Neuro-Oncology Advances

8(1), vdaf255, 2026 | https://doi.org/10.1093/noajnl/vdaf255 | Advance Access publication December 08, 2025

Innovative radiotherapies for the treatment of glioblastoma

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Abstract

Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by rapid progression, extensive inter- and intra-tumoral heterogeneity, and resistance to standard-of-care, including radiotherapy. Radiotherapy remains a cornerstone of GBM management, but its efficacy is limited by complex tumor biology and mechanisms of radioresistance. This review explores the advances in radiotherapy for GBM, focusing on the interplay between tumor biology and emerging treatment strategies. Hallmarks of GBM biology, including hypoxia, the robust DNA repair mechanisms, the immunosuppressive tumor microenvironment (TME), and the extensive plasticity contribute to therapeutic resistance. Innovative approaches in radiotherapy may allow to address these challenges. Charged particle therapies (CPTs), including proton and carbon ion therapy, offer superior precision and enhanced biological effectiveness by delivering lethal doses to tumor cells while sparing surrounding healthy tissue. FLASH therapy, using ultra-high dose rates, could reduce normal tissue toxicity without compromising tumor control. Furthermore, targeted radionuclide therapy harnesses tumor-specific targets to systemically deliver radiopharmaceuticals carrying therapeutic radionuclides directly to cancer cells, improving specificity and reducing off-target effects. This review highlights the promise of these novel radiotherapy modalities to address GBM's inherent heterogeneity and treatment resistance. By integrating advancements in technology with novel insights into GBM biology, these approaches may significantly improve patient outcomes.

Key points

- Glioblastoma is characterized by complex and dynamic tumor biology and extensive radioresistance mechanisms, highlighting the need for innovative (radio)therapies.
- Charged particles, FLASH and targeted radionuclide therapy can address these challenges by increasing or maintaining tumor control while reducing normal tissue toxicity and off-target effects.

Biology of Glioblastoma

Glioblastoma (GBM, IDH-wildtype adult-type diffuse gliomas)[1] is one of the most lethal cancers, with a median survival of less than 2 years. GBM is an astrocyte-like tumor, typically exhibiting invasiveness toward other regions of the brain along with other defining hallmarks that contribute to its aggressiveness and low survival rate.[2]

GBM cells display a high level of plasticity allowing them to easily change phenotype.[3,4] In fact, GBM development strongly resembles normal brain development and partially its hierarchical structure,[5,6] although, the GBM tumor cell population is thought to be much more dynamic and diverse.[4] Unlike neural stem cells which terminally differentiate to cells such as neurons and neuroglia, differentiated GBM cancer stem cells adopt similar phenotypes that still have the potential to dedifferentiate, for instance, in response to radiation.[4,7]

This extreme plasticity underlies another key hallmark of GBM tumors, namely a very high inter- and intra-tumoral heterogeneity. Indeed, bulk genomic and transcriptomic analyses have

Received July 9, 2025; accepted December 2, 2025.

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revealed inter-patient heterogeneity. This led to the currently used molecular classification that distinguishes proneural (PN), classical (CL) or mesenchymal (MES) tumors.[8–10] Lately, single-cell analyses revealed GBM tumors as mixtures of these subtypes, referred to as intratumoral heterogeneity.[11] Moreover, multiple studies have shown how GBM cells can exist in different dynamic cellular states. One of these seminal studies identified states reflecting normal neuronal development, including oligodendrocyte precursor cell-like (OPC-like), neuronal progenitor cell-like (NPC-like), astrocyte-like (AC-like) and MES-like cells.[9] When these different GBM cell populations were isolated and cultured, all populations could induce new tumors that resembled the original tumors' heterogeneity.[9] Since then, additional cell states have been defined along axes of neurodevelopment,[5,6] cellular metabolism,[12] stemness/differentiation[13] or injury response,[14] among others.[15,16] This highlights how GBM heterogeneity comprises a broad phenotypic spectrum of different states rather than a collection of discrete subtypes.

Cellular plasticity and the resulting tumor heterogeneity are thought to be among the major culprits in terms of treatment resistance and dismal patient outcome. For instance, GBM cells can shift from proliferative to migratory phenotypes in a process called proneural-MES transition (PMT) allowing them to further invade surrounding tissue.[17] Resistance mechanisms have been studied extensively in the hopes of finding new potential treatment options, unfortunately without much success. For this review, we will describe mechanisms of resistance to conventional photon radiotherapy (CONV-RT), and how innovative radiotherapies may contribute to more effective treatment of GBM patients.

Standard-of-Care

The standard-of-care treatment for GBM consists of maximal safe surgical resection, followed by CONV-RT with concomitant temozolomide (TMZ) and then adjuvant TMZ.[18] TMZ is an oral alkylating agent that induces DNA damage and works synergistically with CONV-RT by increasing the radiosensitivity of tumor cells by impairing DNA repair through depletion of the DNA repair enzyme O6-methylguanine DNA methyltransferase (MGMT).[19] Its effectiveness is enabled by the methylation of the *MGMT* promoter, making *MGMT* methylation status a commonly used prognostic and predictive biomarker for GBM.[20,21]

CONV-RT is a cornerstone of the management of GBM[22] by enhancing local control. CONV-RT has been used to treat brain tumors since the first half of the 20th century.[23] At first, kilovoltage X-rays and by the 1960s megavoltage X-rays or 60-Co therapy irradiations of the whole brain were used. However, it wasn't until the late 1970s that a survival benefit of RT could be actually demonstrated.[24] Although initially lower doses and dose rates were used, a thorough analysis of the dose-effect relationship for RT in malignant gliomas suggested an optimal dose of 60 Gy that had superior survival over lower doses with no increased toxicity.[25] Above that dose, no further beneficial effects have been observed. Nowadays, the most standard regimen of CONV-RT for GBM is 2-Gy daily fractions, 5 days per week, for 6 weeks, for a total dose of 60 Gy. This regimen is applied to newly diagnosed patients under 70 years in good general and neurologic condition. It should be adapted based on patient characteristics as age and general health.[26] In the elderly, or patients with poor Karnofsky performance status hypofractionation can be used, in which higher daily doses are provided for a shorter period of time. This reduces the overall treatment time, decreases costs to patients and health care systems, while overall survival and quality of life is not affected.[27–29]

Since the establishment of the Stupp protocol as standard of care, advances in the treatment have been few and far between. Yet, there is evidence that since the generalization of this protocol, median survival is rising. However, this may not be attributed to advances in the Stupp protocol itself,[30] but could be due to the introduction of new treatment modalities such as bevacuzimab (for recurrent GBM) and tumor-treating fields (for newly diagnosed and recurrent disease),[31,32] although these are not yet applied routinely across the world and their clinical benefit remains to be definitively established.[26] Besides these, significant advances were made in target delineation and patient positioning and the application of conformal radiation techniques such as intensity-modulated RT or stereotactic RT have helped to reduce the unwanted irradiation of healthy tissue near the tumor.[22] However, some brain structures may still receive high doses resulting in acute or late toxicity and CONV-RT only prolongs survival with several months. Moreover, irradiation itself can induce therapeutic resistance, tumor aggressiveness and recurrence, indicating the need for more advanced therapeutic strategies.

Mechanisms of Radioresistance

Despite intensive chemoradiotherapy after maximal surgery, GBMs almost inevitably recur. This is believed to result from the re-emergence of cancer cells, either via (1) positive selection of intrinsically treatment-resistant primary tumor cells, or (2) adaptation of tumor cells toward a more resistant phenotype.[4] Both pathways are linked to the extreme heterogeneity of cell types and cellular states, where adaptation involves cellular plasticity. Cancers stem cells (in this case glioma stem cells, GSCs[33]) are believed to be at the apex of these processes as they have the properties of initiating the cancer, resist therapy and underlie recurrence. The exact identity of GSCs and their biological characteristics are still debated,[5,34,35] in part due to the lack of adequate biomarkers,[36] but also their high plasticity. Rather than fixed, GSCs are flexible and can adapt to signals from the TME or therapy itself. Likewise, non-GSC GBM cells are capable to adopt any cell state (including GSC) and reconstitute tumor heterogeneity.[9] Therefore, rather than targeting GSCs directly, the inherent cancer cell plasticity may provide an alternative therapeutic target.[37] Resistance itself can be dictated by intrinsic properties, by extrinsic cues, or the cooperation between both. Below, we discuss intrinsic and extrinsic mechanisms known to be involved in GBM radioresistance (Figure 1).

Intrinsic Mechanisms of Radioresistance

One of the earliest identified mechanisms by which GSCs promote radioresistance was a very efficient DNA damage

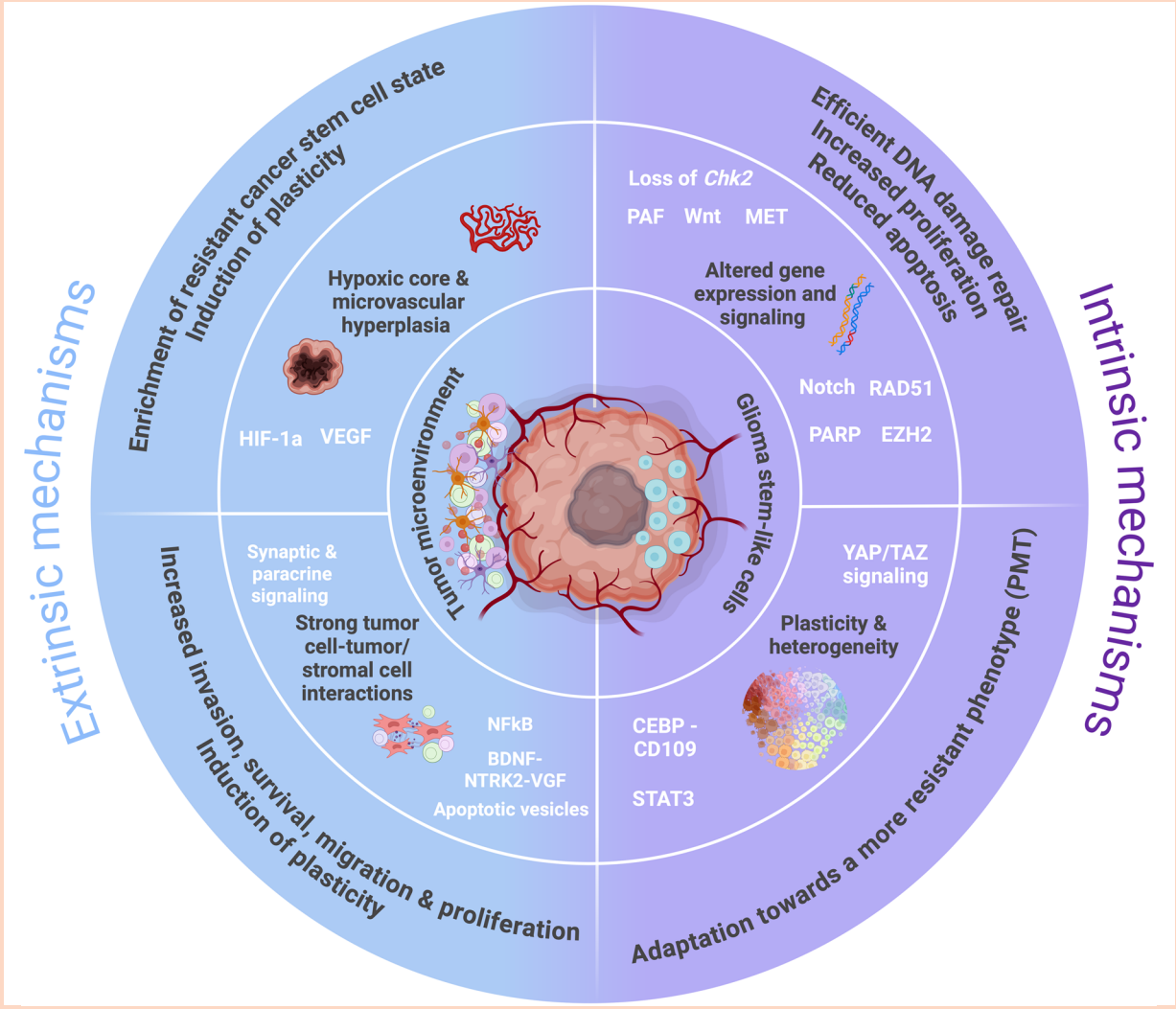


Figure 1. Glioblastoma hallmarks and how they contribute to radioresistance. The TME lies at the base of 2 important hallmarks of GBM, its hypoxic core and microvascular hyperplasia and the strong interactions existing among tumor cells and stromal cells. These 2 hallmarks promote radioresistance through extrinsic mechanisms by enrichment of cancer stem cells, increasing invasion, survival, migration and proliferation, and inducing plasticity toward more resistant states. The heterogeneous glioma stem-like cells with their high plastic abilities, altered gene expression and signaling pathways, on the other hand, lie at the base of intrinsic mechanisms of radioresistance including adaptation (PMT), efficient DNA damage repair, increased proliferation and reduced apoptosis. Extrinsic and intrinsic mechanisms of radioresistance, however, often cooperate which is indicated by the color gradient. Genes, pathways or other important factors involved in these hallmarks and radioresistance, which are discussed above, are indicated in white.

response (DDR)[21] that depended on the activation of checkpoint kinases (ATM, Chk1 and Chk2).[38] This was confirmed in patients treated with high doses of radiation. Recurrences in these patients were enriched in $CD133^+$ GSCs which were rarely seen in the primary tumor before adjuvant treatment.[39] Another DNA damage signaling mechanism involved in GSC radioresistance, stemness and tumorigenicity is the proliferating cell nuclear antigen-associated factor (PAF) which regulates DNA translesion synthesis to facilitate DNA damage bypass.[40] PAF was enriched in GSCs and correlated with poor prognosis, while its depletion resulted in reduced tumorigenesis when combined with radiation. Moreover, expression of RAD51, a central player in the homologous repair pathway, is highly enriched in GSCs and directly correlated with their radiosensitivity. Reduced expression of RAD51 upon differentiation of GSCs coincides with their decreased DNA repair potential. Inhibition of RAD51 enhanced their radiosensitivity, indicating that DNA repair in GSCs is RAD51-dependent and that it could be used as a potential therapeutic target.[41,42] A similar preferential activation of the ATM-Chk2-RAD51-depedent DNA repair was observed in GSCs characterized by high expression of the MET receptor kinase.[43] Finally, inhibition of Poly(ADP-Ribose) polymerase has been proposed as a way to radiosensitize GSCs and to reduce their tumorigenic capacity.[44,45] DNA damage repair mechanisms in glioma and their potential as therapeutic vulnerabilities have been reviewed elsewhere.[21]

