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Brain Dose Burden Is Associated with Treatment-Related Lymphopenia in IDH- Wildtype Glioblastoma: Dosimetric Determinants and Prognostic Impact

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Abstract

Background and purpose

Treatment-related lymphopenia is common during chemoradiotherapy for glioblastoma and has been associated with adverse outcome. Prior studies have implicated whole-brain V20–V25, mean brain dose, and treatment-volume reduction, but the relative contribution of target size and cerebral dose burden remains uncertain. We evaluated the relationship between brain dose-volume exposure, longitudinal lymphocyte depletion, and landmark outcome in 102 patients with IDH-wildtype glioblastoma.

Materials and methods

This retrospective single-center cohort included 102 adults with histologically confirmed IDH-wildtype glioblastoma, paired blood counts, target-volume and brain-DVH data, and clinical follow-up. Absolute lymphocyte count (ALC) was assessed before radiotherapy and at treatment completion. Brain mean dose, V5, V10, V15, V20, V25, integral dose, target volumes, corticosteroid exposure, temozolomide, and clinical-molecular variables were evaluated. V20 was used as the principal clinically interpretable dosimetric exposure, with the remaining dose metrics treated as correlated secondary exposures. Multivariable robust linear regression modeled log2 end-of-radiotherapy ALC after adjustment for baseline ALC and major confounders. Survival analyses were anchored at radiotherapy completion.

Results

Median ALC decreased from 1810 to 1050 cells/μL, corresponding to a median relative decline of 42.6%. Grade ≥ 2 and grade ≥ 3 lymphopenia occurred in 33 (32.4%) and 14 (13.7%) patients, respectively. Greater cerebral dose burden was associated with lower lymphocyte preservation. In adjusted models, mean brain dose (β per 5 Gy, − 0.251; 95% CI, − 0.430 to − 0.071; p = 0.006), V15 (β per 10 percentage points, − 0.198; p = 0.005), V20 (β, − 0.241; p = 0.003), V25 (β, − 0.262; p = 0.004), and integral dose (β per 5 Gy·L, − 0.197; p = 0.001) predicted lower end-of-radiotherapy ALC; V10 was also significant, whereas V5 was not. PTV was strongly correlated with V20 (ρ = 0.78, p < 0.001) but showed little direct association with lymphocyte preservation (ρ=−0.06, p = 0.55). Grade ≥ 2 lymphopenia was independently associated with inferior landmark overall survival (HR, 2.43; 95% CI, 1.30–4.56; p = 0.005) and progression-free survival (HR, 2.42; 95% CI, 1.37–4.29; p = 0.004).

Conclusion

Greater cerebral dose burden is associated with treatment-related lymphocyte depletion beyond the information conveyed by target volume alone. The most consistent associations involve mean brain dose, V15–V25, and integral dose, while clinically relevant grade ≥ 2 lymphopenia identifies patients with

substantially inferior landmark outcome. These findings support prospective evaluation of immune-sparing radiotherapy strategies that reduce unnecessary cerebral dose exposure without compromising target coverage.

Highlights

· Brain dose-volume exposure shows a coherent association with treatment-related lymphocyte depletion.

· The most consistent dosimetric signals involve intermediate-dose exposure (V15–V25), mean brain dose, and integral dose.

· GTV, CTV, and PTV volume alone do not explain lymphocyte depletion, suggesting that dose distribution provides information beyond target size.

· Grade ≥ 2 lymphopenia remains independently associated with inferior landmark overall and progression-free survival.

· The dose–lymphocyte relationship persists after adjustment for baseline ALC, target volume, corticosteroid exposure, temozolomide, infection, and fraction number.

1. Introduction

Glioblastoma, IDH-wildtype, is the most aggressive adult-type diffuse glioma and is defined by integrated histologic and molecular criteria in the 2021 World Health Organization classification [1]. Maximal safe resection followed by conventionally fractionated radiotherapy with concurrent and adjuvant temozolomide remains the standard postoperative treatment for fit patients [2, 3]. Despite multimodal therapy, recurrence is common and long-term survival remains limited.

Treatment-related lymphopenia has emerged as a clinically relevant host factor in glioblastoma. Circulating lymphocytes are highly radiosensitive, and repeated irradiation can expose substantial fractions of the circulating blood pool to biologically meaningful doses [4]. Temozolomide, corticosteroids, baseline immune status, infection, tumor-related inflammation, and radiotherapy may all contribute to lymphocyte depletion [5–10, 19]. Across multiple glioblastoma cohorts, severe lymphopenia or low post-treatment ALC has been associated with inferior survival [6–10].

The potentially modifiable component is radiotherapy-related exposure. Reduced treatment volumes have been associated with lower rates of severe lymphopenia [11], and a randomized phase II comparison of proton versus photon therapy demonstrated a lower probability of grade ≥ 3 lymphopenia with proton therapy, with whole-brain dose-volume exposure emerging as a relevant predictor [12]. Additional series and predictive models have implicated mean brain dose, V20–V25, baseline ALC, sex, and other clinical variables [13–15, 20]. However, target volume and brain DVH metrics are strongly

interrelated, making it difficult to determine whether lymphocyte depletion reflects target size itself or the broader dose distribution through the highly vascularized brain.

