HHS Public Access

Author manuscript

Int J Radiat Oncol Biol Phys. Author manuscript; available in PMC 2026 June 01.

Mechanisms, Microenvironments, and Models: Understanding Therapeutic Resistance in Glioblastoma

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Abstract

Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in adults. Despite aggressive multimodal therapy—including maximal safe resection, radiation therapy, and temozolomide chemotherapy—median survival remains approximately 16 months, and nearly all tumors recur. Over the past two decades, numerous therapies that demonstrated promise in preclinical studies have failed to improve outcomes in randomized clinical trials, underscoring therapeutic resistance that defines this disease. This resistance arises from a convergence of tumor-intrinsic mechanisms, microenvironmental constraints, and limitations of current preclinical models.

In this review, we synthesize advances in understanding the molecular, cellular, and anatomical determinants of resistance to radiation therapy, chemotherapy, targeted therapies, and immunotherapies in adult GBM. We highlight how extensive intra- and intertumoral heterogeneity, transcriptional plasticity, and adaptive reprogramming enable tumor cells to evade cytotoxic stress. Key resistance mechanisms include activation of DNA damage response pathways, exploitation of hypoxic niches, therapy-induced mesenchymal transitions, and evasion of immune surveillance through impaired antigen presentation and a profoundly immunosuppressive tumor microenvironment. We further discuss how glioblastoma exploits the unique immunologic features

Disclosures: AJW, HT, YC, CTdV, TK, and FMW have nothing to disclose. SRF is on the Board of Directors for Round Table Research. SS is a scientific advisory board member for Related Sciences, Arcus Biosciences, Ankyra Therapeutics, Prox Bio, and Repertoire Immune Medicines; is a co-founder of Danger Bio; is a consultant for TAKEDA and Merck; and receives funding for unrelated projects from Leap Therapeutics and iTeos Therapeutics.

of the central nervous system—including the blood–brain barrier, limited antigen burden, and tolerogenic myeloid populations—to blunt the efficacy of immunotherapies. A major focus of this review is the role of preclinical models in shaping our understanding of therapeutic resistance. We critically evaluate established cell lines, patient-derived xenografts, syngeneic models, and genetically engineered mouse models, emphasizing both their strengths and their inability to fully recapitulate defining features of human GBM. Finally, we outline emerging strategies to overcome resistance, including rational combination therapies, adaptive trial designs, improved biomarker-driven stratification, and integrative modeling approaches. Together, these insights provide a framework for translating mechanistic understanding into more effective, durable therapies for glioblastoma.

Introduction

Glioblastomas (GBM) are the most common primary malignant brain tumors in adults and although standard therapy with surgical resection, temozolomide (TMZ) chemotherapy, and radiation therapy (RT) extends survival, these tumors are nearly uniformly fatal.[1,2] Standard therapy with up-front surgical resection and the Stupp protocol consisting of concurrent TMZ and RT followed by adjuvant TMZ results in a median survival of approximately 16 months.[2] The extent of resection is a critical variable, as patients with gross total resections have significantly longer overall survival (OS) than patients with subtotal resections.[3] Despite gross total resections and high-dose local radiation therapy, the vast majority of glioblastomas recur locally within or at the margin of the radiation field.[4] At the time of recurrence, standard treatment options include re-resection, reirradiation, and/or second-line systemic treatment with chemotherapy or bevacizumab.[5]

Over the past two decades, numerous therapies that demonstrated promise in preclinical models have failed to improve outcomes in randomized clinical trials for patients with glioblastoma.[6–9] This translational gap reflects several unique challenges posed by glioblastoma, including its intrinsic resistance to radiation and chemotherapy, extensive intratumoral genetic heterogeneity, and the inability to achieve complete surgical resection. Glioblastomas are particularly difficult to resect due to their highly infiltrative growth pattern, which enables tumor cells to disseminate into surrounding brain parenchyma.[10] Unlike tumors in other organs, wide surgical margins cannot be achieved in the brain without risking profound neurologic deficits. Additionally, the development of new treatments is hindered by an immunosuppressive microenvironment, limitations in preclinical models, and an incomplete understanding of the mechanisms underlying GBM development, progression, and recurrence. The blood-brain barrier (BBB) also poses a significant obstacle, preventing many systemic therapies from reaching therapeutic concentrations within brain tumors. This review summarizes the features of GBMs that pose scientific and therapeutic challenges, reviews the current state of therapy, and highlights promising areas for future investigation (Figure 1).

