BMC Cancer

STUDY PROTOCOL

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Neoadjuvant ultrahypofractionated radiotherapy with simultaneous integrated boost for glioblastoma: study protocol for a phase I multicenter clinical trial

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Abstract

Background The Stupp protocol standardized adjuvant radiotherapy (RT) with temozolomide as the best treatment after glioblastoma (GBM) resection. Later phase III trials found that moderate hypofractionation and ultrahypofractionation were not inferior in elderly or frail patients. As most recurrences occurred near the tumor bed, RT margins could be reduced, favoring ultrahypofractionated RT (UHRT). Neoadjuvant UHRT might provide additional benefits by allowing better margin selection and lowering radionecrosis risk through the surgical removal of the irradiated tissue.

Methods This is a phase I, single-arm, multicenter clinical trial having as primary endpoint the identification of maximum tolerated dose in operable GBM patients. A total dose of 30 Gy delivered in 5 fractions will be prescribed to planning target volume, with a simultaneous integrated boost dose escalation to gross tumor volume from 35 to 50 Gy, with 5 patients at each dose level. Dose to the next patient group will be escalated if toxicity target is met. The assumption is that not more than 30% of patients would present cumulative ≥G3 toxicity (CTCAE v5.0 scale) one month after the end of UHRT. A 30-patient sample size is predicted to result in a 2-sided 95% confidence interval with a width of 0.328 (0.136–0.464) when the sample proportion is 0.300. Four weeks after the end of UHRT, following a brain MRI, the lesion will be surgically removed. A peripheral blood sample will be taken before and after UHRT, up to 6 months, for immunophenotyping.

Discussion The study aims at evaluating MRI-guided dose escalation neoadjuvant UHRT-related toxicity along with potential improvements in overall- and disease-free survival. Other goals are to identify prognostic factors and monitor radiation-treatment related immune effects. An important expected benefit of the neoadjuvant UHRT is

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that it will shorten treatment time, improving patients' quality of life, and facilitate access to specialized treatment centers. Neoadjuvant RT represents a paradigm shift in GBM, and implications for the scientific community should be significant. The study may help identify distribution patterns of aggressive radioresistant areas with a higher probability of recurrence, counteract intrinsic radioresistance, and identify immune correlates of therapeutic efficacy and/or biomarkers of treatment response/toxicity.

Trial registration The study was prospectively registered at clinicaltrials.gov (NCT06551909, 13/08/2024). Protocol version: Version 1.0, 17/06/2024.

Keywords Glioblastoma, Neoadjuvant radiotherapy, Stereotactic radiotherapy, Simultaneous integrated boost, Magnetic resonance imaging, Clinical prospective trial

Introduction

Glioblastoma or malignant glioma is the most common and aggressive primary brain tumor in adults [1]. Prognosis remains poor, with median survival of approximately 15 months, and survival rarely exceeds 2 years after the diagnosis [2]. Despite refinements in neurosurgical technique, radiotherapy (RT) planning, and systemic therapies, clinical outcomes have improved only modestly over two decades. Standard diagnostic evaluation includes contrast-enhanced MRI with advanced sequences (diffusion, perfusion, spectroscopy when available) for localization and surgical planning, MR-based functional mapping when eloquent cortex is involved, and contrast chest/abdomen imaging for staging only when clinically indicated. Histopathological diagnosis must be paired with molecular profiling (IDH1/2, MGMT promoter methylation, TERT, EGFR, 1p/19q codeletion where appropriate, and copy-number alterations) as these markers guide prognosis, eligibility for trials, and increasingly, therapeutic choices [2]. Standard-of-care across suitable patients continues to be maximal safe resection followed by radiotherapy (RT) with concurrent and adjuvant temozolomide (TMZ) [3]. Recent developments include device-based therapies, such as tumor-treating fields (TTFields), increasing molecular stratification (WHO CNS5), intensifying interest in immunotherapy, and technical advances in RT delivery including hypofractionated stereotactic radiotherapy (SRT). Ultrahypofractionated radiotherapy (UHRT) delivered in five or fewer fractions (commonly referred to as 5-fraction stereotactic radiosurgery (SRS)/ stereotactic radiotherapy (SRT) or short-course SRT) has emerged as a pragmatic strategy in glioblastoma (GBM) management with two overlapping clinical roles: (a) upfront/primary-adjuvant therapy as an alternative short-course regimen in selected patients, and (b) re-irradiation for focal recurrence [4, 5]. The approach is motivated by radiobiological and practical considerations: higher fraction doses potentially improve cell kill in radioresistant subpopulations, reduce overall treatment time (important for patient convenience and rapid systemic therapy integration), and concentrate dose to the enhancing target while sparing surrounding brain through steep dose gradients. Nevertheless, concerns about radiation necrosis, normal brain tolerance, and the need to adequately cover microscopic infiltrative disease remain central to the interpretation of outcomes.

UHRT also modulates tumor antigenicity and immunogenicity, converting the tumor mass into an in-situ vaccine and favoring the recruitment of biological effectors at the site of irradiation. Such effector cells have proven active also outside the treatment field, i.e. the so-called abscopal effect [6]. Accordingly, UHRT in neoadjuvant settings, i.e. on the disease, and not on the tumour bed after surgery should maximize the immune-promoting effects, allow the priming of circulating tumor-specific effectors, and thus reduce the risk of recurrence.