We hypothesized that cerebral dose burden would be associated with treatment-related lymphocyte depletion beyond target-volume size alone. The primary objective was to assess the relationship between normal-brain V20 and end-of-radiotherapy lymphocyte depletion after adjustment for baseline ALC and major clinical confounders. Mean brain dose, V10, V15, V25, and integral dose were evaluated as correlated secondary dosimetric exposures. Secondary objectives were to assess grade ≥ 2 lymphopenia as a clinically interpretable endpoint and to examine its prognostic association with landmark outcome.

2. Materials and methods

2.1 Study design and population

This retrospective single-center cohort included 102 adult patients (January 2018 - December 2025) with histologically confirmed glioblastoma, IDH-wildtype. Patients were eligible when paired blood counts before radiotherapy and at treatment completion, target-volume and brain-DVH data, and clinical follow-up were available. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Azienda Ospedaliero-Universitaria Senese, Area Vasta Sud-Est (protocol code GLIOMARKERSOBS_2025-1; approval date 6 May 2025). Informed consent was obtained from all individual participants included in the study.

2.2 Clinical, molecular, and treatment variables

Clinical variables included age, sex, Karnofsky performance status (KPS), focality, extent of surgery, dexamethasone dose at radiotherapy initiation and completion, infection and antibiotic treatment during radiotherapy, treatment interruptions, and temozolomide administration. Extent of surgery was classified as gross total resection (GTR), subtotal resection (STR), or biopsy. Molecular variables included IDH status and MGMT promoter methylation. Radiotherapy was delivered using IMRT, VMAT, or helical tomotherapy. The principal schedules were 60 Gy in 30 fractions and 59.4 Gy in 33 fractions.Target volume delineation was performed according to institutional standards consistent with international guidelines [21]. The gross tumor volume (GTV) included the postoperative resection cavity and any residual contrast-enhancing tumor on T1-weighted MRI. The clinical target volume (CTV) was generated by isotropic expansion of the GTV, typically 1.5 cm, and adjusted to anatomical boundaries. The planning target volume (PTV) was obtained by adding a margin to the CTV to account for setup uncertainties typically 3 mm.

2.3 Dosimetric assessment

GTV, CTV, PTV, brain volume, brain mean dose, V5, V10, V15, V20, and V25 were analyzed. The brain was contoured as the whole intracranial brain parenchyma, excluding the brainstem; target volumes (GTV, CTV, and PTV) were not subtracted from the brain structure. Dose-volume histogram parameters were

therefore expressed as whole-brain metrics. Integral dose was calculated as mean brain dose multiplied by the corresponding brain volume in liters and expressed in Gy·L. The same clinical brain structure was used consistently for Dmean and V5–V25 extraction within each treatment plan. Because whole-brain dose-volume parameters inherently reflect, in part, target-volume size, PTV volume was included as an adjustment variable in the multivariable models. Additional analyses examined the association between V20 and lymphocyte preservation after accounting for PTV and total brain volume. V20 was selected as the principal clinically interpretable dosimetric exposure because of prior literature and its direct relevance to treatment planning; mean dose, V10, V15, V25, and integral dose were treated as correlated secondary exposures. Each dosimetric variable was evaluated in a separate adjusted model to limit multicollinearity.

2.4 Hematologic assessment

ALC and the absolute neutrophil, monocyte, and platelet counts were evaluated before radiotherapy and at treatment completion. NLR, PLR, SII, and PIV were derived. The lymphocyte ratio was defined as end-of-radiotherapy ALC divided by baseline ALC and log2-transformed. Lymphopenia was graded using CTCAE version 5.0 thresholds: grade 1, ALC < 1000 to 800 cells/μL; grade 2, < 800 to 500 cells/μL; grade 3, < 500 to 200 cells/μL; and grade 4, < 200 cells/μL [17].

2.5 Outcomes and landmark definition

Overall survival (OS) was defined from the date of diagnosis to death from any cause or last follow-up. Progression-free survival (PFS) was defined from diagnosis to radiographic or histopathologic progression or death, whichever occurred first. Radiographic progression was retrospectively adjudicated according to the Response Assessment in Neuro-Oncology 2.0 (RANO 2.0) criteria [18]. For newly diagnosed glioblastoma, the first post-radiotherapy MRI was used as the reference baseline for subsequent response assessment, and progression was primarily evaluated on contrast-enhancing disease. Because of the high incidence of pseudoprogression during the early post-treatment period, apparent radiographic progression occurring within 12 weeks after completion of radiotherapy required confirmation on a subsequent MRI or histopathologic evidence of unequivocal viable tumor before being classified as progression. When the distinction between tumor progression and treatment-related changes remained uncertain, cases were reviewed within the multidisciplinary neuro-oncology board, integrating serial imaging findings, clinical evolution, corticosteroid requirement, and, when available, advanced imaging or histopathologic information. For prognostic analyses of biomarkers measured at radiotherapy completion, radiotherapy completion was used as the landmark. Patients who had progressed at or before the landmark were excluded. The landmark cohort comprised 96 patients; 92 complete cases entered the multivariable Cox models, with 65 deaths and 83 progression-or-death events.

2.6 Statistical analysis

Continuous variables were summarized as medians and interquartile ranges and categorical variables as counts and percentages. Paired hematologic values were compared using the Wilcoxon signed-rank test.

Spearman coefficients quantified unadjusted associations between dosimetric variables and the log2 end-to-baseline lymphocyte ratio. The primary dosimetric model used ordinary least-squares regression with HC3 robust standard errors and log2 end-of-radiotherapy ALC as the dependent variable. Models adjusted for sex, age, log2 baseline ALC, PTV, dexamethasone at radiotherapy completion, concurrent temozolomide, infection, and fraction number. Each dosimetric exposure was entered separately. Dose-response nonlinearity was explored using cubic spline terms with 3 degrees of freedom. Grade ≥ 2 lymphopenia was assessed using logistic regression.