Other signaling pathways not directly related to DNA damage repair also influence radiosensitivity in GBM. Wnt signaling caused radioresistance of GBM cells in orthotopic

mouse models.[46] Similarly, inhibition of Notch signaling reduced proliferation and clonogenic survival of CD133$^+$ (but not CD133$^-$) GSCs, while increasing radiation-induced apoptosis.[47] In a GBM explant model containing endothelial cells, similar Notch inhibition decreased self-renewal of CD133$^+$ GSCs partly via the loss of endothelial cells.[48] Finally, the epigenetic regulator EZH2, one of the core components of the Polycomb repressive complex 2, together with its upstream transcriptional regulators MELK and FOXM1, is upregulated in GSCs in response to radiation and induces radioresistance, both *in vitro* and *in vivo*.[49,50]

The impact of genetic evolution toward a radioresistant phenotype is most likely of limited importance in GBM. Indeed, recurrences usually present few genetic changes compared to the primary tumor.[51–53] This contrasts with IDH_{mut} glioma, where hypermutation and changes in DNA methylation in recurrences are more common.[54–56] Here, the hypermutation rate is driven by mutations in mismatch repair genes and dose-dependently associated with TMZ treatment, but not radiotherapy.[57]

Intrinsic cellular plasticity

Besides inherent mechanisms of radioresistance, other intrinsic properties were identified that can induce the adaptation of cells toward a more resistant phenotype.[4] One of the best-known examples of phenotypic plasticity that occurs under radiation pressure is the PMT. PMT is for GBM the equivalent to epithelial-to-mesenchymal transition (EMT), a process that occurs under physiological (eg, embryonic development) and pathological (eg, cancer progression) conditions. EMT is characterized by the downregulation of epithelial features in epithelial cells allowing them to acquire MES phenotypes with enhanced migration and invasion.[58,59] In the case of PMT, PN GBM cells which are generally assumed to be more sensitive to radiation, undergo a phenotypic shift toward the more resistant MES subtype.[11,60] The first evidence that radiation itself could trigger PMT came from a study in PN GSCs in which radiation upregulated the expression of MES markers, while PN markers were reduced.[61] This was soon followed by a study in glioma-bearing mice, in which a shift of PN to MES gene expression profiles was observed within hours after irradiation. This shift was an inherent feature of tumor cells and not evoked by stromal changes.[62] Different cell-intrinsic mechanisms of radiation-induced PMT in GSCs have been proposed, including activation of STAT3[63] and YAP/TAZ-signaling.[64] Furthermore, a clonal analysis of GSCs exhibiting a wide range of sensitivities to different treatments, including TMZ and RT, showed that treatment sensitivity correlated with gene expression signatures along a continuous PN-MES subtype axis, which was associated with DNA methylation alterations.[65] The clinical significance of PMT was evidenced by studies comparing paired primary and recurrent tumors. This showed that overall, primary tumors displayed a more PN phenotype that shifted to a MES phenotype in recurrent tumors.[64,66,67] This suggests that patients undergo PMT at recurrence, as a consequence of treatment. The mechanism was proposed to involve both cell-intrinsic and -extrinsic factors.[66]

Importantly, like EMT, PMT is reversible (MES-to-PN transition) and can also lead to intermediate states.[68] Furthermore, GBM cells can transition to virtually any cell state, including a newly identified neural subtype[67] in a stochastic manner.[4,37,69] Also endothelial transdifferentiation of GSCs to vascular- and pericyte-like cells, supporting tumor recurrence, occurs after radiation.[70] However, not all these transitions are accompanied by an increase in therapeutic resistance. Which transitions occur under therapy, how this is regulated at the molecular level, and whether these transitions can be therapeutically exploited, are important subjects for new studies.

Extrinsic Mechanisms of Radioresistance

Extrinsic mechanisms, often activated by cues originating from the TME, can influence radioresistance too. Recent advances in single-cell and spatial multiomics have contributed greatly to a better understanding of the interplay between GBM cells and the TME.[71] In GBM, the TME composition is defined by different niches, including the perivascular tumor niche, the invasive edge and the and hypoxic-necrotic tumor niche, each with their own characteristics and influences on their respective GBM cells, including their sensitivity to radiation.[72–76]

Hypoxia has long been associated with tumor radioresistance[77] and most studies seem to converge on the observation that it enriches the radioresistant cancer stem cell state.[37] A recent study showed that hypoxia increased the number of quiescent MES-like and radioresistant GBM cells.[78] Spatial analyses demonstrated that it affects proliferation and migration to allow cells to escape the hypoxia-necrotic niche,[71] which is not observed in oxygenated tumor areas or less hypoxic brain tumors.[15] As with radiation itself, hypoxia induces tumor cell plasticity,[79,80] although this does not seem to occur in a cell-intrinsic manner.[81] Altogether, these studies have further indicated that hypoxia is a major determinant of cellular plasticity and radioresistance of GBM tumors.

The exact influence of the different components of the complex GBM TME on radioresistance is a field of active research and some of these processes are only emerging to be discovered. At first, interactions between tumor cells were investigated. These interactions occur via so-called tumor microtubes and enhance the invasion, proliferation and resistance to radiotherapy of interconnected GBM cells as compared to unconnected cells.[82] These networks are further supported via synaptic and paracrine signaling, including mitochondrial transfer, from surrounding neurons and astrocytes which leads to increased proliferation and invasion.[83–85] Bidirectional tumor-tumor or tumor-stromal cell interactions also influence their cellular state and migratory potential. Stationary gap-junction-coupled tumor cells mostly reside in the tumor core and adopt AC-like or MES-like cell states. In contrast, tumor cells that connect to neurons via synapses resemble NPC-like or OPC-like states. These astrocyte-unconnected cells are found at the invasive edge and are the main drivers of diffuse brain invasion.[86,87] On the other hand, indirect crosstalk between cells from the tumor core with the invasive edge via paracrine signaling also promotes infiltrative growth and contributes to the development of radioresistance of the latter.[88] Paracrine

signaling via BDNF-NTRK2-VGF was also found between GSCs and their differentiated progeny, their survival and proliferation.[89] Finally, intercellular paracrine contact exists between dying and surviving GBM cells. Dying cells secrete apoptotic extracellular vesicles containing spliceosome components which induce changes in alternative splicing of DNA repair genes and EMT-related proteins in the recipient cells. This, altogether, induced proliferation, migration and therapy resistance in the surviving neighboring cells.[90]

Other extrinsic signals from the TME that contribute to radioresistance involve immune signaling from macrophages via NFkB, which triggers PMT in GSC cultures.[91] This was confirmed when interactions between macrophages and GBM cells were shown to drive the MES-like state *in vitro* and *in vivo*.[92] Furthermore, signaling from the cerebrospinal fluid induces tumor cell plasticity and chemoradioresistance, which could be pharmacologically prevented.[93] Importantly, RT itself also directly affects all components of the TME,[94] and these changes may cooperate with cell-intrinsic mechanisms that contribute to therapy resistance. For instance, chemoradiotherapy was recently found to reprogram GBM cells to a state that favors blood vessel co-option and brain infiltration preferentially near the perivascular niche. This, in turn, promotes further state transitions, creating a self-perpetuating cycle that drives GBM recurrence.[68]

How to Uncover Mechanisms of Radioresistance?

Research has greatly benefited from recent advances in single-cell (multi-)omics to identify some of the abovementioned mechanisms underlying treatment-induced plasticity and resistance. These methods have been applied in prolonged exposures or dose escalation studies which result in the enrichment of treatment-resistant and drug-tolerant persister cells that could be studied at different time intervals.[95] Moreover, lineage tracing of cells under therapeutic pressure allowed the identification of epigenetic mechanisms contributing to resistance.[96] Such methods have, to our knowledge, not yet been applied to study mechanisms of resistance to radiation specifically. Also, these studies used mostly simple *in vitro* (eg, monolayer or spheroid GSC cultures) and animal models. Therefore, a real understanding of the underlying biology in patients is currently lacking. This is in part because of the low fidelity of many of the preclinical models, which overall poorly represent intra- and inter-tumoral heterogeneity, as well as the slow cycling and quiescent states seen *in vivo*, that promote resistance.[11,95] Some studies have used pre- and post-treatment patient samples (mostly from biopsies at recurrence), for example, from the Glioma Longitudinal AnalySiS (GLASS) consortium,[51] and found evidence of changes in cellular phenotypes, TME and immune environment.[12,66,67,97] However, these are likely not reflective of the real dynamics that tumor cells, including GSCs, may undergo during SoC, that is, following daily fractions of irradiation and chemotherapy for many weeks. To uncover tumor responses to a treatment, different strategies will need to be employed. More advanced preclinical models such as GBM organoids (also called tumoroids),[98] brain-GBM assembloids,[99,100] tumor-on-chip,[101] and orthotopic tumoroid xenografts[102] better recapitulate certain aspects of the tumor biology, including the cellular heterogeneity, plasticity and tumor invasion. Moreover, these models reliably predict the patient's response to therapy.[103–106] Longitudinal studies in such models will allow to dissect mechanisms of resistance to radiation, drugs, or their combination. Perhaps the most relevant to the patient's response will be the use of serial biopsies or longitudinal sampling studies, in which tumor samples are obtained before and at predefined time points after therapy. Although this setup is widely used across many tumor types, this is not yet the case for GBM.[107] The feasibility of such protocols has recently been proven in a study investigating tumor response to oncolytic immunotherapy.[108] This allows the mapping of temporal trajectories of resistance, including epigenetic reprogramming and microenvironmental influences, although singling out mechanisms of radiation resistance is only possible from samples of patients who only received RT as therapy (~10% in the GLASS data).

It is clear that GBM tumor cells and GSCs are exquisitely equipped to adapt to therapy. Many of the abovementioned mechanisms have been (pre)clinically targeted but so far, none of these have resulted in novel treatment options. Below, we will highlight current and emerging RT modalities, their pros and cons and new avenues to consider for a better outcome for GBM patients.

External Beam Radiotherapy

Charged Particle Therapy

CPT refers to the use of protons (proton beam therapy, PBT) or heavy ions, such as carbon ions (carbon ion beam therapy, CIBT), for cancer treatment. PBT and CIBT are currently clinically used to treat various cancers.[109] CPT partly owes its effectiveness to its so-called Bragg peak, a steep increase in the deposition of energy at a certain distance from the source, usually toward the end of the particle range[110] (Figure 2A). This Bragg peak is focused on the tumor, thereby minimizing damage to normal tissue while delivering more damage, usually in the form of complex double-strand DNA breaks (DSBs), to the target.[109,111–113] The charged particles allow multiple beams to be magnetically directed and longitudinally and laterally spread out, resulting in a spread-out Bragg peak (SOBP) (Figure 2A), which allows clinicians to focus the energy deposition on the entire three-dimensional tumor.[114] Around the point of entry, CPT is generally characterized by a lower linear energy transfer (LET). This LET increases with depth as the particle slows down resulting in a high LET at greater depths. Here, CIBT is able to inflict more, often clustered, DNA damage than low-LET radiation such as CONV-RT or PBT (Figure 2A). However, due to nuclear fragmentation, distal tissue receives a, albeit very minimal, dose of energy when irradiated with CIBT.[113] CIBT has a higher relative biological effectiveness (RBE), estimated at 2.5-5.0.[114] Because high-LET radiation more directly targets DNA compared to low-LET radiation, it is less dependent on oxygenation and therefore particularly interesting for hypoxic tumors, such as GBM[115] (Figure 2A). PBT being classified as low-LET radiation, has a lower estimated RBE of 1.1.

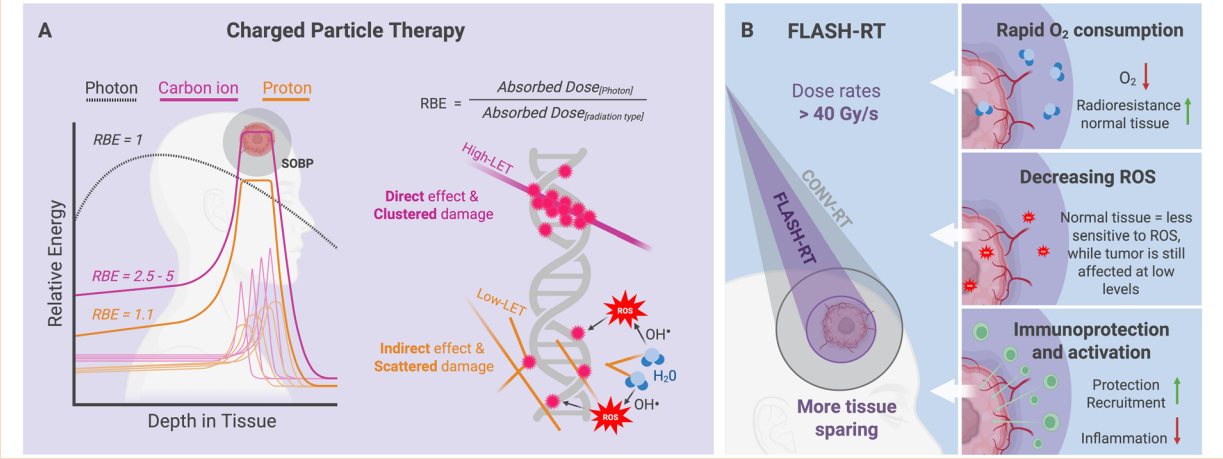


Figure 2. Innovative external beam radiotherapy (EBRT) for glioblastoma. (A) CPT such as CIBT and PBT is characterized by a Bragg peak in the dose-depth curve, allowing a higher energy dose to be delivered to the target site and more sparing of normal tissue. High-LET radiation such as CIBT has a high relative biological effectiveness (RBE) and can induce clustered DNA damage though a direct effect whereas low-LET radiation like PBT and CONV-RT (Photon) have lover RBE's and result in more scattered damage, single-stranded breaks via the formation of reactive oxygen species (ROS). (B) FLASH-RT is characterized by high dose rates above 40 Gy/s. Mechanistically, 3 hypotheses are proposed for the increased normal tissue sparing including (1) rapid O_2 consumption resulting in increased radioresistance of normal tissue; (2) a decrease in ROS resulting in less damage to normal tissue while the tumor is still affected at low levels; and (3) and increase in immunoprotection and activation/recruitment together with a decrease in inflammation.