Methods/design

Aim, design, and setting of the study

This is a multicentric single-arm prospective, non-inferiority interventional dose escalation study, being compared to the literature data, which involves enrolling five patients per dose level, for a total of 30 patients. Since the biologic effective dose (BED), for a α/β ratio of 10, considered useful for GBM is 48 Gy [7], the dose prescribed to the PTV -planning tumor volume, the expansion of the gross tumor volume (GTV) outlined with MRI- will be 30 Gy in 5 fractions (BED10 = 48 Gy). The dose levels for the integrated simultaneous boost (SIB) to GTV will be 35 Gy (BED10 = 59.5 Gy), 40 Gy (BED10 = 72 Gy), already investigated on large volumes in the postoperative setting [8], 42.5 Gy (BED10 = 78.6 Gy), 45 Gy (BED10 = 85.5 Gy), 47.5 Gy (BED10 = 92.6 Gy, close to the BED10 of 96 Gy from studies delivering 60 Gy in 10 fractions), and 50 Gy (BED10 = 100 Gy). These sample sizes were determined based on a dose escalation scheme, with 5 patients at each dose level [8–10]. The dose will be increased, moving to the next group of 5 patients, if no more than 1 patient exhibits grade (G) 4 acute toxicity (intracranial hypertension caused by the treatment may require a shunt surgery even in the case of standard treatment) (CTCAE v5.0 scale [11]). Four weeks after the end of the radiation treatment, following a brain MRI, the patient will undergo removal of the lesion by neurosurgeons,

unless neurological toxicity requires the surgical treatment to be brought forward. The hypothesis is that the proportion of patients free from ≥G3 cumulative acute toxicity 1 month after sequential radiation treatment and surgery should not be greater than that of the standard post-operative treatment [3].

The treatment will be stopped if ≥ 2 cases of G4 toxicity and/or recurrences occur within one month at a given dose level; otherwise, the study will proceed at the next dose level until at least 30 patients are enrolled. Patients who withdraw from the treatment will be replaced by patients enrolled subsequently, to reach the planned sample size. Concomitant treatments, including corticosteroids and antiepileptic drugs, will be permitted as clinically indicated.

If toxicity remains low and the disease control comparable to literature and historical data from our institution, this protocol will be adopted as a standard departmental treatment. The project was approved by the Territorial Ethics Committee 1 of the Lombardy region (identification number CET 292–2024) and registered at clinicaltrials.gov (NCT06551909). The study is supported by the European Union (NextGenerationEU, PNRR-MCNT2-2023-12378239 (AIM2)).

The study protocol was developed in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidelines. A completed SPIRIT checklist is provided as Supplementary File 1. The schedule of enrolment, interventions and assessments is summarized below in Table 1.

Study population

The patient population studied will be represented by men and women between 18 and 80 years old diagnosed with GBM. The healthy donors will have age and sex comparable with patient population.

Participant characteristics and eligibility criteria are summarized in Table 2.

Patients who agree to participate in the study and meet the inclusion and exclusion criteria will be scheduled for the treatment within the following week (the minimum time needed to make the immobilization device and to carry out the exams necessary for defining the target and calculating the treatment plan). Follow-up visits are scheduled to ensure complete data collection. Data will be collected in anonymized electronic databases with secure storage.

Imaging, contouring and treatment planning

As soon as possible, according to organizational needs, and in any case within one week of notification, the thermoplastic mask, computed tomography (CT) and contrast enhanced MRI simulation will be performed, and the treatment plan calculated. T1-, T2-, FLAIR, and volumetric axial T1-weighted MRI with contrast-enhancement will be acquired. Rigid deformable registration, based on bony landmarks, will be performed between MRI sequences and treatment planning non-contrast enhanced CT images.

After CT and MRI image fusion, the structures are automatically generated using the robotic radiosurgery atlas or radiotherapy software programs already in use, and then manually corrected by a Radiation Oncologist, observing the ESTRO-EANO and NRG guidelines [12, 13].

The GTV will be delineated on the contrast volumetric axial T1-weighted sequence and corrected based on T2, FLAIR and other sequences if necessary. A GTV expansion of 0.3 cm in all directions will be used to generate the PTV in order to limit the treated volume and subsequent toxicity. The GTV- CTV (clinical target volume) 0 cm margin is based on the continuous margin reduction in recent years, and the observation that a tight margin of 0.5 cm (tighter than those in use today) determined a similar overall survival to large margins and did not increase marginal relapse rate [7]. An initial treatment dose of 30 Gy in 5 sessions on the PTV, with an escalated dose SIB to GTV from 35 to 50 Gy every five patients, is envisioned. Standard constraints will be used for the various organs at risk (brain – PTV, brainstem, chiasm, optic nerves, eyes, lenses, inner ear, scalp) according to current international guidelines for stereotactic radiotherapy in 5 fractions (see Table 3) [14], in order to obtain the lowest possible dose (ALARA = As low as reasonably achievable -as close as possible to the desired value, even if unreachable).

Furthermore, by applying the Simultaneous Integrated Boost (SIB) technique, the GTV will be treated at progressively increasing dose levels for every 5 patients (if no more than one G4 toxicity is recorded in the previous group) up to 50 Gy in 5 sessions. The role of dose escalation SIB will be to deliver radical stereotactic doses, corresponding to the location of aggressive and hypothetically radioresistant cell clones, to the tumoral lesion.

Treatment delivery will be carried out on five consecutive working days. Total system accuracy of the robotic stereotactic radiotherapy has been reported as submillimetric [15], while intracranial accuracy, using mask and whole skull for matching, is high [16].