Multiplicity across the seven core dosimetric variables (mean dose, V5, V10, V15, V20, V25, and integral dose) was controlled using the Benjamini-Hochberg false-discovery-rate procedure. Landmark Cox models were anchored at radiotherapy completion and adjusted for age, KPS, MGMT status, gross total resection, PTV, mean brain dose, and grade ≥ 2 lymphopenia. A parallel sensitivity model replaced grade ≥ 2 lymphopenia with the continuous end-to-baseline ALC ratio. Two-sided p values < 0.05 were considered statistically significant.

3. Results

3.1 Patient and treatment characteristics

The cohort comprised 102 patients. Median age was 59.7 years (IQR, 53.7–68.3), and median KPS was 90 (IQR, 80–100). Seventy-six patients (74.5%) were male and 61 (59.8%) had MGMT promoter methylation. Gross total resection was performed in 35 patients (34.3%), subtotal resection in 45 (44.1%), and biopsy in 22 (21.6%). Eighty-seven patients (85%) received 60 Gy in 30 fractions and 15 patients received 59.4 Gy in 33 fractions, and 99 (99%) received concurrent temozolomide. Median dexamethasone dose was 8 mg/day at radiotherapy initiation and 4 mg/day at completion.

Median GTV, CTV, and PTV were 33.5 cm^3 (IQR, 20.4–55.0), 135.3 cm^3 (IQR, 98.7–170.1), and 192.4 cm^3 (IQR, 153.0–251.0), respectively (Table 1).

Table 1
Baseline and treatment characteristics of the cohort (n = 102).

Characteristic	Value
Age, years	59.7 (53.7–68.3)
Male sex	76 (74.5%)
KPS	90 (80–100)
MGMT promoter methylated	61 (59.8%)
Gross total resection	35 (34.3%)
Subtotal resection	45 (44.1%)
Biopsy	22 (21.6%)
GTV, cm^3	33.5 (20.4–55.0)
CTV, cm^3	135.3 (98.7–170.1)
PTV, cm^3	192.4 (153.0–251.0)
60 Gy/30 fx or 59.4 Gy/33 fx	102 (100%)
VMAT	48 (47%)
Helical tomotherapy	31 (30%)
IMRT	23 (23%)
Concurrent temozolomide	99 (99%)
Dexamethasone at RT start, mg/day	8 (4–12)
Dexamethasone at RT end, mg/day	4 (0–8)
Overall treatment time, days	44 (41–46)

3.2 Brain dose exposure and hematologic changes

Median brain mean dose was 23.93 Gy (IQR, 18.88–27.89). Median V5, V10, V15, V20, and V25 were 82.9%, 71.8%, 59.9%, 48.0%, and 37.3%, respectively; median integral dose was 33.64 Gy·L (IQR, 27.26–39.60) (Table 2). Median ALC decreased from 1810 cells/μL (IQR, 1405–2375) to 1050 cells/μL (IQR, 715–1500), corresponding to a median relative decline of 42.6% (Fig. 1). Grade ≥ 2 lymphopenia occurred in 33 patients (32.4%) and grade ≥ 3 lymphopenia in 14 (13.7%). NLR increased from a median of 4.47 to 6.15, PLR from 126.5 to 181.6, and SII from 955 to 1214, whereas PIV did not show a consistent paired change.

Table 2
Brain dosimetry and hematologic changes during radiotherapy.

Variable	Median (IQR) or n (%)
Brain mean dose, Gy	23.93 (18.88–27.89)
Brain V5, %	82.9 (68.3–89.0)
Brain V10, %	71.8 (58.1–81.5)
Brain V15, %	59.9 (47.0–72.5)
Brain V20, %	48.0 (35.9–58.5)
Brain V25, %	37.3 (27.4–49.0)
Integral dose, Gy·L	33.64 (27.26–39.60)
ALC before RT, cells/μL	1810 (1405–2375)
ALC at RT completion, cells/μL	1050 (715–1500)
Median relative ALC change	−42.6%
NLR before RT	4.47
NLR at RT completion	6.15
Grade ≥ 2 lymphopenia	33/102 (32.4%)
Grade ≥ 3 lymphopenia	14/102 (13.7%)

3.3 Dosimetric determinants of lymphocyte depletion

Unadjusted analyses showed a coherent inverse relationship between cerebral dose exposure and lymphocyte preservation. The direction was uniform across mean dose and V5–V25, with higher brain exposure associated with a lower end-to-baseline ALC ratio; the relationship is illustrated for V20 in Fig. 2A.

In adjusted models, the association persisted across the intermediate-dose range (Table 3; Fig. 2B). Mean brain dose was associated with lower end-of-radiotherapy ALC (β per 5 Gy, − 0.251; 95% CI, − 0.430 to − 0.071; p = 0.006). V15 (β per 10 percentage points, − 0.198; p = 0.005), V20 (β, − 0.241; p = 0.003), V25 (β, − 0.262; p = 0.004), and integral dose (β per 5 Gy·L, − 0.197; p = 0.001) were significant. V10 was also associated with lower ALC (β, − 0.156; p = 0.018), whereas V5 was not independently significant.

Table 3
Adjusted associations between brain dosimetry and log2 end-of-radiotherapy ALC (n = 102).