Therefore, the biological effects of PBT is comparable to CONV-RT, although PBT better spares the surrounding normal tissue.[116]

CPT for glioblastoma

CPT is considered for GBM due to its theoretically higher tumor-eradicating potential, independence of tumor oxygenation and reduced healthy tissue toxicity[116,111,112] Preclinical studies have shown that CIBT results in a superior eradication of GSCs compared to PBT and CONV-RT, via induction of more complex DNA damage that could not be repaired.[117] In a follow-up *in vivo* study CIBT led to prolonged survival through a potent antiangiogenic effect and loss of hypoxic radioresistant cells. Moreover, differential gene expression analysis revealed that genes associated with EMT, were downregulated after CIBT when compared to sham-treatment.[118] This suggests that CIBT is less likely to induce EMT (and therefore also PMT) than photon radiation, although this remains to be investigated. Stahler et al.[119] reported a decrease in EGFR-mediated migration of GBM cells after CIBT whereas an increase in tumor cell migration was seen after photon irradiation. However, patient-derived GBM cells with dysfunctional ATM signaling showed resistance to both photon and carbon ion irradiation.[120] Moreover, the effect of the combination of CIBT with chemotherapy such as TMZ has not yet been sufficiently studied although a synergistic effect independent of MGMT expression was observed after treatment of GBM cells with CIBT and TMZ.[121,122]

It should be noted that carbon ions have a lower fractionation capacity, which makes the treatment of infiltrative tumors like GBM challenging as these treatments require dose fractionation to spare healthy tissue that lies within the clinical target volume. Therefore, a combination of CONV-RT and PBT has been suggested for optimal treatment efficacy.[111] Combs et al.[123] conducted a retrospective analysis comparing data from a phase I/II trial where patients with GBM or anaplastic astrocytoma (AA) were treated with CONV-RT (total dose 50 Gy), a carbon ion boost (16.8-24.8 gray equivalent [GyE, ie, absorbed dose]) and concurrent nimustine (100 mg/m^2)[124] with data form the Heidelberg Ion Therapy (HIT) center where 32 GBM and 16 AA patients were treated with CONV-RT alone or in combination with TMZ.[125–127] Based on progression-free (PFS) and overall median survival (OS), they suggested that a carbon ion boost could be beneficial for GBM patients. An ongoing randomized phase II trial (NCT01165671, CLEOPATRA) investigates the clinical benefit of delivering a carbon ion boost of 18 Gy in 6 fractions after CONV-RT (48-52 Gy) and concurrent TMZ.[128]

A number of clinical trials have investigated the effects of PBT in GBM, either on its own or in combination with chemoradiotherapy[18] or a CIBT boost. Already in 1993, a small-scale phase I/II clinical trial was performed including 13 patients with AA or GBM that either received a proton boost after CONV-RT up to 86.6 Gy or PBT alone up to 66.8 Gy.[129] No significant clinal benefits were reported. In 1999 a prospective phase II trial was conducted on 23 GBM patients with a proton boost after dose-escalated RT which resulted in increased local tumor control.[130] Mizumoto et al.[131] reported the results of hyper-fractionated RT with a PBT boost up to a total dose of 96.6 GyE in 56 fractions. GBM growth was significantly suppressed and increased local tumor control was observed in regions receiving over 90 GyE. A retrospective analysis based on the CLEOPATRA trial (NCT01165671) investigated 132 high-grade glioma patients who received conventional chemoradiotherapy followed by a proton boost. Compared to chemoradiotherapy, there was no significant increase in PFS or OS but also no high-grade toxicity, whereas grade 3 toxicities were observed in 8% of the photon cohort.[132] A study from 2020[133] investigated the

Table 1. Clinical trials for charged particle therapy in glioblastoma

EBRT type	Trial ID or publication	Phase	Status	Indication	Intervention	End points or outcome
Carbon ion						
	Mizoe *et al. Int J Rad Onc* (2007)	I/II	Completed	Glioblastoma, anaplastic astrocytoma	CONV-RT + carbon ion boost (16.8-24.8 GyE) + nimustine (100 mg/m^2)	Median survival 17 months (GBM) and 35 months (AA)
	NCT01165671	II	Ongoing	Glioblastoma	CONV-RT (48-52 Gy) + TMZ + carbon ion boost (18 Gy)	Overall survival, progression-free survival, toxicity and safety.
Proton						
	Tsjujii *et al. Int J Radiat Oncol Biol Phys* (1993)	I/II	Completed	Glioblastoma, anaplastic astrocytoma	CONV-RT + pronton boost *vs.* proton alone	No significant clinical benefits
	Fitzek *et al. J Neurosurg* (1999)	II	Completed	Glioblastoma	CONV-RT + proton boost	Increased local tumor control
	NCT01854554	II	Completed	Glioblastoma	PBT vs. IMRT	No increased OS or cognitive performance, decrease in adverse effects

clinical potential of PBT combined with a CIBT boost and TMZ on 50 GBM patients and observed considerable increases in PFS and OS compared to chemoradiotherapy. Based on a prospective phase II trial with GBM patients receiving either PBT or intensity-modulated RT (NCT01854554), Brown et al.[134] and Mohan et al.[135] reported no significant increase in OS or improvement in cognitive performance but adverse effects were significantly decreased, including reduced radiation-induced grade 3+ lymphopenia and fatigue. In summary, these clinical trials showed only minor advantages of PBT over CONV-RT regarding the tumor eradicating effect whereas adverse effects were often less severe with PBT due to its healthy tissue sparing abilities.

Nonetheless, PBT for GBM is still being explored further. The above-mentioned CLEOPATRA trial (NCT01165671) additionally investigates the clinical benefit of a proton boost of 10 Gy in 5 fractions after 48-52 Gy CONV-RT and concurrent TMZ, although, it is considered as the standard arm as its biological effects are comparable to those of CONV-RT.[128] A phase III clinical trial (NCT04536649) conducted by the Shanghai Proton and Heavy Ion Center (SPHIC) currently investigates standard dose PBT (60 GyE/30 fractions) plus TMZ with or without a CIBT boost (15 GyE/3 fractions) for the residual tumor. Finally, a phase II clinical trial (NCT02179086, also NRG-BN001) compared dose-escalated photon and proton therapy plus TMZ with conventional chemoradiotherapy for GBM patients. 193 patients were treated in the proton therapy arm and an improvement in survival was observed of 6.8% at 2 years and 4.6% at 3 years, together with no significant difference in high grade toxicities compared to the other treatment arms.[136] An overview of current and past clinical trials is provided in Table 1.

FLASH-RT

FLASH-RT is the term used for the administration of radiotherapy where relatively high doses of radiation (eg, photons or protons) are administered at much higher (ie, > 40 Gy/s) dose rates than those of CONV-RT (ie, < 0.1 Gy/s) in the matter of milliseconds (Figure 2B).[137] This concept was first investigated in the 1960s[138] and has gained increased interest since 2014 when preclinical studies indicated a potential benefit of FLASH over CONV-RT concerning normal tissue toxicity due to its superior healthy tissue-sparing abilities while preserving tumor control resulting in an increased therapeutic index.[139,140] Several mechanisms have been proposed to explain its mode-of-action (Figure 2B): (1) FLASH-RT consumes the oxygen present in healthy tissues leading to an increase in their radioresistance. This effect would be limited in the already-hypoxic tumor[141–144]; (2) FLASH-RT protects normal tissue by decreasing ROS. Healthy cells are less sensitive to ROS than cancer cells, leading to the latter still being affected at lower ROS levels after FLASH-RT[142,145,146]; (3) FLASH-RT is better at protecting circulating immune cells[147] and recruiting them to kill the tumor than CONV-RT[148] and decreases TGF-β-dependent inflammation in normal tissue.[149] Importantly, it was recently shown in head and neck squamous cell carcinoma that rhodotron accelerator-based FLASH-RT could reverse radioresistance and improve tumor control in CONV-RT resistant tumors.[150]

FLASH-RT for glioblastoma

In 2019, the first patient was treated with FLASH-RT. A 75-year-old T-cell lymphoma patient received 15 Gy of FLASH-RT and the clinical outcome was favorable, marking the start of clinical application for FLASH-RT.[151] After that, proton FLASH-RT has been investigated in a clinical setting for bone metastatic cancer in the prospective, nonrandomized FAST-01 trial[152] and, after favorable outcome, the ongoing FAST-02 trial (NCT05524064). Another ongoing, randomized phase II trial (LANCE,[153] NCT05724875) evaluates the curative effect of electron FLASH-RT versus CONV-RT in skin cancer patients. No clinical trials have investigated the therapeutic efficacy of FLASH-RT on GBM patients so far. Nonetheless, several preclinical data support the potential for this treatment modality, especially when it

Table 2. Properties of different modes of decay used in targeted radionuclide therapy

Feature	α decay	β^- decay	Auger emission
Particle emitted	α-particle	Electron	Auger electron
Change in nucleus	Yes	Yes	No
Penetration	Low	Moderate	Very low
Ionization	High	Moderate	High (localized)
Linear energy transfer (LET)	~80 keV/μm	~0.2 keV/μm	~4-26 keV/μm
Relative biological effectiveness (RBE)	5-20	1	3-5
Approximate particle range	40-100 μm in tissue (a few cm in air)	few mm in tissue (tens of cm in air)	nm in tissue (μm in air)
Examples of radionuclides (with half-life)	Astatine-211 (~7.2 hours) Radium-223 (~11.4 days) Actinium-225 (~10 days) Lead-212 (~10.6 hours) Bismuth-213 (~45.6 minutes)	Yttrium-90 (~2.7 days) Iodine-131 (~8 days) Lutetium-177 (~6.7 days)	Technetium-99m (~6 hours) Indium-111 (~2.8 days) Iodine-123 (~13.2 hours) Iodine-125 (~59.4 days)

comes to neuroprotective effects. One study used a murine orthotopic GBM model to investigate the effects of FLASH-RT versus CONV-RT at single doses (10 or 14 Gy) or hypofractionated (4 x 3.5 Gy or 2 x 7 Gy in daily fractions or 3 x 10 Gy with 48 h intervals).[154] The decrease in GBM growth was similar between both RT types but significantly fewer cognitive defects were observed after FLASH-RT. Other studies in healthy mice showed that FLASH-RT protected neurogenesis and limited apoptosis in the hippocampus[155] or to lower the levels of pro-inflammatory microglia[142] whereas CONV-RT increased the release of pro-inflammatory cytokines in the hippocampus.[156] Moreover, structural integrity of dendritic spines was preserved after FLASH-RT compared to CONV-RT.[142,156] As mentioned above, FLASH-RT is proposed to be better at eliciting an immunogenic response than CONV-RT.[148] Iturri et al.[157] used an orthotopic glioma rat model to study the immune response after proton FLASH-RT (25 Gy single dose) and regular PBT. Proton FLASH-RT spared memory function while inducing an effective but comparable recruitment of lymphocytes.[157] A recent study sought to investigate the long-term neurocognitive effects of FLASH-RT on the radiosensitive juvenile mouse brain. Comparing FLASH-RT with CONV-RT using this non-tumor-baring mouse model indicated that FLASH-RT resulted in a superior preservation of cognitive function, synaptic integrity and cerebrovascular structure over the course of 6 months after irradiation.[158] Finally, using an osteosarcoma mouse model, researchers have recently shown that carbon ion FLASH-RT (20 Gy) resulted in fewer normal tissue toxicities, while tumor control and immune activation remained comparable to CONV-RT.[159] Despite these promising results, other animal studies showed no additional benefit of FLASH-RT, both in terms of neuroprotection,[160] tumor control[161] or immune activation.[162] Unfortunately, the ultra-high dose rates used for FLASH-RT impose a major technological challenge. Accurate delivery of FLASH-RT still relies heavily on machine stability and passive dosimetry, as the currently available monitoring chambers are ineffective for these ultra-high dose rates.[163] Therefore, a recent study investigated the stability of an ultra-high dose rate Mobetron (IntraOp, United States) in terms of both short- and long-term output over the course of more than 2 years. In parallel, the phase I IMPulse trial (NCT04986696) was initiated where patients with skin metastases from melanoma were treated with a range of flash doses (22-28 Gy). Results showed favorable short- and long-term stability of the Mobetron system.[164] Concerning treatment efficacy, no concluding findings have been reported, although, initial results (n=8) suggest no dose-limiting toxicities.[165] Clinical translation of this novel EBRT modality will require further investigation on a multidisciplinary level, including a better understanding of the biological mechanisms behind FLASH, technological advances to overcome delivery-related challenges, and further in-depth assessment of the long-term toxicity in adequate preclinical models.[166]

Targeted Radionuclide Therapy

Targeted radionuclide therapy utilizes radiopharmaceuticals binding with high affinity and specificity to disease-specific epitopes, while carrying a radionuclide that emits short-range cytotoxic ionizing radiation in the form of α-particles, β-particles, Auger and conversion electrons (Table 2, Figure 3).[167,168] The targeting moiety consists of a small molecule, peptide, aptamer, antibody, or antibody fragment, often repurposed from regular drug development. This moiety is coupled via a linker to a chelator which complexes radiometals (eg, gallium-68, lutetium-177) or covalently binds to the radionuclide (iodine-131, astatine-211).[169]

TRT offers several advantages in the treatment of brain cancers. By delivering radiation directly to cancer cells or the TME, TRT minimizes damage to surrounding healthy tissues, thereby reducing side effects compared to conventional therapies. TRT can effectively target both primary tumors and minimal residual lesions, offering a comprehensive strategy for managing advanced or hard-to-treat cancers. Notably, one of the main strengths of TRT is the ability to implement a theranostic approach, where the presence of the target is first confirmed with a diagnostic radionuclide (emission of γ- or X-rays). Accurate target quantification by

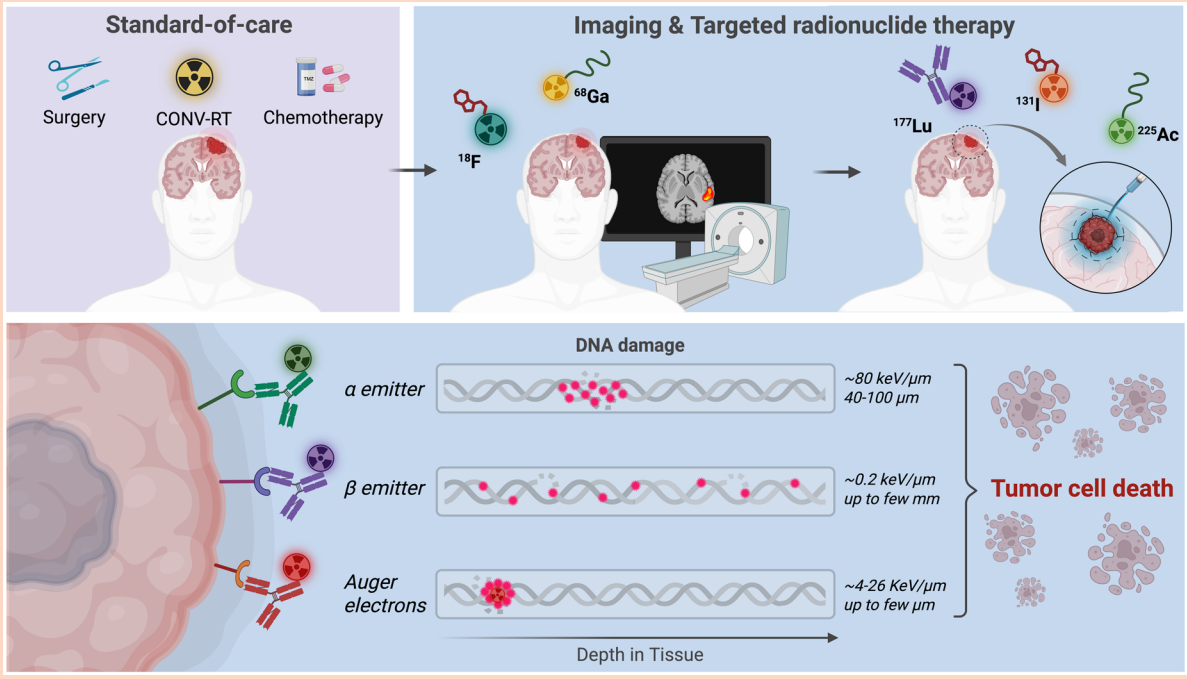


Figure 3. Targeted radionuclide therapy as a novel approach for treatment of GBM. Standard-of-care treatment, including CONV-RT, chemotherapy (TMZ), and surgery, are often insufficient for managing GBM due to its aggressive and infiltrative nature. TRT, combined with imaging for personalized treatment, could serve as an adjunct to standard-of-care. Diagnostic imaging uses radiopharmaceuticals, such as fluorine-18 and gallium-68, to visualize tumors and guide therapy. Therapeutic radionuclides, including iodine-131, lutetium-177, and actinium-225, deliver targeted radiation to GBM cells. These radionuclides induce either single-strand or double-strand DNA damage, as well as cell membrane damage through the emission of α particles, β particles, and Auger/conversion electrons, leading to tumor cell death. Their distinct energy and penetration depth result in different DNA damage profiles: α-particles cause clustered double-strand breaks due to their short range but high energy deposition, β-particles have a longer penetration depth with lower energy, primarily inducing single-strand breaks, and Auger electrons create highly localized, severe DNA damage when near the nucleus.