Tumor heterogeneity

Tumor cell heterogeneity in GBM confounds efforts to stratify tumors into biologically and clinically meaningful subtypes, and thus is one of the major obstacles to developing and testing effective therapeutics. GBMs exhibit extensive intra- and inter-tumoral heterogeneity at the genetic, epigenetic, transcriptomic, and immunologic levels, with subclones exhibiting varying levels of aneuploidy, different morphologies, and different mutations.

GBM is diagnosed in the setting of an astrocytic glial neoplasm with microvascular proliferation or necrosis and wild type isocitrate dehydrogenase (IDH).[11] These tumors often harbor TERT promoter mutations, chromosome 7 gain/chromosome 10 loss, CDKN2A deletion, or EGFR amplification.[12–14] Genomically, GBM is not driven by a single oncogenic mutation, but rather by an array of mutations affecting multiple genes. The most common mutations include TP53, PTEN, NF1, EGFR, ERBB2, RB1, and PIK3CA, with frequent aberrations in receptor tyrosine kinase, p53, and Rb signaling pathways.[15] These mutations cooperate to cause aberrant cell proliferation, cell cycle regulation, apoptosis, and senescence. Intertumoral heterogeneity in GBM reflects substantial variability in genetic, epigenetic, and immunologic features across tumors from different patients, resulting in differential therapeutic vulnerabilities. This diversity poses a major challenge to the efficacy of standardized treatments and contributes to the high failure rate of targeted and immunotherapies in clinical trials.

Molecular profiling efforts have characterized the genomic, transcriptomic, and immunologic landscapes of GBM and revealed significant intertumoral heterogeneity in GBM. Specifically, numerous transcriptional phenotypes found within a single tumor including neural progenitor-like, oligodendrocyte progenitor-like, astrocyte-like, and mesenchymal-like states, which are defined by aberrations and gene expression of EGFR, NF1, and PDGFRA/IDH1, among other signatures.[16,17] These cell states have distinct expression signatures for genes related to cell cycle, hypoxia, complement/immunity, and oligodendrocyte function.[18] Furthermore, emerging evidence indicates that, despite the presence of the four principal transcriptional states in glioblastoma, individual tumors display marked heterogeneity and dynamic plasticity during temporal progression and even across different spatial microenvironments within the same tumor.[19–21] This inability to stratify tumors into distinct subtypes hinders the design of targeted therapies and clinical trials, as patient populations remain heterogeneous even within defined subgroups. Consequently, therapeutic responses are diluted, signal-to-noise ratios in trials are reduced, and promising treatments may fail to demonstrate efficacy due to underlying biological complexity that remains unaccounted for. Thus, a better method for tumor stratification and the identification of predictive biomarkers is essential to design integrative trials that account for the complex tumor biology of glioblastoma.

Modeling the extensive intra- and intertumoral heterogeneity of GBM remains one of the major challenges in preclinical research, which is critical for studying tumor biology and testing therapies. Traditional adherent patient-derived GBM cell lines including U87, U251, and A172 are commonly used for *in vitro* studies and as xenografts in immunodeficient mice. These models have a high rate of engraftment and predictable tumor kinetics, but

cannot be used to model the interactions of GBM with a host immune system. Additionally, studies have reported that these xenografts fail to recapitulate the clinical, mutational, and histologic characteristics of patient glioblastoma, and that genotypes of these models lack the copy-number alterations, epigenetic states, and ecDNA-driven oncogene amplification observed in patient samples.[22–25]

Patient-derived xenografts (PDXs) are created by implanting fresh or minimally cultured human tumor tissue into mice, rather than established cell lines.[26,27] In GBM, this often involves taking a fragment of dissociated cells from a patient's tumor and transplanting them either orthotopically (into the brain of an immunodeficient mouse) or subcutaneously. PDX tumors generally retain key histopathological features of the original GBM including nuclear atypia, pleomorphism, and high mitotic activity, but necrosis and microvascular proliferation are identified less commonly in intracranial PDXs.[28] Additionally, they generally harbor the same mutational spectrum and copy number alterations as the patient's tumor, although in one study 4 of 24 PDX lines gained or lost driver alterations on engraftment, consistent with clonal selection.[28] This fidelity means that molecular subtypes of GBM (proneural, classical, mesenchymal) and specific driver mutations (EGFR amplification, TP53 mutation, etc.) can be studied in the appropriate genomic context using PDX panels. However, PDXs must be grown in immunodeficient hosts and can lose human immune and stromal elements that influence tumor state, or can be grown in mice reconstituted with human immune systems. Organoids have emerged as promising platforms that preserve cellular heterogeneity and lineage hierarchies, but they lack vasculature and exhibit batch-to-batch variability.[29,30]