Dosimetric variable	Unit	β	95% CI	P-value	FDR q-value	Estimated end-ALC change
Mean brain dose	per 5 Gy	−0.251	−0.430 to − 0.071	0.006	0.008	−15.9%
V5	per 10 percentage points	−0.010	−0.204 to 0.185	0.923	0.923	−0.7%
V10	per 10 percentage points	−0.156	−0.286 to − 0.027	0.018	0.021	−10.3%
V15	per 10 percentage points	−0.198	−0.335 to − 0.061	0.005	0.008	−12.8%
V20	per 10 percentage points	−0.241	−0.398 to − 0.083	0.003	0.008	−15.4%
V25	per 10 percentage points	−0.262	−0.443 to − 0.082	0.004	0.008	−16.6%
Integral dose	per 5 Gy·L	−0.197	−0.315 to − 0.080	0.001	0.007	−12.8%

Table 4
Multivariable Cox regression for landmark OS and PFS using grade ≥ 2 lymphopenia (complete-case n = 92).

Variable	Unit	OS HR (95% CI)	OS p-value	PFS HR (95% CI)	PFS p-value
Age	per 10 years	1.20 (0.92–1.56)	0.180	1.12 (0.90–1.39)	0.300
KPS	per 10 points	0.89 (0.74–1.07)	0.210	0.92 (0.79–1.08)	0.300
MGMT unmethylated	yes vs no	2.35 (1.28–4.33)	0.006	2.06 (1.21–3.51)	0.008
Gross total resection	yes vs no	0.49 (0.26–0.91)	0.021	0.52 (0.30–0.90)	0.019
PTV	per 100 cm^3	1.46 (0.91–2.33)	0.110	1.69 (1.05–2.70)	0.030
Mean brain dose	per 5 Gy	1.09 (0.74–1.60)	0.680	1.12 (0.80–1.57)	0.510
Grade ≥ 2 lymphopenia	yes vs no	2.43 (1.30–4.56)	0.005	2.42 (1.37–4.29)	0.002

The coefficients have a direct clinical interpretation. An increase of 5 Gy in mean brain dose corresponds to an estimated 16% lower end-of-radiotherapy ALC, whereas a 10-percentage-point increase in V20 corresponds to approximately 15% lower ALC and a 10-percentage-point increase in V25 to approximately 17% lower ALC, after adjustment for baseline ALC and the other prespecified covariates. This pattern suggests that the biologically relevant signal is not restricted to very low-dose exposure but is concentrated across the low-to-intermediate dose range.

In contrast, GTV, CTV, and PTV volume did not show an independent monotonic association with lymphocyte preservation. PTV was strongly related to brain dose burden, but PTV itself showed little direct relationship with the lymphocyte ratio. This separation between geometry and immune effect is illustrated in Fig. 3: brain V20 remained inversely associated with lymphocyte preservation after adjustment for PTV and brain volume.

In logistic models, grade ≥ 2 lymphopenia was associated with mean brain dose (OR per 5 Gy, 2.19; p = 0.016), V10 (OR per 10 percentage points, 1.58; p = 0.034), V20 (OR, 2.53; p = 0.001), and V25 (OR, 2.46; p = 0.003). Restricted cubic splines were compatible with an approximately linear dose-response over the observed exposure range rather than a sharply defined threshold.

3.4 Landmark survival analysis

Ninety-six patients entered the radiotherapy-completion landmark analysis and 92 were complete for multivariable Cox modeling, with 65 deaths and 83 progression-or-death events. The continuous

lymphocyte ratio showed only a borderline association with OS (HR per doubling, 0.70; p = 0.053) and was not associated with PFS.

By contrast, clinically relevant lymphopenia was a strong prognostic classifier. Grade ≥ 2 lymphopenia was independently associated with inferior OS (HR, 2.43; p = 0.005) and PFS (HR, 2.42; p = 0.004). Grade ≥ 3 lymphopenia produced larger effect estimates, although the smaller number of severe events requires cautious interpretation. End-of-radiotherapy NLR was associated with OS but not PFS.

Median landmark OS was 17.4 months in patients with grade 0–1 lymphopenia versus 9.8 months in those with grade ≥ 2 lymphopenia; corresponding landmark PFS medians were 9.3 and 3.8 months (Fig. 4). In multivariable models, MGMT-unmethylated status and absence of gross total resection remained adverse prognostic factors. PTV was associated with PFS but not clearly with OS, whereas mean brain dose was not directly associated with either survival endpoint. The grade ≥ 2 landmark models are summarized in Fig. 5.

Model discrimination was approximately 0.67 for the clinical OS model, 0.72 after addition of PTV and brain dose, and 0.74 after addition of the continuous lymphocyte ratio. The incremental contribution of the continuous ratio remained modest, reinforcing grade ≥ 2 lymphopenia as the more clinically robust prognostic endpoint.

4. Discussion

This study identifies four findings with potential relevance to immune-sparing radiotherapy. First, cerebral dose burden is consistently associated with lymphocyte depletion after adjustment for baseline ALC and major clinical confounders. Second, the relationship is not confined to a single DVH point: mean dose, V15, V20, V25, and integral dose show concordant effects, suggesting a broader low-to-intermediate dose phenomenon (Fig. 2). Third, target volume alone does not reproduce the same association, indicating that the spatial distribution of dose across the brain may contain immune-relevant information beyond PTV size (Fig. 3). Fourth, clinically relevant grade ≥ 2 lymphopenia remains prognostically informative for both OS and PFS (Figs. 4 and 5), whereas the continuous lymphocyte ratio is less robust after full multivariable adjustment.