PET imaging enables patient selection, dose determination, and monitoring of disease progression/remission. Following pretherapeutic or follow-up imaging, selected patients can be treated with the therapeutic counterpart of the radiopharmaceutical (Figure 3), allowing for a personalized treatment approach that maximizes therapeutic efficacy.[170] In addition to being a targeted radiation approach, a key difference between TRT and EBRT is the dose rate, which may lead to different biological outcomes. EBRT delivers localized high-dose exposures over repeated treatment sessions, often requiring frequent hospital visits, while TRT provides lower dose-rate radiation systemically across a few spaced treatment cycles.[171] Additionally, TRT can also be administered locally, such as via intrathecal injection in the case of GBM.[172]

Personalized dosimetry, which involves estimating absorbed doses in tumors and healthy organs based on injected activity and radiopharmaceutical imaging, is a challenging yet advantageous aspect of TRT. The 2 FDA- and EMA-approved TRTs, [^{177}Lu]Lu-DOTATATE (Lutathera) for gastroenteropancreatic neuroendocrine tumors and [^{177}Lu]Lu-PSMA-617 (Pluvicto) for metastatic castrate-resistant prostate cancer, are currently administered to patients in fixed doses.[173,174] However, ongoing research is exploring the integration of personalized dosimetry to improve treatment efficacy, minimize toxicity, and better tailor therapies to individual patient needs.

Another advantage of TRT over EBRT is its ability to effectively treat small, dispersed lesions through systemic or localized administration of radiopharmaceuticals, allowing the targeting of multiple tumor sites simultaneously.[171] This makes TRT particularly beneficial in managing advanced and aggressive cancers, such as GBM, where localized, untargeted approaches like EBRT may be insufficient to completely eradicate the disease and prevent recurrence.[175]

The Choice of Radionuclide in TRT for Glioblastoma

A diverse array of radionuclides is currently employed in TRT, each offering unique properties based on their particle emission type, energy, range, and physical half-life (see Table 1). Effective treatment planning requires aligning the physical half-life of the radionuclide with the biological half-life of the targeting vector, ensuring optimal tumor accumulation while minimizing exposure to healthy tissues. Particle range is also a critical consideration, longer ranges are beneficial for addressing large or heterogeneous tumors, while shorter ranges are more suitable for localized or microscopic disease.[171]

β-emitting radionuclides, including yttrium-90, iodine-131, and lutetium-177, are the most commonly used in current clinical TRT protocols. Their ability to emit particles over

several millimeters makes them well-suited for treating large or bulky GBM lesions, typically larger than 0.5 cm.[175] This is mainly due to the crossfire effect, which allows β-particles to irradiate surrounding tumor cells that may not have directly taken up the radiopharmaceutical. This phenomenon is particularly valuable in GBM, where heterogeneous expression of the target receptor often limits uniform distribution of the therapeutic agent across the tumor mass.[176] Despite this advantage, β-particles exhibit a relatively low LET of approximately 0.2 keV/µm, which may reduce their efficacy against hypoxic or radioresistant cells that require higher energy deposition for effective DNA damage.[175]

In comparison, α-emitters such as actinium-225, astatine-211, lead-212, and bismuth-213 emit particles with a much higher LET (around 80 keV/µm) and a significantly shorter range of 40 to 100 µm.[177,178] These properties enable highly efficient cell killing and an RBE that can reach up to 20, making them particularly effective against hypoxic tumors, infiltrative residual GBM, as well as cerebral micro-metastases. However, the potent cytotoxicity of α-particles also presents challenges as nonspecific uptake or off-target distribution can damage adjacent healthy tissues. Moreover, recoiling daughter isotopes during decay of actinium-225 may redistribute to non-target organs and contribute to toxicity.[178]

Beyond α- and β-emitters, TRT research is increasingly exploring the therapeutic use of Auger and conversion electron emitters with radionuclides such as technetium-99m, indium-111, iodine-123, and iodine-125, which are more traditionally associated with diagnostic imaging. These isotopes emit electrons with very short travel distances, in the range of nanometers to micrometers, but deliver relatively high LETs (ranging from 4 to 26 keV/µm).[179,180] Their highly localized energy deposition is ideal for inducing lethal DNA damage, provided the radiopharmaceutical reaches the nucleus or other radiation-sensitive intracellular compartments. While targeting internal organelles remains the main strategy for Auger-based therapy, there is growing interest in exploiting these properties for cell membrane targeting as well.[180]

TRT for Glioblastoma

The recent success of TRT in prostate cancer with Pluvicto, and in neuroendocrine tumors with Lutathera, sparked interest in applying TRT for GBM.[176,181,182] Compared to other solid tumors, brain tumors, including GBM, pose the additional challenge of crossing the BBB in case of systemic administration. While rapid tumor expansion or altered angiogenesis can cause leaky vessels and BBB disruption, thereby increasing tumor permeability, this process is heterogeneous and does not guarantee uniform diffusion of radiopharmaceuticals, complicating their systemic administration. Therefore, most recent clinical trials administer radiopharmaceuticals directly into the resection cavity or by using more advanced techniques, such as convection-enhanced delivery, focused ultrasound and microbubbles. Additionally, targeting efficiency can be improved through the use of pre-targeting platforms.[182,183] Different methods of radiopharmaceutical delivery have been reviewed elsewhere.[184] For practical considerations, patients in the maintenance phase of GBM treatment may represent the most suitable population for TRT, as opposed to those with newly diagnosed, where timing and treatment logistics can be more complex.[185] However, integrating TRT into the first-line setting for newly diagnosed GBM is an increasingly explored and promising opportunity in clinical studies (NTC05450744).[184]

Several molecular targets have been extensively studied in GBM, with many of them also evaluated in the context of TRT. On the other hand, the high cellular heterogeneity of GBM implicates non-uniform target expression. This may be addressed by using combination therapies or multitarget therapy. Key clinical trials are listed in Table 3.

Epidermal growth factor receptor

EGFR, a cell-surface tyrosine kinase receptor, is highly overexpressed in GBM and exhibits minimal expression in normal brain tissue, making it a promising therapeutic GBM target.[186] Aberrant EGFR signaling, often driven by gene amplification, mutations (eg, *EGFRvIII*), or overexpression, contributes to the aggressive nature of GBM by promoting uncontrolled tumor growth, resistance to apoptosis, and enhanced invasive potential. Targeting EGFR is an active area of research aimed at improving treatment outcomes in GBM patients, mostly following standard-of-care treatment.[187–189]

One of the first GBM TRT clinical trials (NCT00589706, NCT01317888) using an iodine-125-labelled monoclonal antibody directed against EGFR ([^{125}I]I-mAb-425) showed some increase in median survival.[190] In this phase I/II trial, 118 GBM patients received intravenous or intra-arterial TRT as an adjuvant therapy with a mean total dose of 5.2 Gigabecquerel (GBq), administered after surgery and CONV-RT, with or without chemotherapy. The OS for the GBM group was 13.4 months, with a notably higher OS of 25.4 months observed in a subgroup of patients under 40 years of age. Of note, it was not specified whether these patients were IDH-wildtype positive and therefore correctly classified as GBM according to the WHO2021 guidelines. Acute toxicity was reported in only one patient, highlighting the safety profile of the treatment. This trial first demonstrated the potential of EGFR-targeted TRT as a promising approach in GBM treatment, offering improved survival with limited toxicity, particularly for younger patients. Another phase II TRT trial involved 192 newly diagnosed GBM patients receiving intravenous injections of 1.8 GBq of [^{125}I]I-mAb-425 each week for 3 weeks for a total of 4.44-5.55 GBq, after debulking surgery and CONV-RT. The results showed no grade 3/4 toxicity and an OS of 15.7 months with an increase to 20.2 months for the group receiving both TRT and TMZ.[191] In contrast, a randomized prospective study with [^{125}I]I-mAb-425 combined with CONV-RT (8 patients) versus CONV-RT alone (10 patients) after surgery, and found no improvement in disease-free or total survival in the former group. The same study also performed immunohistochemical analysis of EGFR in 100 patients of grade III and IV gliomas and found positive expression in 68% and 50% of tumors, respectively.[192]

Despite these initial promising data, EGFR-targeted TRT did not advance in GBM clinical studies, likely due to the complexity of treatment administration and limited overall efficacy. However, the continuous development of novel

Table 3. Overview of TRT and diagnostic companions in GBM clinical trials

Target	Trial ID	TRT agent	Phase	Status	Indication	Intervention	Administration	End points/outcome
EGFR								
	NCT00589706	[^{125}I]I-mAb-425	II	Completed	Glioblastoma	TRT	IV	Improved PFS
	NCT01317888	[^{125}I]I-mAb-425	NA	Completed	Glioblastoma	TRT	IV	No significant overall survival benefit
SSTR2								
	NCT05109728	[^{177}Lu]Lu-DOTATATE, [^{68}Ga]Ga-DOTATATE	I	Recruiting	Newly diagnosed and recurrent glioblastoma	TRT ± EBRT and temozolomide	IV	Safety, tolerability, and efficacy
LAT1								
	NCT03849105 EudraCT 2018-002262-39	[^{131}I]IPA	I/II	Completed	Recurrent glioblastoma	TRT + EBRT	IV	Well tolerated; PFS 4.3 months; OS 13 months
	NCT05450744 EudraCT 2021-006426-43	[^{131}I]IPA	I	Recruiting	Newly diagnosed glioblastoma	TRT + Stupp regimen	IV	Dose-finding; safety and tolerability
	NCT07100730	[^{131}I]IPA	III	Not yet recruiting	Recurrent glioblastoma (first recurrence)	TRT + lomustine *vs.* lomustine	IV	Not yet available
Tenascin C								
	NCT00003461	[^{211}At]At-ch81C6	I/II	Completed	Primary or metastatic malignant brain tumors	Intracavitary TRT	Intralesional (via catheter)	Dose escalation study; toxicity and therapeutic response OS 54 weeks versus 23-31 weeks
GRPR								
	NCT03872778	[^{177}Lu]-Lu-NeoB, [^{68}Ga]-Ga-NeoB	I/II	Ongoing	Glioblastoma	TRT	IV	Safety, tolerability, distribution, and efficacy
	NCT05739942	[^{177}Lu]-Lu-NeoB, [^{68}Ga]-Ga-NeoB	I	Recruiting	Glioblastoma & recurrent glioblastoma	TRT + temozolomide	IV	Safety and targeting
CA XII								
	NCT05533242	[^{177}Lu]-Lu-6A10-Fab	I	Recruiting	Glioblastoma after standard treatment	Intracavitary TRT	Locoregional, intracavitary radioimmunotherapy	Efficacy
PSMA								
	NCT07052877	[^{68}Ga]-Ga-PSMA-11	Observational	Recruiting	Glioblastoma	Bevacizumab + PSMA PET/CT imaging	IV	PSMA expression post-VEGF blockade
	NCT06241391	[^{68}Ga]-Ga-PSMA-11	NA	Recruiting	Glioma recurrence	PSMA PET/CT imaging	IV	Diagnostic imaging study

radioligands and optimized administration methods could significantly progress the application of this therapy in GBM.

Somatostatin receptors

Somatostatin receptors (SSTRs) are G-protein–coupled receptors with 5 subtypes (SSTR1–5), of which SSTR2 is notably overexpressed in various cancers, particularly those of neuroendocrine origin.[193] This led to the development of [^{177}Lu]Lu-DOTATATE (Lutathera), the first clinically-approved TRT.[194] SSTRs are also expressed in gliomas, including GBM, though different studies report contrasting results.[195–197] Three patients with recurrent GBM were treated with 1.7-2.2 GBq of [^{90}Y]Y-DOTATOC administered locally over 3 or 4 cycles.[198] SSTR expression was pre-therapeutically assessed with [^{68}Ga]Ga-DOTATOC PET. One patient showed complete remission, while 2 patients showed partial remission with improved quality of life and increased Karnofsky performance of 10%-40%. These findings highlight the potential of SSTR-targeted TRT in GBM. However, the variability in reported SSTR expression across studies underscores the need for further investigation. The ongoing NCT05109728 trial will provide crucial insights into the efficacy and safety of (Lutathera) in combination with standard-of-care in different GBM patient groups, as well as the utility of [^{68}Ga]Ga-DOTATATE PET for patient selection and treatment monitoring.