Immunophenotyping

Peripheral blood mononuclear cells (PBMCs) and sera will be collected and bio-banked before and after RT, and 1, 3 and 6 months after surgery to identify putative immune correlate of therapy efficacy and/or acute biomarkers predictive of response/toxicity to therapy. A longitudinal flow-cytometry analysis will assist the characterization of biological/immune correlate of therapy

Table 1 Study Procedures

	Enrollment	Pre-treament Phase	Treatment Phase					Post-treatment Phase									
	Enrollment	Simulation(C)	SRT (R)	Re-evaluation before surgery(1 month)(C)	Surgery(C)	Re-evaluation before chemotherapy (1 month)(C)	Chemotherapy(C)	FOLLOW-UP Months (C)									
								3	6	9	12	15	18	21	24	27	30
Informed consent(C)	X																
Demographics(C)	X																
Inclusion and exclusion criteria evaluation(R)	X																
Medical History(C)	X																
Physical examination and anamnesis(C)	X			X		X		X	X	X	X	X	X	X	X	X	X
Performance status(C)	X			X		X		X	X	X	X	X	X	X	X	X	X
Laboratory tests: Haematologic, Biochemistry(C)	X			X		X		X	X	X	X	X	X	X	X	X	X
PMBC withdrawal (R)			X*	X				X	X								
Termoplastic mask, simulation CT, simulation contrast-enhanced MRI(C)		X															
Treatment delivery(C)			X		X		X		X	X	X**	X**					
Evaluation CE MRI (+/- amino-acids PET for diagnostic doubts)(C)	X			X		X		X	X	X	X	X	X	X	X	X	X
Quality of life questionnaires# (R)	X		X	X		X		X	X	X	X	X	X	X	X	X	X
AE evaluation (CTCAE v 5.0)(C)			X	X		X	X	X	X	X	X	X	X	X	X	X	X

X*: PMBC withdrawal at the beginning and end of the treatment

X** 6-or 12-month TMZ decided by the attending oncologist

Table 2 Patients characteristics and eligibility criteria

Eligibility	Criteria
Inclusion criteria	1) Age between 18 and 80 years 2) ECOG performance score 1–2 (defined during the first visit) 3) Diagnosis of GBM 4) Surgically removable lesion (according to operability criteria established by the Neurosurgery Unit)
Exclusion criteria	1) Previous stroke 2) Presence of another primary and/or metastatic tumor

Table 3 Planning objectives and constraints for organs at risk

Organ	Volume	Dose (Gy)
PTV (V 100)	95%	27 (90%)
Planning-PTV	95%	28.5 (95%)
Whole brain-PTV	D 0.03 cc	100%
Whole brain	D mean desired	≤ 5
	D mean accepted	≤ 20
Brainstem	D 0.03 cc	31
	< 0.5 cc	23
Optic chiasm	D 0.03 cc	25
	< 0.5 cc	23
Optic nerves	D 0.03 cc	25
	< 0.5 cc	23
Eye	D 0.03 cc	22
	D mean	≤ 20
Crystalline	D 0.03 cc	≤ 5
Internal ear	D0.03 cc	25
Scalp	D 0.03 cc	39.5
	< 10 cc	36.5

efficacy. High dimensional flow-cytometry will be performed to scrutinize T cell phenotypes, indicative of RT-induced T cell priming. A 24-color panel was validated, allowing for the identification of immune cell subsets and the deep characterization of T cell subtypes and phenotypes. Relative expressions of T-cell activation/exhaustion markers (including, but not restricted to: CD4, CD8, CD45RA, CD25, CD137, CTLA-4, PD-1, ICOS) will be analyzed to investigate RT-induced effects. Sera will be analyzed for cytokine content.

Study tasks

- pre-clinical investigation of procedure feasibility using ablative doses delivered using SRT by means of robotic radiosurgery, or Image Guided-Intensity Modulated Radiotherapy (IG-IMRT), to establish protocol procedures.
- registration of maximum acute toxicity at the end of treatment, 1 month after SRT (before surgery), 1 month after surgery (before starting temozolomide chemotherapy) and subsequently during the 6-month chemotherapy delivery, to verify overall toxicity of the protocol.
- identification of local, intra-cranial and distant relapses through complete brain MRI, aminoacid PET/CT and possible biopsy, in case of local (near the original tumor site) or as new (lesions in different brain areas) recurrence. Local control, cancer specific survival and overall survival will be calculated at the end of the planned 2-year follow-up.
- registration of late toxicity incidence until the end of follow-up.
- immune monitoring– longitudinal evaluation of representation and phenotype of immune cell subsets in the peripheral blood (multiparametric flow cytometry approach including but not restricted to CD4, CD8, CD45RA, CD25, CD137, CTLA-4, PD-1, ICOS, etc.)
- identification of clinical and dosimetric factors that influence the outcome, and of the radiomic features predictive of results.

Study endpoints

Primary endpoint: the evaluation of acute toxicity in the entire patient sample.

Secondary endpoints include local control, survival, intracranial relapse, chronic toxicity, quality-of-life (with EORTC QLQ BN20, FACT, FACIT and MMSE questionnaires), identification of clinical, imaging and laboratory prognostic factors for an aggressive GBM phenotype, evaluation of radiomic features on CT and MRI images in relation to clinical and histological parameters and their predictive role regarding treatment response.

Statistical analysis: definitions, analyses

Relapse-free survival (local, intracranial, or distant)

Time interval from the end of radiotherapy to the date of local relapse (LR), intracranial relapse (IR), or distant relapse (metastases outside the brain, DM). Local relapse refers to recurrence at the original site, intracranial relapse includes marginal or nearby recurrences, and distant relapse involves metastases outside the brain (rare but possible events). Patients who survive without events will be censored at their last follow-up date. Deceased patients will be censored at the date of death.

Disease-free survival

Measures the cumulative incidence over time; the time to event is calculated as the interval between the end of treatment and the date of the first of the following events: local relapse, intracranial relapse, distant relapse, or death from any cause. For patients who survive without

events, the data will be censored at their last follow-up date.

Cause-specific survival

Measured as the time from the end of treatment to death attributable to tumor progression. If a patient dies from other causes, including other tumors not related to GBM or subsequent local, intracranial, or distant relapse, he/she will be censored at the date of death, but this will not be counted as an event. Surviving patients will be censored at their last follow-up.

Overall survival

Measured as the time from diagnosis to death, or to the last available follow-up date.