The most novel observation is the apparent separation between target size and cerebral dose burden. PTV is expected to correlate strongly with mean brain dose and V20–V25 because larger targets expose more brain. However, GTV, CTV, and PTV were not themselves associated with lymphocyte preservation, whereas the brain DVH metrics were. This argues against a purely volumetric explanation and raises the possibility that planning geometry, beam arrangement, conformity, and the volume of vascularized brain receiving repeated intermediate doses influence lymphocyte exposure. From a radiotherapy perspective, this distinction is important because target size is largely dictated by disease, whereas dose spill outside the target is at least partly modifiable.

The dose range implicated by the analysis is also informative. V5 shows an unadjusted association with depletion but loses its effect after covariate adjustment, whereas V15–V25 remain consistently associated with end-of-treatment ALC. Mean dose and integral dose show the same direction. Together, these findings point toward cumulative low-to-intermediate cerebral dose burden rather than a single very-low-dose threshold. This is biologically plausible because circulating lymphocytes repeatedly traverse the cerebral vascular bed over 30–33 fractions, and both the fraction of blood exposed and the dose delivered during each transit may contribute to cellular injury [4].

The results align with previous reports linking brain V20–V25 and treatment-volume reduction to radiation-induced lymphopenia. Rudra et al. reported lower rates of acute severe lymphopenia with reduced-volume chemoradiotherapy and identified brain V25 as a relevant predictor [11]. In the randomized phase II proton-versus-photon study, proton therapy reduced high-grade lymphopenia and whole-brain V20 was among the strongest dosimetric variables [12]. Byun et al. and Ye et al. similarly incorporated cerebral dose metrics into predictive models [13, 14], and a recent multi-institutional nomogram further supports the feasibility of integrating clinical and dosimetric information [15]. The present analysis adds a potentially important nuance: the immune signal may be better understood as a distributed brain-dose effect rather than as a consequence of PTV volume per se.

Integral dose deserves particular attention because it combines mean dose and irradiated volume and therefore represents global energy deposition within the contoured brain structure. It is less familiar clinically than V20 or V25 and should not immediately be proposed as a treatment constraint, but it may be an attractive research metric for immune-aware planning. A practical strategy for future validation would be to prespecify V20 as the principal clinically interpretable DVH variable while treating V15, V25, mean dose, and integral dose as a coherent family of secondary metrics.

The logistic results strengthen the clinical interpretation. A 10-percentage-point increase in V20 corresponds to approximately 2.5-fold higher odds of grade ≥ 2 lymphopenia, and V25 produces a similar effect. This does not establish a safe threshold. The spline analysis does not support a sharp inflection point within the exposure range, and the findings are therefore better interpreted as a continuous dose-response. Prospective work should determine whether a clinically useful constraint can be identified and whether reducing V20–V25 without compromising target coverage genuinely lowers severe lymphopenia.

The survival results refine the biological narrative. The continuous lymphocyte ratio is directionally favorable but only borderline for OS and nonsignificant for PFS. In contrast, grade ≥ 2 lymphopenia is independently associated with both endpoints. This may indicate a clinically relevant level of immune suppression that captures reduced physiologic reserve, impaired treatment tolerance, infection susceptibility, or broader host vulnerability. Alternatively, categorical lymphopenia may be more robust to measurement noise than a ratio constructed from two laboratory values. Grade ≥ 3 lymphopenia shows larger hazard ratios, but the smaller number of severe events warrants cautious interpretation.

The lack of a direct independent association between mean brain dose and survival should not be viewed as contradictory. Dosimetry is upstream of several competing biological and clinical processes, while survival is influenced by MGMT status, extent of surgery, tumor burden, subsequent treatment, and salvage options. The data are compatible with a pathway in which greater brain dose burden contributes to treatment-related immune depletion, and clinically relevant immune depletion identifies patients with worse outcome. However, the present retrospective framework cannot establish formal causal mediation. Demonstrating such a pathway would require temporally resolved blood sampling, preferably weekly ALC and lymphocyte subsets, cumulative corticosteroid exposure, and a larger dataset suitable for causal mediation analysis.

The findings have practical implications for immune-sparing radiotherapy. They do not support reducing target coverage or compromising established oncologic principles. Rather, they suggest that plan optimization might incorporate the goal of limiting unnecessary intermediate-dose exposure to uninvolved brain once target coverage and standard organ-at-risk constraints are satisfied. This could favor more conformal beam arrangements, reduced margins when oncologically appropriate, and technologies such as proton therapy in selected patients. The concept is particularly relevant as immunotherapeutic and vaccine-based strategies continue to be investigated in glioblastoma, where preservation of systemic immune competence may become increasingly important.

Several limitations should be considered in a final clinical study. The retrospective single-center design limits causal inference and external generalizability. Blood counts at limited time points may not capture the true nadir or recovery kinetics. Dexamethasone exposure is represented by point doses rather than cumulative exposure. Brain DVHs remain a surrogate for dose delivered to circulating blood; cerebral-vessel or blood-pool modeling may provide a more mechanistically specific measure [16]. Finally, the dosimetric variables are highly collinear, so a confirmatory analysis should prespecify a primary exposure rather than interpret every significant Vx as an independent biological discovery.

The principal strength of the study is its integrated design: paired longitudinal hematology, molecularly defined disease, target volumes, brain dosimetry, treatment variables, and landmark survival are evaluated within the same framework. The internally coherent V15–V25, mean-dose, and integral-dose signal provides a strong hypothesis-generating basis for prospective immune-sparing planning studies.