L-type amino acid transporter 1

L-type amino acid transporter 1 (LAT1), expressed on both the luminal and abluminal sides of the BBB, transports phenylalanine into the brain.[199] LAT1 is also responsible for the uptake of o-(2-[^{18}F]-fluoroethyl)-L-tyrosine ([^{18}F]FET), widely used in gliomas for primary diagnosis, recurrence and *in vivo* grading.[200] Iodine-coupled derivatives of *L*-phenylalanine (4-*L*-[^{123}I]iodophenylalanine, 4-*L*-[^{131}I]iodophenylalanine (IPA)) have been evaluated preclinically[201,202] and recently advanced to clinical trials for recurrent GBM (NCT03849105, EudraCT 2018-002262-39 (IPAX-1)) in combination with CONV-RT. In these trials, stable disease was observed in 44% of patients 3 months post-treatment, leading to [^{131}I]IPA receiving orphan drug designation for glioma treatment from both EMA (designation no. EU/3/06/363) and FDA (designation no. 10-3287).[202] Current phase I clinical trials (NCT05450744, EudraCT 2021-006426-43 (IPAX-2)) explore the safety, tolerability and efficacy of [^{131}I]IPA in combination with standard-of-care for newly diagnosed GBM. The latest clinical trial (NCT07100730) will assess safety and radiation dosing and compare combination therapy versus standard of care. [^{211}At]At-phenylalanine has shown a dose-dependent tumor growth inhibition,[203] with similar results observed in intracranial GBM-bearing rats.[204] These findings suggest that α-therapy holds promise for GBM treatment, though potential side effects must be considered. Further studies are needed to refine its clinical application, but early results provide a strong foundation for advancing LAT1-targeted TRT in GBM.

Tenascin-C

Tenascin-C is a glycoprotein expressed in the extracellular matrix of GBM lesions as well as GSC niches, with its expression being positively correlated with higher tumor grades.[205] First clinical trials of TRT against Tenascin-C in GBM patients used iodine-131-radiolabelled murine antibodies BC-2 and BC-4[206,207] which were administered by means of a removable or an indwelling catheter placed into the postoperative cavity. These trials demonstrated the tolerability of such treatments with the potential for tumor stabilization and partial or complete remissions in some patients. In another trial (NCT00003461), 18 patients with primary or metastatic brain tumors (14 with GBM) received 71–347 MBq of [^{211}At]At-ch81C6, into a surgically created resection cavity, followed by salvage chemotherapy.[208] No patient experienced dose-limiting toxicity, and the OS with TRT was 54 weeks compared with 23-31 weeks observed for patients receiving conventional therapies, although the number of patients in this study was too small to indicate a clear dose-response. These early studies underscored the promise of Tenascin-C-based TRT, particularly with α therapies. However, these trials primarily included patients with low residual tumor volumes and were conducted post-surgery. Thus, the observed outcomes may not be fully representative of broader patient populations.[209,210] Future studies will be essential to determine whether similar therapeutic benefits can be achieved with less selective patient inclusion criteria and after accounting for residual tumor burden.[182]

Innovative targets

Other targets are being explored in ongoing clinical trials of TRT for GBM, including gastrin-releasing peptide receptor (NCT03872778 and NCT05739942), and carbonic anhydrase XII (NCT05533242).[211] PSMA has been investigated due to its expression on the endothelium of highly vascularized tumors,[212] though only one proof-of-concept study has been conducted in a GBM patient.[213] Noteworthy, PSMA PET/CT studies (NCT07052877 and NCT06241391) will evaluate PSMA expression after VEGF block and assess if [^{68}Ga]Ga-PSMA-11 can be used as a diagnostic tool in glioma recurrences. CXCR4-targeted TRT in GBM has also shown potential in preclinical models, offering a promising avenue for further investigation.[214] Similarly, the increased interest in radiolabeled fibroblast activation protein inhibitors (FAPi) as a pan-cancer tool targeting the TME has resulted in a proof-of-concept study using [^{177}Lu]Lu-DOTAGA.Glu.(FAPi)$_2$ in a GBM patient.[215]

Combination Therapies to Enhance Radiosensitivity

Besides novel RT methods, several pharmacological combination therapies are being explored to enhance the radiosensitivity of GBM, and to overcome the treatment resistance, high level of recurrence and poor prognosis that characterize this cancer. Poly ADP-ribose polymerase (PARP) inhibitors are a very interesting class of agents to induce radiosensitivity of tumors via the inhibition of DNA repair, and they have been safely combined with either CONV-RT, TMZ or the combination in early phase clinical trials.[216–218] However, no clinical benefit was found for the use of veliparib in a randomized phase II trial of 125 patients (84 in the experimental arm) with unmethylated *MGMT* GBM.[219] PARP inhibition remains under investigation as a possible avenue for combination therapies for GBM. Another DNA damage pathway that is under investigation is the ataxia telangiectasia mutated (ATM) kinase which plays an essential role in ionizing

Table 4. Comparison of radiation modalities for GBM

GBM radiotherapy	Type	Current application in GBM	Advantages	Disadvantages
CONV-RT	External	Clinically approved—Standard-of-care	High energy, effective for localized disease	Not effective for invasion, untargeted, side effects
CIBT	External	Preclinical/early clinical	High RBE, effective for localized disease, high tissue sparing potential, better for pediatric tumors or tumors near vital brain structures	Not effective for invasion, untargeted, very limited access, high cost
PBT	External	Preclinical/early clinical	Effective for localized disease, high tissue sparing potential, better for pediatric tumors or tumors near vital brain structures	Not effective for invasion, untargeted, limited access, high cost
FLASH	External	Preclinical/early clinical	Effective for localized disease, high tissue sparing potential, better for pediatric tumors or tumors near vital brain structures	Not effective for invasion untargeted, limited access, High cost
TRT	Internal targeted (α, β, Auger/electron conversion)	Preclinical/early clinical	Targeted, improved quality of life, low toxicity, Versatile, effective for residual disease/invasion, amenable for personalized medicine	Supply, high costs, heterogeneous target expression, complex administration routes, specialized administration center

radiation-induced DSB repair by activating the downstream kinase CHK2 and eventually p53. AZD1390 is a BBB penetrable ATM inhibitor that achieves acceptable pharmacokinetics after oral administration[220] and has been found to be well tolerated in early phase studies of newly diagnosed and recurrent GBM[221,222] but no data about its clinical effectiveness are currently available. Several clinical trials for use of AZD1390 in brain tumor patients are in the recruitment phase at the time of writing. WEE1 kinase inhibitors are another class of molecules that may act as radiosensitizers for GBM. WEE1 is an important regulator of the G2/M checkpoint and its inhibition forces cells with DNA damage (eg, after irradiation) to undergo mitotic catastrophe.[223] In a phase 0 clinical trial a good tumor penetration was found after single oral dose of the WEE1 inhibitor AZD1775, which led to a dose-dependent abrogation of G2 arrest, increased DNA damage and apoptosis, albeit that the effects of a dose of 200 mg were more pronounced than 400 mg.[224]

Immunotherapy, alone or in combination with CONV-RT or chemotherapy is increasingly being exploited to treat a variety of cancers. Current immunotherapy strategies under investigation for GBM include immune checkpoint inhibitors, adoptive T-cell therapies, tumor vaccines and oncolytic viral therapies.[225] GBM, however, is a locally immunosuppressive tumor and clinical trials using immunotherapy have so far mostly failed.[226] This is in part because both CONV-RT and TMZ may impact the tumor's response to immunotherapy.[225] Nevertheless, the combination with CONV-RT may also prove to be beneficial as radiation can induce a special form of apoptosis named immunogenic cell death, in which dying cells release neoantigens favoring cancer cell recognition by immune cells.[227] Multiple clinical trials are ongoing to evaluate the efficiency of immunotherapy combined with SoC which seems to be well tolerated in the recurrent setting.[228]

Perspectives and Conclusions

The treatment of GBM is particularly challenging due to the aggressive and heterogeneous nature of the disease. The past decade has seen big advances in the use of various radiation types to treat primary and recurrent GBM patients, with many ongoing clinical trials evaluating improved protocols and novel approaches. However, each RT modality brings its own advantages and drawbacks (Table 4). Despite the (pre)clinically suggested benefits of CPT therapy over CONV-RT, especially in terms of limiting adverse effects, there is a reason why CPT is not used as standard-of-care. Worldwide there are 141 operational CPT facilities of which only 17 for CIBT. Moreover, clinical trials performed so far have limitations.[229,230] For example, a major challenge remains the poor prognosis of GBM making long-term follow-up almost impossible, leaving uncertainty regarding the late toxic effect and potential delayed detrimental effect on cognitive function.[116] In addition, the CPT treatment arm has a lack of conformity across different clinical trials, or the standard treatment regimen was often not respected. Finally, *IDH* mutation and/or *MGMT* methylation status was often not considered.[116]

Based on preclinical data, also FLASH-RT has significant advantages over CONV-RT when it comes to sparing normal tissue and thereby reducing long-term cognitive dysfunction in the context of brain tumors. However, like PBT, the tumor eradicating effects seem to be comparable to CONV-RT. Moreover, clinical data on FLASH-RT for GBM patients is still lacking and more research is needed on other long-term effects involving recurrent tumors. Also, the potential combined effects of FLASH-RT and TMZ must be considered. Furthermore, most of the current gantries are too slow to match the high speed needed for FLASH-RT and new RT

devices, including new treatment planning systems, are needed. Recently, researchers proposed a treatment plan involving 3D-conformal very-high electron therapy for FLASH-RT in GBM and lung cancer patients.[231] Similarly to CPT, this also means that the available infrastructure and the high cost remain the main bottle necks for the further development of FLASH-RT in a clinical setting. Future endeavors might focus on delivering FLASH-RT using CONV-RT facilities, thereby making this promising radiation modality more accessible to patients. However, there are also some shortcomings that have to be considered. The current lack of sufficient therapeutic centers for CPT and FLASH-RT implies a geographical constraint on clinical trials. Unlike for drug-based therapies, reimbursement procedures are also not standardized, again limiting the accessibility for patie nts.[229,230,232] The establishment of new treatment centers is capital-intensive and will require substantial funding. Unfortunately, the development of radiation modalities faces far weaker patentability and patent protection than drug development as they mainly rely on hardware, protocols and devices, making them weaker business assets.

TRT has evolved beyond being solely useful in a palliative setting, as it now also prolongs survival and improves the quality-of-life for cancer patients.[167] Current clinical trials show that newly diagnosed and recurrent GBM patients show limited adverse effects to TRT. However, studies either have small patient populations or lack efficacy data, highlighting the need for large, randomized trials to generate the necessary level of evidence for TRT in GBM.[182] Despite the success of TRT implementation for some cancers, there is still a lack of universal guidelines and requirements addressing the use of TRT, particularly in brain tumors. Given the very high recurrence rates in GBM patients, TRT may prove more effective if given immediately after standard therapy or immediately after diagnosis of recurrence.[233] Additionally, to fully use TRT's theranostic potential, future studies should investigate the optimal standardized uptake values at baseline during pretreatment scans, as these may help predict favorable outcomes and guide patient selection.[185] Other challenges include limitations in radionuclide supply, the need for specialized facilities, and the complexity of treatment planning and dosimetry, which require careful consideration in clinical practice.[167]

All these challenges will require systemic solutions, such as the development of centralized treatment centers and the establishment of collaborative networks to pool resources and expertise, as specialized work force is needed for their implementation. Moreover, there is a need for coordinated, multi-institutional research efforts to accelerate clinical translation. Preclinical innovations, such as patient-specific organoids and *in silico* treatment simulations, will become essential in bridging the gap between laboratory findings and clinical applications. Nonetheless, before translational and clinical advancements can be made, a profound understanding of GBM biology and the mechanisms behind (radio)resistance is imperative as we will need to overcome this potential resistance to increase the success of these novel treatment modalities. As our understanding of GBM's radiation biology deepens and breakthroughs in radiation physics, advanced clinical imaging, and targeted drug delivery systems continue to shape neuro-oncology, the potential for novel RT modalities to revolutionize GBM management grows exponentially, paving the way to transform patient outcomes and quality-of-life.

Keywords

FLASH radiotherapyglioblastomaparticle therapyradiotherapytargeted radionuclide therapy

Funding

L.V.E. received a PhD fellowship from the Research Fund—Flanders (11B2323N and 11B2325N). I.D.B. received a PhD Fellowship from the Bijzondere Onderzoeksfonds UGent (01D07923).

Conflict of interest statement

None declared.

Author Contributions

Drafting of manuscript: L.V.E., A.W., R.Q., I.P., K.V.; preparation of figures: L.V.E., I.D.B.; revision of manuscript: all authors; approval of final manuscript: all authors.

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References

1. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23:1231-1251.
2. Ludwig K, Muthukrishnan SD, Alvarado AG, Kornblum HI. Overview of glioblastoma biological hallmarks and molecular pathology. In: R Paulmurugan, TF Massoud, eds. *Glioblastoma Resistance to Chemotherapy: Molecular Mechanisms and Innovative Reversal Strategies*. San Diego: Elsevier; 2021:1-15.
3. Horak V, Mishory A, Baisiwala S, Ahmed A. *Understanding the Role of Plasticity in Glioblastoma*. In: Kenakin T, Riddick DS, eds. Comprehensive Pharmacology. Amsterdam: Elsevier; 2022;6:126–145.
4. Yabo YA, Niclou SP, Golebiewska A. Cancer cell heterogeneity and plasticity: a paradigm shift in glioblastoma. *Neuro Oncol*. 2022;24:669-682.