Toxicity will be assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (National Cancer Institute, Bethesda, MD, USA). Continuous data will be expressed as median values with interquartile ranges (IQRs). Categorical variables will be analyzed using Fisher's exact test, while continuous variables will be evaluated using Wilcoxon rank sum test or Kruskal-Wallis rank sum test, as appropriate. Survival rates will be estimated using the Kaplan-Meier method. Survival calculations will be based on the time from RT completion to the registered event, death, or last follow-up. Statistical significance is set at $P \leq 0.05$ (two-tailed). Analyses will be performed using R statistical software (version 4.5.1, https://cran.r-project.org/index.html).

Trial status

At the time of manuscript submission, the study has received ethical approval and patient recruitment has started. The first patient was enrolled in 25/11/2024, and recruitment is expected to be completed by 02/2027. Patients will be recruited from all participating centers. The study will be monitored by the coordinating center.

Ethical aspects

The study will be conducted according to the Declaration of Helsinki/Tokyo and to Good Clinical Practice guidelines. The protocol has been presented to and approved by the Territorial Ethics Committee 1 of the Lombardy region, Italy. Patients signing the written informed consent for participation in the trial after a complete explanation of the objectives and modalities of the study will be included. Patient data will be anonymized and handled according to GDPR regulations.

Discussion

Ultrahypofractionation

The α/β ratio for GBM is estimated at 8–10 Gy, suggesting potential benefit from BED escalation. SRT provides steep dose gradients, spares normal tissue, and reduces treatment time, representing advantages for patients with poor prognosis or those at risk for Radiation-Induced Leukoencephalopathy (RIL). Prospective studies and randomized comparisons have recently investigated regimens delivering 25–40 Gy in 5 fractions for newly diagnosed and recurrent GBM. A randomized phase II investigation comparing 35 Gy in 5 fractions versus 25 Gy in 5 fractions in the reirradiation setting failed to show a clear survival advantage for the higher dose, highlighting the balance between dose escalation and toxicity in heavily pretreated tissue [17]. Earlier phase I/II data explored concurrent temozolomide with 5-fraction SRT, establishing a maximum tolerated dose of 40 Gy in 5 fractions (with 5-mm margins) and acceptable acute toxicity in carefully selected patients [18]. Multiple institutional series and meta-analyses report median overall survival (OS) after focal SRT for recurrent GBM generally in the range of several months to > 1 year in well-selected younger patients with longer recurrence intervals and small target volumes, with low rates of grade ≥ 3 radiation necrosis when modern planning and image guidance are used [19, 20]. Recent comparative analyses that include MRI-LINAC adaptive 5-fraction regimens suggest comparable oncologic outcomes versus conventional fractionation in select cohorts while markedly reducing patient visits and cumulative hospital burden [21].

In our protocol the treatment includes neoadjuvant UHRT in order to irradiate the tumor before resection, targeting the visible GTV with high precision. The rationale includes improved target delineation before postoperative changes, potential sterilization of tumor margins, and immediate treatment initiation. Early-phase trials (NCT04699930, NCT05030298) show feasibility and safety, with close coordination between radiation oncology and neurosurgery. Typical scheduling delivers SRT within 3–7 days prior to surgery. In our study, the decision to perform surgery 4 weeks after the end of radiotherapy was based on the clinical observation that patients not operated on but rather treated with radiotherapy alone, generally showed disease progression 2 months after the end of treatment, still leaving a minimum period for radiotherapy to provide an immunogenic effect.

Toxicity

The dominant late toxicity concerns include radiation necrosis and symptomatic radionecrosis requiring steroids or surgery. Risk correlates with volume of high-dose exposure (e.g., V12-V20), concurrent systemic agents (notably some targeted drugs and immune modulators), and anatomic location [18, 22]. Prospective dose-finding studies and modern normal tissue dose constraints adapted from brain metastasis and reirradiation literature have been used to guide safe practice; nevertheless,

careful candidacy selection (KPS, lesion size, and proximity to critical structures) remains essential [18, 22]. Multidisciplinary review and routine use of advanced dose-painting and planning optimization, integrated with MRI-based target delineation and perfusion imaging where available, are recommended to reduce toxicity risk.

The protocol we propose takes into account the literature data and suggests the implementation of a neoadjuvant treatment that we believe provides several advantages over an adjuvant treatment, also regarding toxicity. Indeed, administering radiotherapy, even at high doses in the neoadjuvant setting, would enable better definition of the lesion contours visible on preoperative MRI images. This will consequently allow the refining of very narrow margins. In addition, the subsequent surgical removal of the lesion should better protect against radionecrosis, as all tissue irradiated at high doses would be surgically removed.

In summary, the factors considered fundamental include: (1) meticulous target delineation that respects the infiltrative biology of GBM while balancing the need for conformality; (2) margin policy—many protocols use tight margins (e.g., 5 mm) when imaging and planning are of high quality; (3) robust image guidance (daily cone-beam CT, robotic stereotactic tracking); (4) stringent normal tissue dose constraints; (5) consideration of fractionation choices tailored to tumor size and localization and (6)Multidisciplinary coordination with neurosurgery .

Patient selection and indications

Best outcomes in the literature accrue to patients with favorable prognostic features: good performance status (KPS ≥ 60–70), small target volumes, and limited, removable lesion, not multifocal disease [3, 7, 18]. In the newly diagnosed setting, short-course stereotactic regimens have been tested in suitable patients seeking shorter treatment courses, or those for whom conventional protracted RT might not be feasible; for elderly or frail patients, even shorter hypofractionated schedules (e.g., 25 Gy in 5 fractions) have demonstrated non-inferiority to standard fractionation in certain settings, and are often combined with temozolomide depending on MGMT status [4, 17]. Our protocol includes patients of all ages because, as reported, hypofractionation is well tolerated by young patients in good general condition, but can be of even greater benefit for elderly and frail patients by considerably reducing the burden of problems related to a radiation treatment characterized by a greater number of fractions.