5. Conclusions

In patients with IDH-wildtype glioblastoma, greater low-to-intermediate cerebral dose burden is associated with treatment-related lymphocyte depletion independently of baseline immune status and target volume. The most consistent dosimetric signals involve mean brain dose, V15–V25, and integral dose, whereas target size alone does not explain the immune effect. Clinically relevant grade ≥ 2 lymphopenia is independently associated with substantially worse landmark overall and progression-free survival. The practical message is clear: once tumor coverage and established organ-at-risk constraints

are secured, reducing unnecessary cerebral dose exposure should be prospectively tested as a strategy to preserve systemic immune competence during glioblastoma radiotherapy.

Declarations

Ethics approval:

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Azienda Ospedaliero-Universitaria Senese, Area Vasta Sud-Est (protocol code GLIOMARKERSOBS_2025-1; approval date 6 May 2025).

Consent to participate:

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

Competing interests:

The authors declare no competing interests.

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Author Contribution

Author contributions: Conceptualization, P.T. and F.D.; methodology, P.T., F.D., E.V. and F.B.B.; investigation, F.D., G.B., P.P., G.R., T.C., M.V., A.M.D. and E.V.; data curation, F.D., M.V., A.M.D. and E.V.; validation, P.T., E.V. and F.B.B.; visualization, P.T. and F.D.; writing – original draft, P.T. and F.D.; writing – review and editing, all authors; supervision, P.T. and F.B.B.; project administration, P.T. All authors read and approved the final version of the manuscript.

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Data availability:

The data presented in this study are not publicly available due to privacy and institutional restrictions. De-identified data may be available from the corresponding author on reasonable request and subject to

approval by the local Ethics Committee and institutional policies.

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Figures

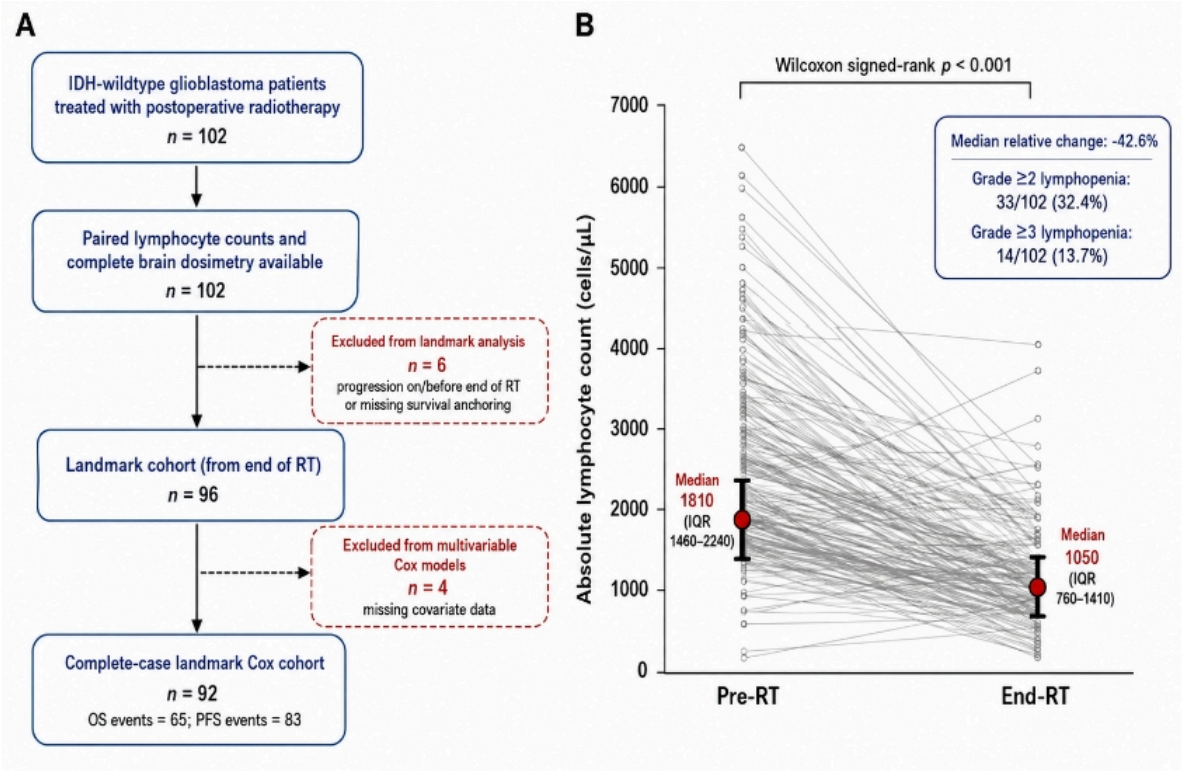

Figure 1

Study workflow and longitudinal lymphocyte depletion. A, cohort flow from the 102-patient analytic cohort to the radiotherapy-completion landmark and complete-case Cox population. B, paired absolute lymphocyte counts before radiotherapy and at treatment completion, showing the within-patient decline in ALC and the frequency of grade ≥2 and grade ≥3 lymphopenia.