5. Bhaduri A, Di Lullo E, Jung D, et al. Outer radial glia-like cancer stem cells contribute to heterogeneity of glioblastoma. *Cell Stem Cell*. 2020;26:48-63.e46.
6. Couturier CP, Ayyadhury S, Le PU, et al. Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. *Nat Commun*. 2020;11:3406.
7. Dahan P, Martinez Gala J, Delmas C, et al. Ionizing radiations sustain glioblastoma cell dedifferentiation to a stem-like phenotype through survivin: possible involvement in radioresistance. *Cell Death Dis*. 2014;5:e1543.
8. Wang Q, Hu B, Hu X, et al. Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. *Cancer Cell*. 2017;32:42-56.e6.
9. Neftel C, Laffy J, Filbin MG, et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell*. 2019;178:835-849.e21.
10. Verhaak RG, Hoadley KA, Purdom E, et al. Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17:98-110.
11. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 2014;344:1396-1401.
12. Garofano L, Migliozzi S, Oh YT, et al. Pathway-based classification of glioblastoma uncovers a mitochondrial subtype with therapeutic vulnerabilities. *Nat Cancer*. 2021;2:141-156.
13. Castellan M, Guarnieri A, Fujimura A, et al. Single-cell analyses reveal YAP/TAZ as regulators of stemness and cell plasticity in glioblastoma. *Nat Cancer*. 2021;2:174-188.
14. Richards LM, Whitley OKN, MacLeod G, et al. Gradient of developmental and injury response transcriptional states defines functional vulnerabilities underpinning glioblastoma heterogeneity. *Nat Cancer*. 2021;2:157-173.
15. Greenwald AC, Darnell NG, Hoefflin R, et al. Integrative spatial analysis reveals a multi-layered organization of glioblastoma. *Cell*. 2024;187:2485-2501.e26.
16. Nomura M, Spitzer A, Johnson KC, et al. The multilayered transcriptional architecture of glioblastoma ecosystems. *Nat Genet*. 2025;57:1155-1167.
17. Fedele M, Cerchia L, Pegoraro S, Sgarra R, Manfioletti G. Proneural-mesenchymal transition: phenotypic plasticity to acquire multitherapy resistance in glioblastoma. *Int J Mol Sci*. 2019;20:2746.
18. Stupp R, Mason WP, van den Bent MJ, et al. National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987-996.
19. Tolcher AW, Gerson SL, Denis L, et al. Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br J Cancer*. 2003;88:1004-1011.
20. Khabibov M, Garifullin A, Boumber Y, et al. Signaling pathways and therapeutic approaches in glioblastoma multiforme (review). *Int J Oncol*. 2022;60:69.
21. Rahman R, Shi DD, Reitman ZJ, et al. DNA damage response in brain tumors: a society for neuro-oncology consensus review on mechanisms and translational efforts in neuro-oncology. *Neuro Oncol*. 2024;26:1367-1387.
22. Niyazi M, Andratschke N, Bendszus M, et al. ESTRO-EANO guideline on target delineation and radiotherapy details for glioblastoma. *Radiother Oncol*. 2023;184:109663.
23. Bouchard JJL. *Radiation Therapy of Tumors and Diseases of the Nervous System. Philadelphia*: Lea & Febiger; 1966.
24. Walker MD, Alexander EJr, Hunt WE, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg*. 1978;49:333-343.
25. Walker MD, Strike TA, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys*. 1979;5:1725-1731.
26. Weller M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol*. 2021;18:170-186.
27. Gzell C, Back M, Wheeler H, Bailey D, Foote M. Radiotherapy in glioblastoma: the past, the present and the future. *Clin Oncol (R Coll Radiol)*. 2017;29:15-25.
28. Mann J, Ramakrishna R, Magge R, Wernicke AG. Advances in radiotherapy for glioblastoma. *Front Neurol*. 2017;8:748.
29. Keime-Guibert F, Chinot O, Taillandier L, et al. Association of French-Speaking Neuro-Oncologists. Radiotherapy for glioblastoma in the elderly. *N Engl J Med*. 2007;356:1527-1535.
30. Sferruzza G, Desingu K, Cubero Cruz A, Finocchiaro G. Assessing time-trend bias in glioblastoma prognosis over 2 decades of clinical trials. *Neuro Oncol*. 2025;
31. Price M, Low JT, Chavez G, et al. Improvements in survival for glioblastoma in the post-Stupp protocol era. *Neuro Oncol*. 2025;27:2484-2486.
32. Sheikh S, Radivoyevitch T, Barnholtz-Sloan JS, Vogelbaum M. Long-term trends in glioblastoma survival: implications for historical control groups in clinical trials. *Neurooncol Pract*. 2020;7:158-163.
33. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res*. 2003;63:5821-5828.
34. Patel KS, Dudley L, Kornblum HI. Give me a sign(ature): converging on the CSC of glioblastoma. *Neuron*. 2024;112:3987-3988.
35. Lee JH, Lee JE, Kahng JY, et al. Human glioblastoma arises from subventricular zone cells with low-level driver mutations. *Nature*. 2018;560:243-247.
36. Osuka S, Van Meir EG. Overcoming therapeutic resistance in glioblastoma: the way forward. *J Clin Invest*. 2017;127:415-426.
37. Dirkse A, Golebiewska A, Buder T, et al. Stem cell-associated heterogeneity in glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment. *Nat Commun*. 2019;10:1787.
38. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444:756-760.
39. Tamura K, Aoyagi M, Wakimoto H, et al. Accumulation of CD133-positive glioma cells after high-dose irradiation by Gamma Knife surgery plus external beam radiation: Clinical article. *J Neurosurg*. 2010;113:310-318.
40. Ong DST, Hu B, Ho YW, et al. PAF promotes stemness and radioresistance of glioma stem cells. *Proc Natl Acad Sci USA*. 2017;114:E9086-E9095.
41. Balbous A, Cortes U, Guilloteau K, et al. A radiosensitizing effect of RAD51 inhibition in glioblastoma stem-like cells. *BMC Cancer*. 2016;16:604.
42. King HO, Brend T, Payne HL, et al. RAD51 is a selective DNA repair target to radiosensitize glioma stem cells. *Stem Cell Reports*. 2017;8:125-139.
43. De Bacco F, D'Ambrosio A, Casanova E, et al. MET inhibition overcomes radiation resistance of glioblastoma stem-like cells. *EMBO Mol Med*. 2016;8:550-568.
44. Dungey FA, Löser DA, Chalmers AJ. Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-Ribose) polymerase: mechanisms and therapeutic potential. *Int J Radiat Oncol Biol Phys*. 2008;72:1188-1197.
45. Venere M, Hamerlik P, Wu Q, et al. Therapeutic targeting of constitutive PARP activation compromises stem cell phenotype and survival of glioblastoma-initiating cells. *Cell Death Differ*. 2014;21:258-269.
46. Kim Y, Kim KH, Lee J, et al. Wnt activation is implicated in glioblastoma radioresistance. *Lab Invest*. 2012;92:466-473.
47. Wang J, Wakeman TP, Lathia JD, et al. Notch promotes radioresistance of glioma stem cells. *Stem Cells*. 2010;28:17-28.

48. Hovinga KE, Shimizu F, Wang R, et al. Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. *Stem Cells*. 2010;28:1019-1029.
49. Kim SH, Joshi K, Ezhilarasan R, et al. EZH2 protects glioma stem cells from radiation-induced cell death in a MELK/FOXM1-dependent manner. *Stem Cell Rep*. 2015;4:226-238.
50. Lee Y, Kim KH, Kim DG, et al. FoxM1 promotes stemness and radio-resistance of glioblastoma by regulating the master stem cell regulator Sox2. *PLoS One*. 2015;10:e0137703.
51. Barthel FP, Johnson KC, Varn FS, et al. GLASS Consortium. Longitudinal molecular trajectories of diffuse glioma in adults. *Nature*. 2019;576:112-120.
52. Körber V, Yang J, Barah P, et al. Evolutionary trajectories of IDH(WT) glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis. *Cancer Cell*. 2019;35:692-704.e12.
53. Aldape K, Amin SB, Ashley DM, et al. Glioma through the looking GLASS: molecular evolution of diffuse gliomas and the glioma longitudinal AnalySiS consortium. *Neuro Oncol*. 2018;20:873–884.
54. Sottoriva A, Spiteri I, Piccirillo SG, et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc Natl Acad Sci USA*. 2013;110:4009-4014.
55. Kim J, Lee IH, Cho HJ, et al. Spatiotemporal evolution of the primary glioblastoma genome. *Cancer Cell*. 2015;28:318-328.
56. de Souza CF, Sabedot TS, Malta TM, et al. A distinct DNA methylation shift in a subset of glioma CpG island methylator phenotypes during tumor recurrence. *Cell Rep*. 2018;23:637-651.
57. Touat M, Li YY, Boynton AN, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature*. 2020;580:517-523.
58. Yang J, Antin P, Berx G, et al. EMT International Association (TEMTIA). Guidelines and definitions for research on epithelial–mesenchymal transition. *Nat Rev Mol Cell Biol*. 2020;21:341-352.
59. França GS, Baron M, King BR, et al. Cellular adaptation to cancer therapy along a resistance continuum. *Nature*. 2024;631:876-883.
60. Carro MS, Lim WK, Alvarez MJ, et al. The transcriptional network for mesenchymal transformation of brain tumours. *Nature*. 2010;463:318-325.
61. Mao P, Joshi K, Li J, et al. Mesenchymal glioma stem cells are maintained by activated glycolytic metabolism involving aldehyde dehydrogenase 1A3. *Proc Natl Acad Sci USA*. 2013;110:8644-8649.
62. Halliday J, Helmy K, Pattwell SS, et al. In vivo radiation response of proneural glioma characterized by protective p53 transcriptional program and proneural-mesenchymal shift. *Proc Natl Acad Sci USA*. 2014;111:5248-5253.
63. Lau J, Ilkhanizadeh S, Wang S, et al. STAT3 blockade inhibits radiation-induced malignant progression in glioma. *Cancer Res*. 2015;75:4302-4311.
64. Minata M, Audia A, Shi J, et al. Phenotypic plasticity of invasive edge glioma stem-like cells in response to ionizing radiation. *Cell Rep*. 2019;26:1893-1905.e7.
65. Segerman A, Niklasson M, Haglund C, et al. Clonal variation in drug and radiation response among glioma-initiating cells is linked to proneural-mesenchymal transition. *Cell Rep*. 2016;17:2994-3009.
66. Wang L, Jung J, Babikir H, et al. A single-cell atlas of glioblastoma evolution under therapy reveals cell-intrinsic and cell-extrinsic therapeutic targets. *Nat Cancer*. 2022;3:1534-1552.
67. Kim KH, Migliozzi S, Koo H, et al. Integrated proteogenomic characterization of glioblastoma evolution. *Cancer Cell*. 2024;42:358-377.e358.
68. Pichol-Thievend C, Anezo O, Pettiwala AM, et al. VC-resist glioblastoma cell state: vessel co-option as a key driver of chemoradiation resistance. *Nat Commun*. 2024;15:3602.
69. Panovska D, Nazari P, Cole B, et al. Single-cell molecular profiling using ex vivo functional readouts fuels precision oncology in glioblastoma. *Cell Mol Life Sci*. 2023;80:147.
70. Muthukrishnan SD, Kawaguchi R, Nair P, et al. P300 promotes tumor recurrence by regulating radiation-induced conversion of glioma stem cells to vascular-like cells. *Nat Commun*. 2022;13:6202.
71. Ravi VM, Will P, Kueckelhaus J, et al. Spatially resolved multi-omics deciphers bidirectional tumor-host interdependence in glioblastoma. *Cancer Cell*. 2022;40:639-655.e613.
72. Hambardzumyan D, Bergers G. Glioblastoma: defining tumor niches. *Trends Cancer*. 2015;1:252-265.
73. Ali MY, Oliva CR, Noman ASM, et al. Radioresistance in glioblastoma and the development of radiosensitizers. *Cancers (Basel)*. 2020;12:2511.
74. Alves ALV, Gomes INF, Carloni AC, et al. Role of glioblastoma stem cells in cancer therapeutic resistance: a perspective on antineoplastic agents from natural sources and chemical derivatives. *Stem Cell Res Ther*. 2021;12:206.
75. Sharma P, Aaroe A, Liang J, Puduvalli VK. Tumor microenvironment in glioblastoma: current and emerging concepts. *Neurooncol Adv*. 2023;5:vdad009.
76. Uribe D, Niechi I, Rackov G, Erices JI, San Martín R, Quezada C. Adapt to persist: glioblastoma microenvironment and epigenetic regulation on cell plasticity. *Biology (Basel)*. 2022;11:
77. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer*. 1955;9:539-549.
78. Tejero R, Huang Y, Katsyv I, et al. Gene signatures of quiescent glioblastoma cells reveal mesenchymal shift and interactions with niche microenvironment. *EBioMedicine*. 2019;42:252-269.
79. Joseph JV, Conroy S, Pavlov K, et al. Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α–ZEB1 axis. *Cancer Lett*. 2015;359:107-116.
80. Karsy M, Guan J, Jensen R, Huang LE, Colman H. The impact of hypoxia and mesenchymal transition on glioblastoma pathogenesis and cancer stem cells regulation. *World Neurosurg*. 2016;88:222-236.
81. Schmitt MJ, Company C, Dramaretska Y, et al. Phenotypic mapping of pathologic cross-talk between glioblastoma and innate immune cells by synthetic genetic tracing. *Cancer Discov*. 2021;11:754-777.
82. Osswald M, Jung E, Sahm F, et al. Brain tumour cells interconnect to a functional and resistant network. *Nature*. 2015;528:93-98.
83. Venkataramani V, Tanev DI, Strahle C, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature*. 2019;573:532-538.
84. Venkatesh HS, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of glioma into neural circuits. *Nature*. 2019;573:539-545.
85. Watson DC, Bayik D, Storevik S, et al. GAP43-dependent mitochondria transfer from astrocytes enhances glioblastoma tumorigenicity. *Nat Cancer*. 2023;4:648-664.
86. Taylor KR, Monje M. Invasive glioma cells: the malignant pioneers that follow the current. *Cell*. 2022;185:2846-2848.
87. Venkataramani V, Yang Y, Schubert MC, et al. Glioblastoma hijacks neuronal mechanisms for brain invasion. *Cell*. 2022;185:2899-2917.e31.
88. Bastola S, Pavlyukov MS, Yamashita D, et al. Glioma-initiating cells at tumor edge gain signals from tumor core cells to promote their malignancy. *Nat Commun*. 2020;11:4660.
89. Wang X, Prager BC, Wu Q, et al. Reciprocal signaling between glioblastoma stem cells and differentiated tumor cells promotes malignant progression. *Cell Stem Cell*. 2018;22:514-528.e5.
90. Pavlyukov MS, Yu H, Bastola S, et al. Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors. *Cancer Cell*. 2018;34:119-135.e10.
91. Bhat KPL, Balasubramaniyan V, Vaillant B, et al. Mesenchymal differentiation mediated by NF-kappaB promotes radiation resistance in glioblastoma. *Cancer Cell*. 2013;24:331-346.