Radiotherapy and lymphopenia

Severe treatment-related lymphopenia (TRL) — often defined as absolute lymphocyte count (ALC) < 500 cells/μL — is common in patients undergoing chemoradiation for GBM, with reported rates of grade 3–4 lymphopenia of ≈ 20–50% depending on definitions and cohorts [23, 24]. TRL has been associated with worse progression-free and overall survival in multiple series and meta-analyses, and is of particular concern in the era of immunotherapy, as lymphocyte depletion may blunt anti-tumor immune responses [25, 26]. Key mechanistic contributors include: (1) direct irradiation of circulating lymphocytes as they traverse high-dose regions during each fraction; (2) irradiation of hematopoietic bone marrow niches (skull base and vertebral bodies) and secondary lymphoid organs; (3) systemic myelosuppression from concurrent agents (notably TMZ); and (4) treatment-related corticosteroid exposure. Modeling studies estimate mean dose to circulating lymphocytes across a typical GBM plan to be on the order of ~ 1–2 Gy with nearly the entire blood pool receiving at least 0.5 Gy cumulatively, plausibly explaining persistent lymphopenia after RT [27, 28]. Multiple clinical and dosimetric predictors have been reported: larger planning target volumes (PTV) and higher integral brain dose correlate with increased TRL risk; IMRT and proton therapy reduce low-dose bath and may lower TRL incidence; early baseline lymphocyte count and patient factors (age, sex) also influence risk. Rudra et al. and Byun et al. have shown that limiting treatment volumes and using IMRT were associated with lower rates of severe lymphopenia (SLP) and potentially improved outcomes [29, 30]. Recent multi-institutional nomograms incorporate mean brain dose and baseline ALC to estimate SLP risk [31]. TRL is associated with inferior survival and may reduce the efficacy of immune checkpoint inhibitors or vaccines if administered during profound lymphocyte nadirs. Timing of immunotherapy relative to RT, lymphocyte-sparing RT planning, and supportive strategies (e.g., IL-7 administration) are active areas of investigation into the preservation of immune competence while delivering effective local therapy [32, 33]. In our protocol to reduce the incidence of SLP, practical mitigation strategies include: reducing unnecessary elective brain irradiation and PTV margins; using highly conformal techniques (robotic SRT/helical IMRT with optimization to spare normal brain, including venous sinuses), hypofractionation and stereotactic approaches to reduce total number of blood exposures, and careful steroid stewardship. Dosimetric constraints related to lymphocyte preservation remain investigational, but planning to minimize mean brain dose and low-dose volumes (e.g., V5–V20) is reasonable [29, 34].

Radiomics in GBM

Radiomics extracts quantitative imaging features (shape, intensity, texture, wavelet-derived metrics) from multiparametric MRI and uses machine learning models to

link imaging phenotypes to molecular status, prognosis, and treatment response [35]. The workflow requires standardized acquisition, robust segmentation (manual/AI), feature harmonization (IBSI standards), and external validation to be clinically useful.

Combining HSRT with radiomics-informed target definition offers a route to tighten margins where infiltrative patterns are low-risk, and to boost high-risk subregions while minimizing mean brain dose. Several studies currently underway are evaluating the effectiveness of radiomics in indicating the regions of the lesion where the radiation dose should be increased, defining the molecular characteristics of the disease in a non-invasive manner, and predicting the possibility of lymphopenia in relation to the dosimetric characteristics of the planning [35–37]. Our protocol also includes a radiomics study using initial and pre-intervention MR images to evaluate the presence of features that may be predictive of response.

For the radiomics study, our planned workflow follows the classical steps recommended by the Image Biomarker Standardization Initiative (IBSI) [38]. First, images will undergo preprocessing to resample them to an identical cubic voxel size, thereby eliminating acquisition-related differences, and enabling reliable comparison of the extracted features. The next step is multi-observer lesion segmentation, typically performed by three independent medical readers. Features extracted from each segmentation set will then be compared using ranking tests and intraclass correlation coefficients (ICC) to identify the most robust features with respect to segmentation variability.

Once a reference segmentation set has been defined, its features will be subjected to post hoc harmonization using the ComBat method [39], in order to minimize variability arising from different scanners and acquisition protocols. After these steps, the most robust features will be carried forward and tested for association with toxicity and clinical outcomes, using appropriate statistical methods.

In our context, given the relatively small patient cohort, the most reliable strategy consists of logistic regression analysis followed by ROC curve evaluation to identify the best cut-off value of potential predictive biomarkers. All analyses will be performed using the SPAARC software [40], which is fully IBSI-compliant. Validation of models will be performed using the recommendations of the TRIPOD guidelines on small patient cohorts [41], which suggest the use of bootstrap methods to estimate model performance, and reduce the risk of overfitting.