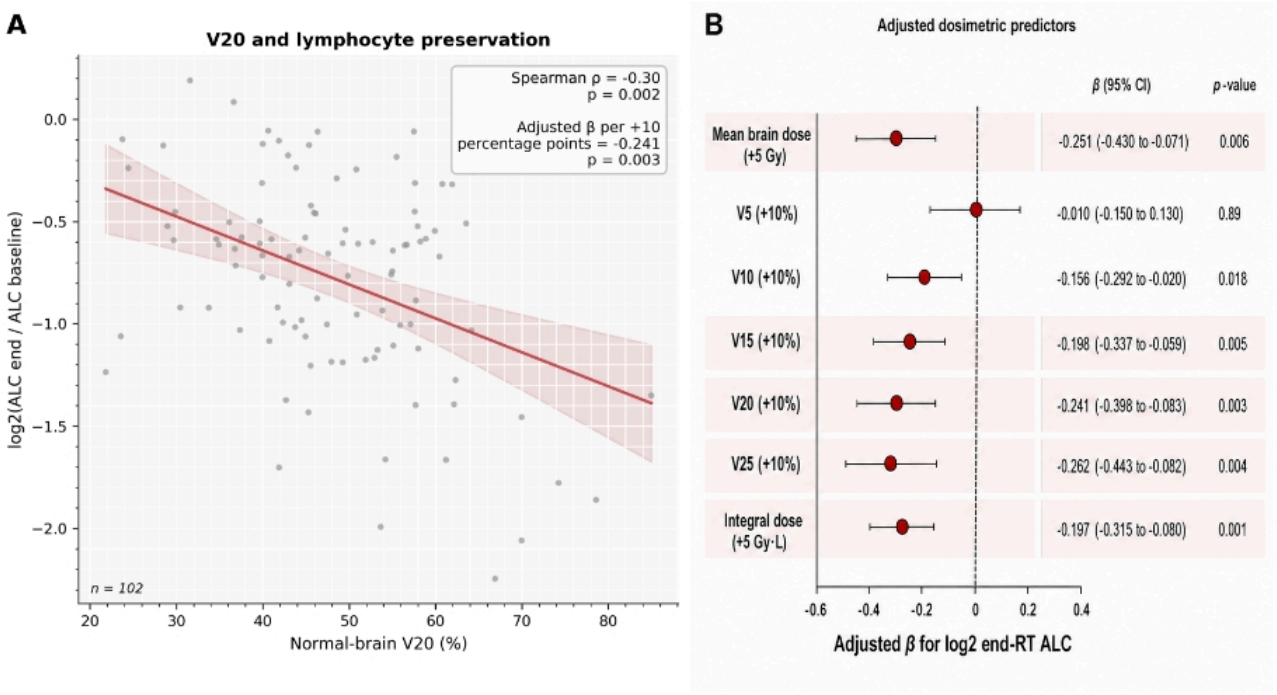

Figure 2

Association between cerebral dose burden and treatment-related lymphocyte depletion. A, relationship between whole brain V20 and lymphocyte preservation, expressed as log2(end-of-radiotherapy ALC/baseline ALC). B, adjusted coefficients and 95% confidence intervals for the principal dosimetric exposures evaluated in separate multivariable models. Negative coefficients indicate lower end-of-radiotherapy ALC with increasing brain dose exposure.

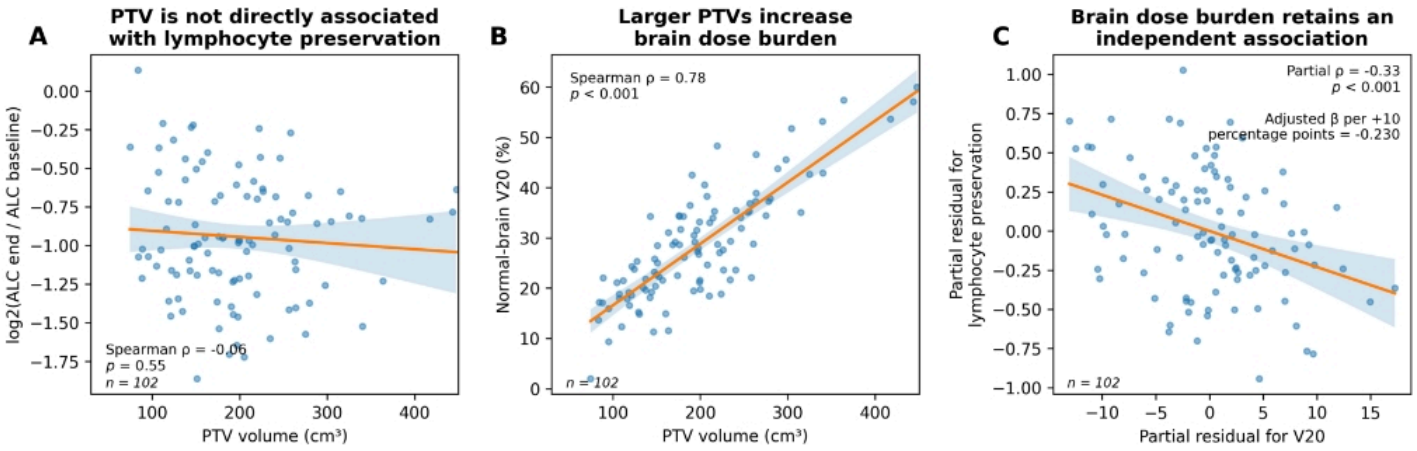

Figure 3

Disentangling target size from cerebral dose burden in treatment-related lymphocyte depletion. A, PTV volume showed little direct association with lymphocyte preservation. B, increasing PTV volume was strongly associated with greater whole brain V20. C, the inverse association between V20 and lymphocyte preservation persisted after adjustment for PTV and brain volume, suggesting that cerebral dose distribution contains information beyond target size alone.