92. Hara T, Chanoch-Myers R, Mathewson ND, et al. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. *Cancer Cell*. 2021;39:779-792.e11.
93. Stringer BW, De Silva MI, Greenberg Z, et al. Human cerebrospinal fluid affects chemoradiotherapy sensitivities in tumor cells from patients with glioblastoma. *Sci Adv*. 2023;9:eadf1332.
94. Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nat Rev Cancer*. 2015;15:409-425.
95. Liau BB, Sievers C, Donohue LK, et al. Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. *Cell Stem Cell*. 2017;20:233-246.e7.
96. Eyler CE, Matsunaga H, Hovestadt V, Vantine SJ, van Galen P, Bernstein BE. Single-cell lineage analysis reveals genetic and epigenetic interplay in glioblastoma drug resistance. *Genome Biol*. 2020;21:174.
97. Jeon H-M, Kim J-Y, Cho HJ, et al. Tissue factor is a critical regulator of radiation therapy-induced glioblastoma remodeling. *Cancer Cell*. 2023;41:1480-1497.e9.
98. Jacob F, Salinas RD, Zhang DY, et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. *Cell*. 2020;180:188-204.e22.
99. Ogawa J, Pao GM, Shokhirev MN, Verma IM. Glioblastoma model using human cerebral organoids. *Cell Rep*. 2018;23:1220-1229.
100. Linkous A, Balamatsias D, Snuderl M, et al. Modeling patient-derived glioblastoma with cerebral organoids. *Cell Rep*. 2019;26:3203-3211.e5.
101. Yi H-G, Jeong YH, Kim Y, et al. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. *Nat Biomed Eng*. 2019;3:509-519.
102. Golebiewska A, Hau A-C, Oudin A, et al. Patient-derived organoids and orthotopic xenografts of primary and recurrent gliomas represent relevant patient avatars for precision oncology. *Acta Neuropathol*. 2020;140:919-949.
103. Verduin M, Hoosemans L, Vanmechelen M, et al. Patient-derived glioblastoma organoids reflect tumor heterogeneity and treatment sensitivity. *Neurooncol Adv*. 2023;5:vdad152.
104. Mangena V, Chanoch-Myers R, Sartore R, et al. Glioblastoma cortical organoids recapitulate cell-state heterogeneity and intercellular transfer. *Cancer Discov*. 2025;15:299-315.
105. Peng F, Wang Y, Cheng L, et al. Chemical reprogramming of human blood cells to pluripotent stem cells. *Cell Stem Cell*. 2025;32:1192-1199.e11.
106. Soubéran A, Jiguet-Jiglaire C, Toutain S, et al. Brain tumoroids: treatment prediction and drug development for brain tumors with fast, reproducible, and easy-to-use personalized models. *Neuro Oncol*. 2025;27:415-429.
107. Singh K, Hotchkiss KM, Parney IF, et al. Correcting the drug development paradigm for glioblastoma requires serial tissue sampling. *Nat Med*. 2023;29:2402-2405.
108. Ling AL, Gantchev J, Prabhu MC , et al. Accelerating GBM Therapies TeamLab. Serial multiomics uncovers anti-glioblastoma responses not evident by routine clinical analyses. *Sci Transl Med*. 2025;17:eadv2881.
109. Matsumoto Y, Fukumitsu N, Ishikawa H, Nakai K, Sakurai H. A critical review of radiation therapy: from particle beam therapy (proton, carbon, and BNCT) to beyond. *J Pers Med*. 2021;11:825.
110. Wilson RR. Radiological use of fast protons. *Radiology*. 1946;47:487-491.
111. Bennan ABA, Unkelbach J, Wahl N, Salome P, Bangert M. Joint optimization of photon & carbon ion treatments for glioblastoma. *Int J Radiat Oncol Biol Phys*. 2021;111:559-572.
112. Malouff TD, Mahajan A, Krishnan S, Beltran C, Seneviratne DS, Trifiletti DM. Carbon ion therapy: a modern review of an emerging technology. *Front Oncol*. 2020;10:82.
113. Durante M, Orecchia R, Loeffler JS. Charged-particle therapy in cancer: clinical uses and future perspectives. *Nat Rev Clin Oncol*. 2017;14:483-495.
114. Mohamad O, Sishc BJ, Saha J, et al. Carbon ion radiotherapy: a review of clinical experiences and preclinical research, with an emphasis on DNA damage/repair. *Cancers (Basel)*. 2017;9:66.
115. Weyrather WK, Debus J. Particle beams for cancer therapy. *Clin Oncol*. 2003;15:S23-S28.
116. Wang Y, Liu R, Zhang Q, et al. Charged particle therapy for high-grade gliomas in adults: a systematic review. *Radiat Oncol*. 2023;18:29.
117. Chiblak S, Tang Z, Campos B, et al. Radiosensitivity of patient-derived glioma stem cell 3-Dimensional cultures to photon, proton, and carbon irradiation. *Int J Radiat Oncol Biol Phys*. 2016;95:112-119.
118. Chiblak S, Tang Z, Lemke D, et al. Carbon irradiation overcomes glioma radioresistance by eradicating stem cells and forming an antiangiogenic and immunopermissive niche. *JCI Insight*. 2019;4:
119. Stahler C, Roth J, Cordes N, Taucher-Scholz G, Mueller-Klieser W. Impact of carbon ion irradiation on epidermal growth factor receptor signaling and glioma cell migration in comparison to conventional photon irradiation. *Int J Radiat Biol*. 2013;89:454-461.
120. Dokic I, Mairani A, Brons S, et al. High resistance to X-rays and therapeutic carbon ions in glioblastoma cells bearing dysfunctional ATM associates with intrinsic chromosomal instability. *Int J Radiat Biol*. 2015;91:157-165.
121. Combs SE, Bohl J, Elsässer T, et al. Radiobiological evaluation and correlation with the local effect model (LEM) of carbon ion radiation therapy and temozolomide in glioblastoma cell lines. *Int J Radiat Biol*. 2009;85:126-137.
122. Harrabi S, Combs SE, Brons S, Haberer T, Debus J, Weber KJ. Temozolomide in combination with carbon ion or photon irradiation in glioblastoma multiforme cell lines—does scheduling matter? *Int J Radiat Biol*. 2013;89:692-697.
123. Combs SE, Bruckner T, Mizoe J-E, et al. Comparison of carbon ion radiotherapy to photon radiation alone or in combination with temozolomide in patients with high-grade gliomas: Explorative hypothesis-generating retrospective analysis. *Radiother Oncol*. 2013;108:132-135.
124. Mizoe J-E, Tsujii H, Hasegawa A, Organizing Committee of the Central Nervous System Tumor Working Group, et al. Phase I/II clinical trial of carbon ion radiotherapy for malignant gliomas: combined X-Ray radiotherapy, chemotherapy, and carbon ion radiotherapy. *Int J Radiat Oncol Biol Phys*. 2007;69:390-396.
125. Combs SE, Nagy M, Edler L, et al. Comparative evaluation of radiochemotherapy with temozolomide versus standard-of-care postoperative radiation alone in patients with WHO grade III astrocytic tumors. *Radiother Oncol*. 2008;88:177-182.
126. Combs SE, Wagner J, Bischof M, et al. Radiochemotherapy in patients with primary glioblastoma comparing two temozolomide dose regimens. *International Journal of Radiation OncologyBiologyPhysics*. 2008;71:999-1005.
127. Combs SEMD, Gutwein S, Schulz-Ertner D, et al. Temozolomide combined with irradiation as postoperative treatment of primary glioblastoma multiforme: Phase I/II study. *Strahlenther Onkol*. 2005;181:372-377.
128. Combs SE, Kieser M, Rieken S, et al. Randomized phase II study evaluating a carbon ion boost applied after combined radiochemotherapy with temozolomide versus a proton boost after radiochemotherapy with temozolomide in patients with primary glioblastoma: the CLEOPATRA trial. *BMC Cancer*. 2010;10:478.
129. Tsujii H, Tsuji H, Inada T, et al. Clinical results of fractionated proton therapy. *Int J Radiat Oncol Biol Phys*. 1993;25:49-60.
130. Fitzek MM, Thornton AF, Rabinov JD, et al. Accelerated fractionated proton/photon irradiation to 90 cobalt gray equivalent for glioblastoma multiforme: results of a phase II prospective trial. *J Neurosurg*. 1999;91:251-260.
131. Mizumoto M, Yamamoto T, Takano S, et al. Long-term survival after treatment of glioblastoma multiforme with hyperfractionated concomitant boost proton beam therapy. *Pract Radiat Oncol*. 2015;5:e9-e16.

132. Adeberg S, Bernhardt D, Harrabi SB, et al. Sequential proton boost after standard chemoradiation for high-grade glioma. *Radiother Oncol*. 2017;125:266-272.

133. Kong L, Wu J, Gao J, et al. Particle radiation therapy in the management of malignant glioma: early experience at the Shanghai proton and heavy ion center. *Cancer*. 2020;126:2802-2810.

134. Brown PD, Chung C, Liu DD, et al. A prospective phase II randomized trial of proton radiotherapy vs intensity-modulated radiotherapy for patients with newly diagnosed glioblastoma. *Neuro Oncol*. 2021;23:1337-1347.

135. Mohan R, Liu AY, Brown PD, et al. Proton therapy reduces the likelihood of high-grade radiation-induced lymphopenia in glioblastoma patients: phase II randomized study of protons vs photons. *Neuro Oncol*. 2021;23:284-294.

136. Mehta MPPS, Mahajan A, Shih HA, et al. Signal-seeking P2R trial of proton or IMRT dose intensification in GBM: NRG BN001. Radiat Oncol Biol Phys. 2025;123:1204.

137. Bourhis J, Montay-Gruel P, Gonçalves Jorge P, et al. Clinical translation of FLASH radiotherapy: why and how? *Radiother Oncol*. 2019;139:11-17.

138. Dewey DL, Boag JW. Modification of the oxygen effect when bacteria are given large pulses of radiation. *Nature*. 1959;183:1450-1451.

139. Liu J, Zhou G, Pei H. The clinical prospect of FLASH radiotherapy. *Radiat Med Prot*. 2023;4:190-196.

140. Marcu LG, Bezak E, Peukert DD, Wilson P. Translational research in FLASH radiotherapy-from radiobiological mechanisms to in vivo results. *Biomedicines*. 2021;9:

141. Adrian G, Konradsson E, Lempart M, Bäck S, Ceberg C, Petersson K. The FLASH effect depends on oxygen concentration. *Br J Radiol*. 2019;93:20190702.

142. Montay-Gruel P, Acharya MM, Petersson K, et al. Long-term neurocognitive benefits of FLASH radiotherapy driven by reduced reactive oxygen species. *Proc Natl Acad Sci USA*. 2019;116:10943-10951.

143. Epp ER, Weiss H, Djordjevic B, Santomasso A. The radiosensitivity of cultured mammalian cells exposed to single high intensity pulses of electrons in various concentrations of oxygen. *Radiat Res*. 1972;52:324-332.

144. Weiss H, Epp ER, Heslin JM, Ling CC, Santomasso A. Oxygen depletion in cells irradiated at ultra-high dose-rates and at conventional dose-rates. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1974;26:17-29.

145. Aykin-Burns N, Ahmad IM, Zhu Y, Oberley LW, Spitz DR. Increased levels of superoxide and H2O2 mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. *Biochem J*. 2009;418:29-37.

146. Spitz DR, Buettner GR, Petronek MS, et al. An integrated physico-chemical approach for explaining the differential impact of FLASH versus conventional dose rate irradiation on cancer and normal tissue responses. *Radiother Oncol*. 2019;139:23-27.

147. Jin J-Y, Gu A, Wang W, Oleinick NL, Machtay M, Kong F-M. Ultra-high dose rate effect on circulating immune cells: a potential mechanism for FLASH effect? *Radiother Oncol*. 2020;149:55-62.

148. Kim Y-E, Gwak S-H, Hong B-J, et al. Effects of ultra-high doserate FLASH irradiation on the tumor microenvironment in lewis lung carcinoma: role of myosin light chain. *Int J Radiat Oncol Biol Phys*. 2021;109:1440-1453.

149. Favaudon V, Caplier L, Monceau V, et al. Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. *Sci Transl Med*. 2014;6:245ra93.

150. Li H-S, Tang R, Shi H-S, et al. Ultra-high dose rate radiotherapy overcomes radioresistance in head and neck squamous cell carcinoma. *Signal Transduct Target Ther*. 2025;10:82.

151. Bourhis J, Sozzi WJ, Jorge PG, et al. Treatment of a first patient with FLASH-radiotherapy. *Radiother Oncol*. 2019;139:18-22.

152. Mascia AE, Daugherty EC, Zhang Y, et al. Proton FLASH radiotherapy for the treatment of symptomatic bone metastases: the FAST-01 nonrandomized trial. *JAMA Oncol*. 2023;9:62-69.

153. Kinj R, Gaide O, Jeanneret-Sozzi W, et al. Randomized phase II selection trial of FLASH and conventional radiotherapy for patients with localized cutaneous squamous cell carcinoma or basal cell carcinoma: a study protocol. *Clin Transl Radiat Oncol*. 2024;45:100743.

154. Montay-Gruel P, Acharya MM, Gonçalves Jorge P, et al. Hypofractionated FLASH-RT as an effective treatment against glioblastoma that reduces neurocognitive side effects in mice. *Clin Cancer Res*. 2021;27:775-784.

155. Montay-Gruel P, Bouchet A, Jaccard M, et al. X-rays can trigger the FLASH effect: ultra-high dose-rate synchrotron light source prevents normal brain injury after whole brain irradiation in mice. *Radiother Oncol*. 2018;129:582-588.

156. Simmons DA, Lartey FM, Schüler E, et al. Reduced cognitive deficits after FLASH irradiation of whole mouse brain are associated with less hippocampal dendritic spine loss and neuroinflammation. *Radiother Oncol*. 2019;139:4-10.

157. Iturri L, Bertho A, Lamirault C, et al. Proton FLASH radiation therapy and immune infiltration: evaluation in an orthotopic glioma rat model. *Int J Radiat Oncol Biol Phys*. 2023;116:655-665.

158. Allen BD, Alaghband Y, Kramár EA, et al. Elucidating the neurological mechanism of the FLASH effect in juvenile mice exposed to hypofractionated radiotherapy. *Neuro Oncol*. 2023;25:927-939.

159. Tinganelli W, Puspitasari-Kokko A, Sokol O, et al. FLASH Bragg-peak irradiation with a therapeutic carbon ion beam: first In vivo results. *Int J Radiat Oncol Biol Phys*. 2025;121:1282-1292.

160. Williams MT, Regan SL, Fritz AL, et al. Effects of whole brain proton irradiation at conventional or ultra-high dose rate (FLASH), in adult male Sprague Dawley rats. *Sci Rep*. 2025;15:10602.

161. Liljedahl E, Konradsson E, Linderfalk K, et al. Comparable survival in rats with intracranial glioblastoma irradiated with single-fraction conventional radiotherapy or FLASH radiotherapy. *Front Oncol*. 2023;13:1309174.

162. Liljedahl E, Konradsson E, Gustafsson E, et al. Long-term anti-tumor effects following both conventional radiotherapy and FLASH in fully immunocompetent animals with glioblastoma. *Sci Rep*. 2022;12:12285.

163. Romano F, Bailat C, Jorge PG, Lerch MLF, Darafsheh A. Ultra-high dose rate dosimetry: challenges and opportunities for FLASH radiation therapy. *Med Phys*. 2022;49:4912-4932.

164. Gonçalves Jorge P, Geyer R, Kinj R, et al. Machine stability and dosimetry for ultra-high dose rate FLASH radiotherapy human clinical protocol. *J Appl Clin Med Phys*. 2025;26:e70102.

