immuno-oncological therapies. Lymphocyte reservoirs play an important role in the planning of immunotherapies, including vaccines, oncolytic viral therapies, and immune checkpoint inhibitors.[33,34] HypoRT prevents LDT, which, in turn, could preserve a patient's immunity. With the emergence of second-line immunotherapy for GBM, HypoRT can be adopted for patients with GBM planning for chemoRT.

The current study provides novel information regarding the association between LDT and Hypo-RT. However, this study has several inherent limitations. Owing to its single-center retrospective design, there might be unrecognized biases that were not completely addressed by the multivariable analysis. The small sample size of the HypoRT group represents a selection bias as the patients in each group were chosen based on physician preference. This may have led to overestimation of the potential benefits of HypoRT. In addition, it could result in a loss of statistical power in the multivariable and subgroup analyses. Since the subtypes of lymphocytes could not be analyzed, tumor-infiltrating lymphocytes, $CD8^+$ or $CD4^+$ effector T-cells, which mediate the antitumor immune responses, could

not be analyzed as well. Therefore, our findings should be interpreted with caution.

In conclusion, HypoRT with 58.5 Gy and 50 Gy in 25 fractions for newly diagnosed GBM treated with temozolomide appears to elicit comparable oncologic outcomes and reduced ALC changes during treatment. In this study, HypoRT decreased the occurrence LDT during the period of Stupp treatment, which could positively impact PFS and OS outcomes. Overall, this study is the first to report the potential benefits of HypoRT in reducing the risk of LDT. Further randomized controlled studies are warranted to confirm these findings.

AUTHOR CONTRIBUTIONS

Conceptualization: Nalee Kim and Do Hoon Lim. Data curation: all authors. Formal analysis: Nalee Kim and Do Hoon Lim. Investigation: all authors. Methodology: Nalee Kim and Do Hoon Lim. Project administration: Do Hoon Lim. Resources: all authors. Software: Nalee Kim. Supervision: Do Hoon Lim. Validation: Nalee Kim and Do Hoon Lim. Visualization: Nalee Kim. Writing—original draft: Nalee Kim and Do Hoon Lim. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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