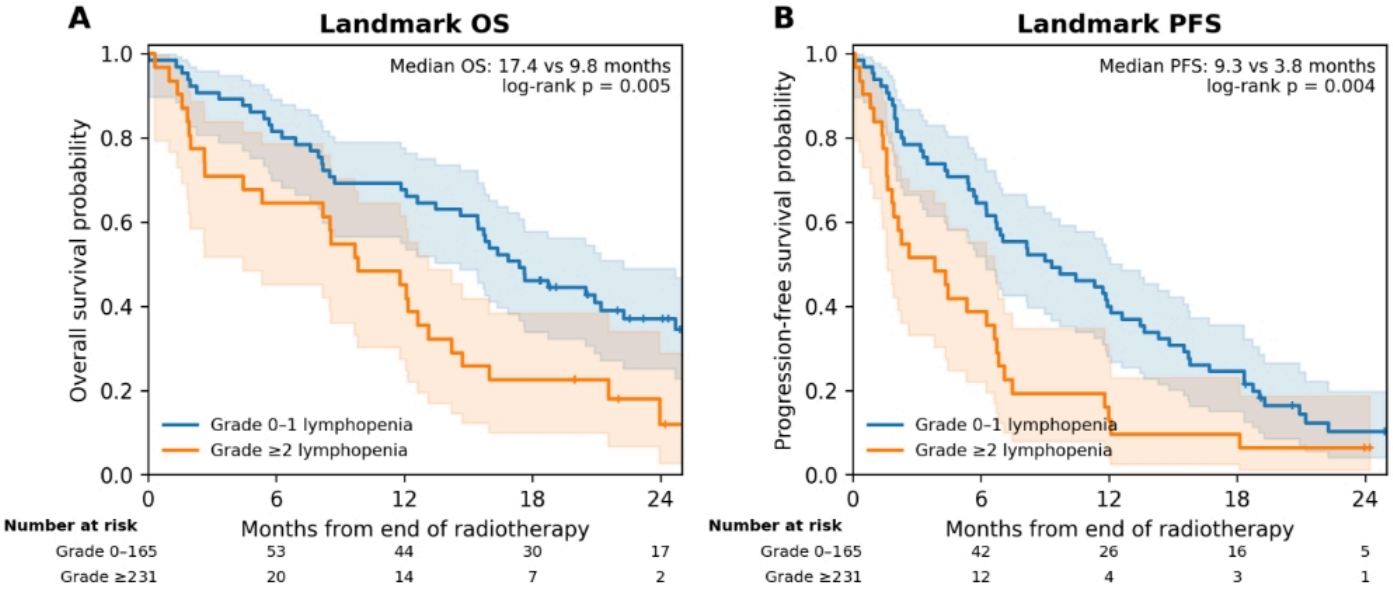

Figure 4

Landmark survival according to lymphopenia severity at radiotherapy completion. A, overall survival. B, progression-free survival. Patients with grade ≥2 lymphopenia had shorter landmark OS and PFS than patients with grade 0–1 lymphopenia; numbers at risk are displayed below each curve.

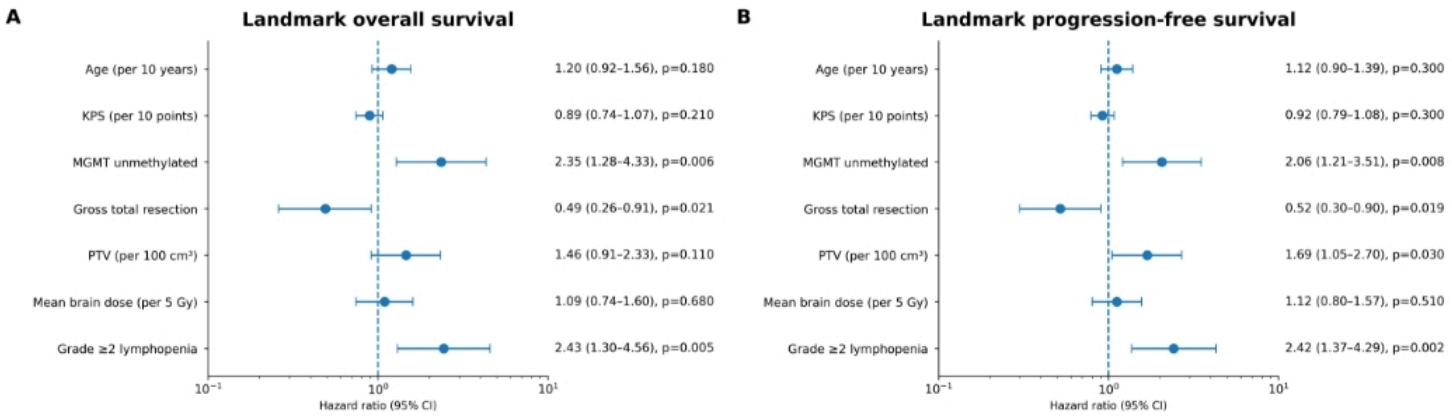

Figure 5

Multivariable landmark survival models using clinically relevant lymphopenia. A, adjusted hazard ratios for overall survival. B, adjusted hazard ratios for progression-free survival. Grade ≥2 lymphopenia remained associated with inferior outcome after adjustment for major clinical, molecular, volumetric, and dosimetric factors.