Immunomodulating effects of radiotherapy

Radiotherapy induces a wide range of changes within the tumor ecosystem, not only causing direct cancer cell killing, but also modulating the local microenvironment. Among RT modalities, hypofractionated regimens have been proven to modulate tumor antigenicity and immunogenicity. Mechanistically, RT-induced tumor cell death was associated with the release of tumor-associated antigens, favoring local dendritic cell activation, T cell priming in secondary lymphoid tissues, and effector T cell responses within the tumor [42]. Radiation also promotes infiltration of cytotoxic T lymphocytes and favors the upregulating MHC class I molecules, enabling local antitumor effects [43]. Through these mechanisms, RT may convert "cold" (non-T cell inflamed) into "hot" (T cell inflamed) tumors [44]. Although the brain was once considered "immune privileged," recent studies indicate that it is "immune peculiar". Under non-disease conditions, the brain is largely immunologically quiescent, with the blood-brain barrier (BBB) limiting the infiltration of circulating immune cells [45]. Cancer and other inflammatory conditions, however, alter the BBB permeability, allowing immune cell infiltration. In GBM, most infiltrating cells are represented by myeloid cells, and tissue-resident microglia and bone marrow–derived macrophages [46]. An immunosuppressive microenvironment, and the paucity of infiltrating T cells, likely account for poor reactivity to the tumor and resistance to immunotherapy, including immune checkpoint inhibitors. Neoadjuvant radiotherapy has the potential to overcome this scenario, instruct T cell priming in secondary lymphoid tissues and intratumoral reactivity, and possibly synergize with neoadjuvant pembrolizumab [47]. The peripheral compartment represents a reservoir of T cells with potential reactivity to the tumor. Accordingly, the phenotype and activation status of immune cells has been used to assess baseline and therapy-mediated changes in local and peripheral cellular immunome in patients with cancer, including pancreatic cancer, localized clear cell renal cell carcinoma, non–small cell lung cancer, and melanoma [48]. Assessing the immune fitness of patient immunity and the peripheral reaction during treatment might represent valuable biomarkers of response to radiotherapy and possibly help identify GBM patients eligible for combinatorial immunotherapy approaches. Thus, understanding the immune fitness of patients at baseline and over the course of therapy might help trace response to therapy, and individuate eligibility for its further combination with immunotherapy, including immune checkpoint blockers (ICB). Accordingly, high dimensional flow cytometry analyses of patient PBMCs allowed defining systemic RT-induced immunomodulatory effects, and the synergy with anti-CTLA-4 antibodies in patients with metastatic lung cancer [43]. We speculate that, while RT is usually employed in GBM patients in adjuvant setting to sterilize tumor bed upon surgical resection, neoadjuvant UHRT would represent the ideal condition to

sensitize the patient against its tumor, helping to monitor for tumor recurrence. Patient immunomonitoring might also provide diagnostic and prognostic biomarkers for therapeutic outcomes.

Thus, we will attempt to understand if stereotactic radiotherapy could be a promising strategy for glioblastoma, offering precise, shorter treatments with potential benefits in local control and immune preservation. Neoadjuvant UHRT may enhance radiosensitivity and immune response (and immunomonitoring peripheral blood should help us to better understand the potential benefits of this treatment). Five-fraction SRT is a practical option for selected GBM patients, providing short treatments with acceptable toxicity and some disease control. However, optimal dosing, combination strategies, and biomarker-guided selection still require confirmation in further studies, including among the outcomes cognitive abilities and quality of life.

Abbreviations

ALARA	As low as reasonably achievable
ALC	Absolute lymphocyte count
BBB	Blood-brain barrier
BED	Biological effective dose
CT	Computed tomography
CTV	Clinical target volume
DL	Distant relapse
GBM	Glioblastoma
GTV	Gross tumor volume
IBSI	Image biomarker Standardisation Initiative
ICB	Immune checkpoint blockers
ICC	Intraclass correlation coefficients
IG-IMRT	Image Guided-Intensity Modulated Radiotherapy
IR	Intracranial relapse
LR	Local relapse
RIL	Radiation induced Leukoencephalopathy
RT	Radiotherapy
SIB	Simultaneous integrated boost
SLP	Severe lymphopenia
SRS	Stereotactic radiosurgery
SRT	Stereotactic radiotherapy
TRL	Treatment-related lymphpenia
UHRT	Ultrahypofractionated radiotherapy

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-026-15950-2.

Supplementary Material 1.

Acknowledgements

We thank Carmen Gigliotti and Lucia Perna, Medical Physics, IRCCS San Raffaele Scientific Institute, Dr. Nicola Boari, Dr Stefania Acerno, Department of Neurosurgery, IRCCS San Raffaele Institute, Dr. Mario Petrazzuoli, Department of Radiation Oncology, IRCCS Istituto Nazionale dei Tumori Fondazione G. Pascale, for their contribution in the implementation of this protocol.

Authors' contributions

Prof. Nadia Di Muzio, Dr. Anna Mondino, Dr. Andrei Fodor contributed to the study conception and design. The first draft of the manuscript was written by prof. Nadia Di Muzio and Andrei Fodor and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

Funding

This research is Funded by the European Union - Next Generation EU - NRRP M6C2 - Investment 2.1 Enhancement and strengthening of biomedical research in the NHS. The funding was obtained through a national competition, with international peer-review, coordinated by the Italian Ministry of Health. The project is entitled "Radio-immunotherapy in solid tumors" (PNRR-MCNT2-2023-12378239- Aim2).

Data availability

The data that support the findings of this study (anonymized individual participant data) are available on request from the corresponding author to researchers who provide a methodologically sound proposal. Requests made to the corresponding author (MT) will be evaluated by the IRCCS San Raffaele Scientific Institute Ethics Committee. Study results will be disseminated through peer-reviewed publications, conference presentations, and updates in the clinical trial registry.

Declarations

Ethics approval and consent to participate

All procedures performed in the present study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies using animals performed by any of the authors. This study has been approved by the Institutional Scientific Board of the IRCCS San Raffaele Scientific Institute (registration number CET 292-2024). All participants will sign an Informed Consent. Any protocol amendments will be communicated to ethics committees.

Consent for publication

Not applicable.

Competing interests

Miriam Torrisi received a speaker honorarium from Accuray Inc. independently from the current work. Laura Giannini received a research fellowship from AIRC: IG 25951 - project "Toward clinical use of PET in selecting patients with pancreatic cancer for local treatment intensification". Miriam Torrisi received a research grant from the Next Generation EU (PNRR-MCNT2-2023-12378239), with a project entitled "Radio-immunotherapy in solid tumors" (CUP Code: C43C24000320005). Andrei Fodor participated in a Johnson & Johnson Advisory Board not related to this paper.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The remaining authors declare no conflicts of interest.