Genetically engineered mouse models (GEMMs) can be used to generate autochthonous tumors to study tumor development, progression, interaction with the host immune system, and therapeutic responses. GEMM approaches often involve activation of oncogenes such as mutant epidermal growth factor receptor (EGFR), Ras, or overexpression of platelet-derived growth factor (PDGF) ligands and loss of tumor suppressors including Nf1, Pten, Trp53, and Rb1. These mutations can be initiated with a cell-type specific Cre recombinase, inducible Cre recombinase, lentiviral vectors, or adenoviral vectors.[31,32] Importantly, deletion of tumor suppressors in different stem and progenitor cell lineages leads to histologically similar yet molecularly distinct GBMs with unique biological behaviors and tumor microenvironments.[32,33] Another method of GEMM initiation includes the replication-competent ASLV long terminal repeat with splice acceptor (RCAS) system that utilizes Rous sarcoma viral vectors that can only cause infection in cells expressing the TVA receptor, which can be controlled under cell-type specific promoters.[31] Alternatively, autochthonous tumors can be generated in immunocompetent mice via delivery of plasmid DNA (often encoding CRISPR or transposase machinery, which is more flexible and requires less breeding of conditional alleles) into cells of the developing mouse brain followed by *in utero* electroporation.[34–36] Mouse age at tumor initiation, the Cre driver of choice, and the combinations of genetic perturbations utilized can lead to varying phenotypes, penetrance, latency, and growth rates.

GEMMs can effectively recapitulate the histopathologic and microenvironmental features of GBM, although distinct tumor microenvironments arise when GBMs originate from different tumor-initiating cells.[32,33] However, many of the recurrent genetic alterations

found in GBM, such as CDKN2A deletion and loss of tumor suppressors, other hallmark genomic features of human GBM remain far more challenging to recapitulate in mice. Copy number alterations including chromosome 7 gain and chromosome 10 loss, along with focal, high-level EGFR amplification, are among the defining diagnostic features of IDH–wildtype GBM, yet these large-scale chromosomal events cannot be readily engineered into the murine genome. Even in autochthonous models, which more faithfully reflect endogenous tumor evolution, introducing broad aneuploidy or human-like amplification patterns is technically difficult and often incompatible with embryonic development or lineage-restricted tumor initiation. As a result, GEMMs frequently rely on overexpression or knock-in strategies that approximate but do not fully reproduce the structural complexity, dosage effects, and clonal dynamics driven by these chromosomal alterations in patients. This limitation underscores the need for complementary model systems and highlights why certain aspects of GBM biology, particularly those linked to large-scale genomic instability and extrachromosomal EGFR amplification,[37] remain difficult to study in traditional mouse models.

Resistance to radiation therapy

RT for GBM typically involves a dose of up to 60 Gy delivered in 30 fractions to the radiographically-defined tumor with a wide margin to include tumor cells that infiltrate surrounding normal tissue.[2,38] Patients with less favorable baseline characteristics including age >70 and poor performance status are often treated with hypofractionated RT.[39] Although radiation therapy is one of the most effective therapies for GBM, tumor recurrence after focal radiation therapy suggests that at least a subpopulation of tumor cells is resistant to RT.

Ionizing radiation primarily functions by inducing lethal DNA double-strand breaks, but GBM cells often upregulate DNA damage response pathways that counteract the efficacy of RT. Glioma stem cells preferentially activate the DNA damage checkpoint kinases Chk1 and Chk2, leading to G2 cell-cycle arrest and increased repair of RT-induced DNA damage.[40] This enhanced capacity for DNA repair leads to radioresistance, but inhibiting Chk1/2 can resensitize GSCs to RT.[40] Additionally, upregulation of the DNA damage kinase (DNA-PK), which mediates non-homologous end joining (NHEJ), provides an alternative pathway for double-strand break repair and further reinforces radioresistance.[41] Mutations in TP53 and loss of PTEN, both common in GBM, impair apoptotic responses and promote tumor cell survival after DNA damage,[42] while the methylation status of MGMT influences repair of alkylating damage and may also affect radiosensitivity indirectly. Inhibition of poly(ADP-ribose) polymerase (PARP), which plays a role in detection and repair of single-strand DNA breaks, is actively being investigated in GBM as a strategy to exploit defects in DNA repair and enhance therapeutic vulnerability, often in combination with other DNA damage response (DDR) inhibitors.[43–45]