165. Yang XX, Luo H, Zhang JJ, Ge H, Ge L. Clinical translation of ultra-high dose rate flash radiotherapy: opportunities, challenges, and prospects. *World J Radiol*. 2025;17:105722.

166. Vozenin MC, Bourhis J, Durante M. Towards clinical translation of FLASH radiotherapy. *Nat Rev Clin Oncol*. 2022;19:791-803.

167. Bodei L, Herrmann K, Schöder H, Scott AM, Lewis JS. Radiotheranostics in oncology: current challenges and emerging opportunities. *Nat Rev Clin Oncol*. 2022;19:534-550.

168. Primac I, Tabury K, Tasdogan A, Baatout S, Herrmann K. The molecular blueprint of targeted radionuclide therapy. *Nat Rev Clin Oncol*. 2025;22:869-894.

169. Zhang T, Lei H, Chen X, et al. Carrier systems of radiopharmaceuticals and the application in cancer therapy. *Cell Death Discov*. 2024;10:16.

170. Gholamrezanezhad A, Shooli H, Jokar N, Nemati R, Assadi M. Radioimmunotherapy (RIT) in brain tumors. *Nucl Med Mol Imaging*. 2019;53:374-381.

171. Sgouros G, Bodei L, McDevitt MR, Nedrow JR. Radiopharmaceutical therapy in cancer: clinical advances and challenges. *Nat Rev Drug Discov*. 2020;19:589-608.

172. Rebischung C, Hoffmann D, Stefani L, et al. First human treatment of resistant neoplastic meningitis by intrathecal administration of MTX plus (125)IUdR. *Int J Radiat Biol*. 2008;84:1123-1129.

173. Piwowarska-Bilska H, Kurkowska S, Birkenfeld B. Individualization of radionuclide therapies: challenges and prospects. *Cancers (Basel)*. 2022;14:

174. Dieudonné A, Bailly C, Cachin F, et al. Dosimetry for targeted radionuclide therapy in routine clinical practice: experts advice vs. clinical evidence. *Eur J Nucl Med Mol Imaging*. 2024;51:947-950.

175. Pinto CIG, Guerreiro JF, Silva F, Mendes F, Paulo A. Radiopharmaceuticals for molecular imaging and theranostics of glioblastoma. In: C Vitorino, C Balaña, C Cabral, eds. *New Insights Into Glioblastoma*: Academic Press, 2023: 667-705.

176. Bolcaen J, Kleynhans J, Nair S, et al. A perspective on the radiopharmaceutical requirements for imaging and therapy of glioblastoma. *Theranostics*. 2021;11:7911-7947.

177. Bruland ØS, Larsen RH, Baum RP, Juzeniene A. Targeted alpha particle therapy in oncology. *Front Med (Lausanne)*. 2023;10:1165747.

178. Zhang S, Wang X, Gao X, et al. Radiopharmaceuticals and their applications in medicine. *Signal Transduct Target Ther*. 2025;10:1.

179. Donoghue JAO, Wheldon TE. Targeted radiotherapy using Auger electron emitters. Phys Med Biol. 1996;41:1973–1992.

180. Ku A, Facca VJ, Cai Z, Reilly RM. Auger electrons for cancer therapy—a review. *EJNMMI Radiopharm Chem*. 2019;4:27.

181. Cimini A, Ricci M, Russo F, et al. Peptide receptor radionuclide therapy and primary brain tumors: an overview. *Pharmaceuticals (Basel)*. 2021;14:872.

182. Tolboom N, Verger A, Albert NL, et al. Theranostics in neurooncology: heading toward new horizons. *J Nucl Med*. 2024;65:167-173.

183. Bellettato CM, Scarpa M. Possible strategies to cross the blood–brain barrier. *Ital J Pediatr*. 2018;44:131.

184. Zhao X, Jakobsson V, Tao Y, et al. Targeted radionuclide therapy in glioblastoma. *ACS Appl Mater Interfaces*. 2024;16:40391-40410.

185. Weller M, Albert NL, Galldiks N, et al. Targeted radionuclide therapy for gliomas: Emerging clinical trial landscape. *Neuro Oncol*. 2024;26:S208-S214.

186. Saadeh FS, Mahfouz R, Assi HI. EGFR as a clinical marker in glioblastomas and other gliomas. *Int J Biol Markers*. 2018;33:22-32.

187. Ezzati S, Salib S, Balasubramaniam M, Aboud O. Epidermal growth factor receptor inhibitors in glioblastoma: current status and future possibilities. *Int J Mol Sci*. 2024;25:2316.

188. An Z, Aksoy O, Zheng T, Fan Q-W, Weiss WA. Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. *Oncogene*. 2018;37:1561-1575.

189. Taylor TE, Furnari FB, Cavenee WK. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. *Curr Cancer Drug Targets*. 2012;12:197-209.

190. Emrich JG, Brady LW, Quang TS, et al. Radioiodinated (I-125) monoclonal antibody 425 in the treatment of high grade glioma patients: ten-year synopsis of a novel treatment. *Am J Clin Oncol*. 2002;25:541-546.

191. Li L, Quang TS, Gracely EJ, et al. A phase II study of anti–epidermal growth factor receptor radioimmunotherapy in the treatment of glioblastoma multiforme: clinical article. *J Neurosurg*. 2010;113:192-198.

192. Wygoda Z, Kula D, Bierzyńska-Macyszyn G, et al. Use of monoclonal anti-EGFR antibody in the radioimmunotherapy of malignant gliomas in the context of EGFR expression in grade III and IV tumors. *Hybridoma (Larchmt)*. 2006;25:125-132.

193. Reubi JC, Waser B, Mäcke H, Rivier J. Highly increased ^{125}I-JR11 antagonist binding In vitro reveals novel indications for sst_2 targeting in human cancers. *J Nucl Med*. 2017;58:300-306.

194. Strosberg J, El-Haddad G, Wolin E, et al. NETTER-1 Trial Investigators. Phase 3 trial of (177)Lu-Dotatate for midgut neuroendocrine tumors. *N Engl J Med*. 2017;376:125-135.

195. Dutour A, Kumar U, Panetta R, et al. Expression of somatostatin receptor subtypes in human brain tumors. *Int J Cancer*. 1998;76:620-627.

196. Lange F, Kaemmerer D, Behnke-Mursch J, Brück W, Schulz S, Lupp A. Differential somatostatin, CXCR4 chemokine and endothelin a receptor expression in WHO grade I–IV astrocytic brain tumors. *J Cancer Res Clin Oncol*. 2018;144:1227-1237.

197. Mawrin C, Schulz S, Pauli SU, et al. Differential expression of sst1, sst2A, and sst3 somatostatin receptor proteins in low-grade and high-grade astrocytomas. *J Neuropathol Exp Neurol*. 2004;63:13-19.

198. Heute D, Kostron H, von Guggenberg E, et al. Response of recurrent high-grade glioma to treatment with (90)Y-DOTATOC. *J Nucl Med*. 2010;51:397-400.

199. Huttunen J, Peltokangas S, Gynther M, et al. L-Type amino acid transporter 1 (LAT1/Lat1)-utilizing prodrugs can improve the delivery of drugs into neurons, astrocytes and microglia. *Sci Rep*. 2019;9:12860.

200. Vettermann FJ, Diekmann C, Weidner L, et al. L-type amino acid transporter (LAT) 1 expression in (18)F-FET-negative gliomas. *EJNMMI Res*. 2021;11:124.

201. Israel I, Blass G, Reiners C, Samnick S. Validation of an amino-acid-based radionuclide therapy plus external beam radiotherapy in heterotopic glioblastoma models. *Nucl Med Biol*. 2011;38:451-460.

202. Pichler J, Traub-Weidinger T, Spiegl K, et al. Results from a phase I study of 4-I-[131I]iodo-phenylalanine ([(131)I]IPA) with external radiation therapy in patients with recurrent glioblastoma (IPAX-1). *Neurooncol Adv*. 2024;6:vdae130.

203. Watabe T, Kaneda-Nakashima K, Shirakami Y, et al. Targeted alpha therapy using astatine ((211)At)-labeled phenylalanine: a preclinical study in glioma bearing mice. *Oncotarget*. 2020;11:1388-1398.

204. Borrmann N, Friedrich S, Schwabe K, et al. Systemic treatment with 4-211Atphenylalanine enhances survival of rats with intracranial glioblastoma. *Nuklearmedizin*. 2013;52:212-221.

205. Fu Z, Zhu G, Luo C, et al. Matricellular protein tenascin C: implications in glioma progression, gliomagenesis, and treatment. *Front Oncol*. 2022;12:971462.

206. Riva P, Arista A, Tison V, et al. Intralesional radioimmunotherapy of malignant gliomas. An effective treatment in recurrent tumors. *Cancer*. 1994;73:1076-1082.

207. Riva P, Arista A, Sturiale C, et al. Treatment of intracranial human glioblastoma by direct intratumoral administration of 131I-labelled anti-tenascin monoclonal antibody BC-2. *Int J Cancer*. 1992;51:7-13.

208. Zalutsky MR, Reardon DA, Akabani G, et al. Clinical experience with alpha-particle emitting 211At: treatment of recurrent brain tumor patients with 211At-labeled chimeric antitenascin monoclonal antibody 81C6. *J Nucl Med*. 2008;49:30-38.

209. Akabani G, Reardon DA, Coleman RE, et al. Dosimetry and radiographic analysis of 131I-Labeled anti–tenascin 81C6 murine monoclonal antibody in newly diagnosed patients with malignant gliomas: a phase II study. *J Nucl Med*. 2005;46:1042-1051.

210. Reardon DA, Akabani G, Coleman RE, et al. Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J Clin Oncol*. 2006;24:115-122.

211. Tolboom N, Verger A, Albert N, et al. EANM position paper: theranostics in brain tumours—the present and the future. *Eur J Nucl Med Mol Imaging*. 2023;51:202-205.

212. Holzgreve A, Biczok A, Ruf VC, et al. PSMA expression in glioblastoma as a basis for theranostic approaches: a retrospective, correlational

panel study including immunohistochemistry, clinical parameters and PET imaging. *Front Oncol*. 2021;11:646387.

213. Kumar A, Ballal S, Yadav MP, et al. 177Lu-/68Ga-PSMA theranostics in recurrent glioblastoma multiforme: proof of concept. *Clin Nucl Med*. 2020;45:e512-e513.
214. Waked A, Crabbé M, Neirinckx V, et al. Preclinical evaluation of CXCR4 peptides for targeted radionuclide therapy in glioblastoma. *EJNMMI Radiopharm Chem*. 2024;9:52.
215. Ballal S, Yadav MP, Raju S, et al. Lu]Lu-DOTAGA.Glu.(FAPi)(2) Radionuclide therapy: a new treatment option for patients with glioblastoma multiforme. *Nucl Med Mol Imaging*. 2024;58:32-34.
216. Fulton B, Short SC, James A, et al. PARADIGM-2: two parallel phase I studies of olaparib and radiotherapy or olaparib and radiotherapy plus temozolomide in patients with newly diagnosed glioblastoma, with treatment stratified by MGMT status. *Clin Transl Radiat Oncol*. 2018;8:12-16.
217. Hanna C, Kurian KM, Williams K, et al. Pharmacokinetics, safety, and tolerability of olaparib and temozolomide for recurrent glioblastoma: results of the phase I OPARATIC trial. *Neuro Oncol*. 2020;22:1840-1850.
218. Stefan D, Lesueur P, Lequesne J, et al. Olaparib, temozolomide, and concomitant radiotherapy for partially resected or biopsy-only glioblastoma first-line treatment: results from the OLA-TMZ-RTE-01 Phase I study. *Clin Cancer Res*. 2025;31:1212-1222.
219. Sim HW, McDonald KL, Lwin Z, et al. A randomized phase II trial of veliparib, radiotherapy, and temozolomide in patients with unmethylated MGMT glioblastoma: the VERTU study. *Neuro Oncol*. 2021;23:1736-1749.
220. Colclough N, Orton AL, Martin S, et al. Achieving human brain exposure with the oral ataxia-telangiectasia mutated kinase inhibitor AZD1390, a substrate of aldehyde oxidase. *Drug Metab Dispos*. 2025;53:100107.
221. Yang JT, Wen PY, Imber BS, et al. Safety and preliminary efficacy of AZD1390 + radiation therapy for glioblastoma. *Int J Radiat Oncol Biol Phys*. 2024;120:S119-S120.
222. Kennedy WR, Sanai N, Desai S, et al. A combined phase 0/1b trial of ATM inhibitor AZD1390 in combination with radiation in patients with newly-diagnosed, MGMT-unmethylated glioblastoma. *Int J Radiat Oncol Biol Phys*. 2024;120:e242-e243.
223. Hirai H, Iwasawa Y, Okada M, et al. Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. *Mol Cancer Ther*. 2009;8:2992-3000.
224. Sanai N, Li J, Boerner J, et al. Phase 0 trial of AZD1775 in first-recurrence glioblastoma patients. *Clin Cancer Res*. 2018;24:3820-3828.
225. Liu Y, Zhou F, Ali H, Lathia JD, Chen P. Immunotherapy for glioblastoma: current state, challenges, and future perspectives. *Cell Mol Immunol*. 2024;21:1354-1375.
226. McAleavey PG, Walls GM, Chalmers AJ. Radiotherapy-drug combinations in the treatment of glioblastoma: a brief review. *CNS Oncol*. 2022;11:Cns86.
227. Awada H, Paris F, Pecqueur C. Exploiting radiation immunostimulatory effects to improve glioblastoma outcome. *Neuro Oncol*. 2023;25:433-446.
228. Sahebjam S, Forsyth PA, Tran ND, et al. Hypofractionated stereotactic re-irradiation with pembrolizumab and bevacizumab in patients with recurrent high-grade gliomas: results from a phase I study. *Neuro Oncol*. 2021;23:677-686.
229. Lazar AA, Schulte R, Faddegon B, Blakely EA, Roach M.3rd., Clinical trials involving carbon-ion radiation therapy and the path forward. *Cancer*. 2018;124:4467-4476.
230. Mohan R, Grosshans D. Proton therapy—present and future. *Adv Drug Deliv Rev*. 2017;109:26-44.
231. Böhlen TT, Germond J-F, Traneus E, et al. 3D-conformal very-high energy electron therapy as candidate modality for FLASH-RT: a treatment planning study for glioblastoma and lung cancer. *Med Phys*. 2023;50:5745-5756.
232. Lin B, Gao F, Yang Y, et al. FLASH radiotherapy: history and future. *Front Oncol*. 2021;11:644400.
233. Królicki L, Kunikowska J, Bruchertseifer F, et al. 225Ac- and 213Bi-substance P analogues for glioma therapy. *Semin Nucl Med*. 2020;50:141-151.