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Received: 27 October 2025 / Accepted: 26 March 2026
Published online: 06 April 2026

References

1. Wirsching HG, Galanis E, Weller M, Glioblastoma. Handb Clin Neurol. 2016;134:381–97. https://doi.org/10.1016/B978-0-12-802997-8.00023-2, indexed in Pubmed: 26948367.
2. Roger S, Mason Warren P, van den Bent Martin J, et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N Engl J Med. 2005;352(10):987–96.
3. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987–96. https://doi.org/10.1056/NEJMoa043330.
4. Roa W, Kepka L, Kumar N, et al. International Atomic Energy Agency Randomized Phase III Study of Radiation Therapy in Elderly and/or Frail Patients With Newly Diagnosed Glioblastoma Multiforme. J Clin Oncol. 2015;33(35):4145–50. https://doi.org/10.1200/JCO.2015.62.660.
5. Andratschke N, Heusel A, Albert NL, et al. ESTRO/EANO recommendation on reirradiation of glioblastoma. Radiother Oncol. 2025;204:110696. https://doi.org/10.1016/j.radonc.2024.110696.
6. Demaria S, Formenti SC. The abscopal effect 67 years later: from a side story to center stage. Br J Radiol. 2020;93:20200042. https://doi.org/10.1259/bjr.20200042).
7. Mendoza MG, Azoulay M, Chang SD, et al. Patterns of progression in patients with newly diagnosed glioblastoma treated with 5-mm margins in a phase ½ trial of 5 -fraction stereotactic radiosurgery with concurrent and adjuvant temozolomide. Pract Rad Oncol. 2023;13:e239–45.
8. Jiang C, Mogilevsky C, Belal Z, et al. Hypofractionation in glioblastoma: an overview of palliative, definitive and exploratory uses. Cancers. 2023;15:5650.
9. Omura GA. Phase 1 Dose-finding trials and Fibonacci. Clin Cancer Res. 2006;12:321. 10.1158/1078 – 0432.CCR-05-1762.
10. Omura GA. Modified Fibonacci search. J Clin Oncol. 2003;21:3177–79. https://doi.org/10.1200/JCO.2003.99.058.
11. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. 2017.
12. Niyazi M, Andratschke N, Bendszus M, et al. ESTRO-EANO guideline on target delineation and radiotherapy details for glioblastoma. Radiother Oncol. 2023;184:109663. https://doi.org/10.1016/j.radonc.2023.109663.
13. Kruser TJ, Bosch WR, Badiyan SN, et al. NRG Brain Tumor Specialists Consensus Guidelines for Glioblastoma Contouring. J Neurooncol. 2019;143(1):157–66. https://doi.org/10.1007/s11060-019-03152-9.
14. Milano MT, Grimm J, Niemierko A, et al. Single and multi-fraction stereotactic radiosurgery dose/volume tolerances of the brain. Int J Radiat Oncol Bio Phys. 2020;110(1):68–86.
15. U.S.Food and Drug Administration. 510(k) Premarket Notification: K102650. AccessData FDA. 2010. https://www.accessdata.fda.gov/cdrh_docs/pdf10/k102650.pdf.
16. You W, Chen C, Chu H, et al. Quantifying thermoplastic mask quality for precision radiotherapy in head and neck cancer: A 3D stress test and 6D-axis error analysis. Medicine. 2024;103(44):e40365.
17. ChenA et al. Prospective Randomized Phase 2 Trial of Hypofractionated Stereotactic Radiation Therapy of 25 Gy in 5 Fractions Compared With 35 Gy in 5 Fractions in the Reirradiation of Recurrent Glioblastoma International Journal of Radiation Oncology, Biology, Physics. 2024;119:1122–32. https://doi.org/10.1016/j.ijrobp.2024.01.013.
18. AzoulayM, et al. A phase I/II trial of 5-fraction stereotactic radiosurgery with 5-mm margins with concurrent temozolomide in newly diagnosed glioblastoma. Neuro Oncol Pract. 2020;22(8):1182–9. https://doi.org/10.1093/neuonc/noaa019.
19. Yilmaz MT et al. Hypofractionated stereotactic re-irradiation for progressive glioblastoma: multicenter outcomes review. J Neurooncol. 2024;167(2):295–303. https://doi.org/10.1007/s11060-024-04607-4.
20. Kim MS et al. Re-irradiation for recurrent glioblastoma: contribution to prognosis and outcomes. Front Oncol. 2020;8(1):29–35.; (PMC7221471).
21. Comparative outcomes of standard RT and 5-fraction adaptive stereotactic RT (Adv Radiat Oncol / Advances in Radiation Oncology). 2025. recent comparative analysis.
22. Cureus. Impact of Reirradiation Utilizing Fractionated Stereotactic Radiotherapy for Recurrent Glioblastoma. 2023.
23. Grossman SA, Ye X, Lesser G, Sloan A, Carraway H, Desideri S, et al. Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. Clin Cancer Res. 2011;17(16):5473–80. https://doi.org/10.1158/1078-0432.CCR-11-1061.
24. Byun HK, Kim N, Yoon HI, Kang SG, Kim SH, Cho J, et al. Clinical predictors of radiation-induced lymphopenia in patients receiving chemoradiation for glioblastoma: Clinical usefulness of intensity modulated radiotherapy in the immuno-oncology era. Radiat Oncol. 2019;14(1):51. https://doi.org/10.1186/s13014-019-1256-6.
25. Wild AT, Ye X, Ellsworth SG, et al. The association between chemoradiation-related lymphopenia and clinical outcomes in pancreatic adenocarcinoma. Am J Clin Oncol. 2015;38(3):259–65. (Context on lymphopenia across cancers).
26. Campian JL, Ye X, Brock M, Grossman SA. Treatment-related lymphopenia in patients with stage III non–small-cell lung cancer. Cancer Immunol Res. 2013;1(5):313–8. https://doi.org/10.1158/2326-6066.CIR-13-0062.
27. Yovino S, Kleinberg L, Grossman SA, Narayanan M, Ford E. The etiology of treatment-related lymphopenia in patients with malignant gliomas: Modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. Cancer Invest. 2013;31(2):140–4. https://doi.org/10.3109/07357907.2012.762780.
28. Jin JY, Hu C, Xiao Y, Zhang H, Ellsworth S, Schild SE, et al. Higher radiation dose to immune system is correlated with poorer survival in patients with stage III non-small cell lung cancer: A secondary study of a phase 3 cooperative group trial (NRG Oncology RTOG 0617). Int J Radiat Oncol Biol Phys. 2017;99(2):S151.
29. Rudra S, Hui C, Rao YJ, Samson P, Lin AJ, Chang X, et al. Effect of radiation treatment volume reduction on lymphopenia in patients receiving chemoradiotherapy for glioblastoma. Int J Radiat Oncol Biol Phys. 2018;101(1):217–25. https://doi.org/10.1016/j.ijrobp.2018.01.069.
30. Mell LK, Sirák I, Wei L, Tarnawski R, Mahantshetty U, Yashar CM, et al. Bone marrow–sparing intensity modulated radiation therapy: rationale and early clinical experience (example in cervical cancer). Int J Radiat Oncol Biol Phys. 2017;97(3):536–45. (Context on marrow sparing).
31. KimN et al. Nomogram for radiation-induced lymphopenia in patients receiving IMRT-based chemoradiation for newly diagnosed glioblastoma: A multi-institutional study. lin Transl Radiat Oncol. 2024;47:100799. https://doi.org/10.1016/j.ctro.2024.100799.
32. Sportès C, Szabolcs P, Kohn DB, et al. Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by expanding naive CD4 T cells. J Clin Invest. 2008;118(7):2272–9. https://doi.org/10.1172/JCI34487.
33. Lim M, Xia Y, Bettegowda C, Weller M. Current state of immunotherapy for glioblastoma. Nat Rev Clin Oncol. 2018;15(7):422–42. https://doi.org/10.1038/s41571-018-0003-5.
34. Ladbury CJ, Rusthoven CG, Camidge DR, Kavanagh BD, Nath SK. Impact of radiation dose to the host immune system on tumor control and survival for Stage III NSCLC treated with definitive RT. Int J Radiat Oncol Biol Phys. 2019;105(2):346–55. https://doi.org/10.1016/j.ijrobp.2019.05.064.
35. Aerts HJWL, Velazquez ER, Leijenaar RTH, Parmar C, Grossmann P, Carvalho S, et al. Decoding tumour phenotype by noninvasive imaging using a quantitative radiomics approach. Nat Commun. 2014;5:4006. https://doi.org/10.1038/ncomms5006.
36. Park JE, Kim HS, Moon SH, et al. Radiomic profiling of glioblastoma: identifying an imaging predictor of patient survival with improved performance over established clinical and radiologic risk models. Radiology. 2016;280(3):880–9. https://doi.org/10.1148/radiol.2016160845.
37. Kickingereder P, Bonekamp D, Nowosielski M, Kratz A, Sill M, Burth S, et al. Radiogenomics of glioblastoma: machine learning-based classification of molecular characteristics by using multiparametric and multiregional MR imaging features. Radiology. 2016;281(3):907–18. https://doi.org/10.1148/radiol.2016161382.
38. Zwanenburg A, Vallières M, Abdalah MA, Aerts HJWL, Andrearczyk V, Apte A, van Griethuysen JJM. The Image Biomarker Standardization Initiative: Standardized Quantitative Radiomics for High-Throughput Image-Based Phenotyping. Radiology. 2020;295(2):328–38. https://doi.org/10.1148/radiol.2020191145.
39. Orlhac F, Eertink JJ, Cottereau AS, Zijlstra JM, Thieblemont C, Meignan M, Boellaard R, Buvat I. A Guide to ComBat Harmonization of Imaging Biomarkers in Multicenter Studies. J Nucl Med. 2022;63(2):172–9. https://doi.org/10.2967/jnumed.121.262464. Epub 2021 Sep 16. PMID: 34531263; PMCID: PMC8805779.
40. Oyibo P, Brynolfsson P, Spezi E. Integrating Radiomic Image Analysis in the Hero Imaging Platform. Proceedings of the Cardiff University School of Engineering Research Conference 2024, Cardiff, UK. 2024. https://doi.org/10.18573/conf3.g.

41. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): the TRIPOD statement. Ann Intern Med. 2015;162(1):55–63. doi: 10.7326/M14-0697. Erratum in: Ann Intern Med. 2015;162(8):600. https://doi.org/10.7326/L15-0078-4. PMID: 25560714.
42. Cytlak UM, Dyer DP, Honeychurch J, et al. Immunomodulation by radiotherapy in tumour control and normal tissue toxicity. Nat Rev Immunol. 2022;22(2):124–38. https://doi.org/10.1038/s41577-021-00568-1.
43. Formenti SC, Rudqvist NP, Golden E, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. Nat Med. 2018;24(12):1845–51. https://doi.org/10.1038/s41591-018-0232-2).
44. Awada H, Paris F, Pecquesr C. Exploiting radiation immunostimulatory effects to improve glioblastoma outcome. Neuro Oncol. 2023;25(3):433–46. https://doi.org/10.1093/neuonc/noac239.
45. Alvarez JI, Dodelet-Devillers A, Kebir H, et al. The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. Science. 2011;334(6063):1727–31. https://doi.org/10.1126/science.1206936.
46. Arrieta VA, Najem H, Petrosyan E, et al. The Eclectic Nature of Glioma-Infiltrating Macrophages and Microglia. Int J Mol Sci. 2021;22(24):13382. https://doi.org/10.3390/ijms222413382.
47. Cloughesy TF, Mochizuki AY, Orpilla JR, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med. 2019;25(3):477–86. https://doi.org/10.1038/s41591-018-0337-7.
48. Holl EK, Frazier VN, Landa K, et al. Examining Peripheral and Tumor Cellular Immunome in Patients With Cancer. Front Immunol. 2019;10:1767. https://doi.org/10.3389/fimmu.2019.01767.

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