A growing body of work also highlights the role of senescence in radiation resistance. Radiotherapy can induce senescence in both stromal and tumor cells, and stromal cells can adopt the so-called senescence-associated secretory phenotype (SASP), secreting several factors including HGF (the ligand of the receptor tyrosine kinase Met). In preclinical models, senolytic therapy can decrease GBM growth and recurrence by selectively

eliminating senescent cells and attenuating SASP.[46–48] In fact, Met signaling can lead to hyper-activation of *Ataxia telangiectasia mutated* (ATM), a key component of the DNA double-strand break repair machinery in GBM stem-like cells, which promotes a radioresistant phenotype.[49–51]

GBM is characterized by highly disorganized and aberrant vasculature, resulting in regions of hypoxia where the efficacy of radiation is diminished due to reduced oxygen-mediated free radical formation. In these hypoxic niches, stabilization of hypoxia-inducible factor 1α (HIF-1α) promotes the expression of pro-survival genes and DNA repair pathways, contributing to a more invasive and radioresistant tumor phenotype.[52–54] HIF-1α–driven upregulation of factors such as vascular endothelial growth factor (VEGF) and C-X-C chemokine receptor type 4 (CXCR4) enhances tumor cell motility, angiogenesis, and resistance to therapy.[55,56] Over time, hypoxia can promote selective pressure that favors the outgrowth of more aggressive, treatment-resistant clones, which may partly explain why recurrent GBMs following chemoradiation are often more diffuse, infiltrative, and refractory to additional radiation therapy.[57–59]

In a process termed adaptive radioresistance, radiation stress can also drive GBM cells to adapt their phenotype. Work in *in vitro* systems and preclinical models has shown that repeated fractional irradiation activates NF-κB signaling to drive expression of cell survival and DNA repair genes that lead to increased radioresistance.[60,61] The result of such reprogramming is often a shift toward a mesenchymal phenotype – a cell state associated with therapy resistance and poor prognosis.[62–64] Mesenchymal-transitioned GBM cells are less proliferative but more resistant to RT, better able to invade surrounding brain tissue, and may drive tumor relapse.[64–66] Additionally, irradiated GBMs undergo a rapid lipid metabolic switch, accumulating unsaturated fatty acids to form lipid droplets that buffer ER stress and limit RT-induced apoptosis.[67]

Strategies to overcome GBM resistance to RT include the use of ablative radiation doses, tailored systemic therapies, and tumor radiation sensitization. Stereotactic radiosurgery (SRS) and hypofractionated stereotactic RT (HFSRT), in which highly conformal high-dose radiation therapy is delivered over a small number of fractions, is one approach to overcome radioresistance. RTOG 9305 was the original study that tested the role of SRS in GBM. This trial randomized patients with GBM to receive 60 Gy in 30 fractions with BCNU with or without adjuvant SRS. There was no improvement in OS, with a median OS of 13.5 months in patients who received SRS and 13.6 months for those who did not. However, this study does not reflect contemporary treatment practices, including the alkylating agent of choice and the use of concurrent and adjuvant TMZ.[68]

The emerging paradigm of preoperative radiation therapy is currently in early-phase trials in GBM (NCT03582514).[69,70] A potential advantage of preoperative RT, particularly preoperative SRS, is the ability to treat smaller volumes with more precise target delineation, potentially decreasing treatment-related morbidity. Moreover, intact tissues typically have higher oxygen levels, which may enhance the initiation of RT-induced DNA double-strand breaks, improving the overall effectiveness of the RT. Of course, implementation of this strategy would be limited by the need to obtain additional tissue for diagnostic purposes, an

increased risk of radiation necrosis, the urgency of symptom management, and the potential for worsening cerebral edema.

Numerous radiosensitizers have shown early but promising results, including PARP inhibitors, ATM inhibitors, and agents targeting DNA-PK.[71–75] However, translation into clinical benefit has been largely unsuccessful, often due to blood-brain barrier limitations, tumor heterogeneity, and insufficient therapeutic windows.

Preclinical models have enabled detailed study of DNA damage responses that underlie radioresistance, yet each model captures only a subset of the clinically relevant biology. GBM stem cell (GSC)-enriched cultures are widely used to interrogate Chk1/Chk2 activation, NHEJ activity via DNA-PK, and other DNA repair pathways; however, these *in vitro* systems lack the hypoxic gradients, metabolic constraints, and microenvironmental cues found *in vivo*. Syngeneic models including the carcinogen-induced GL261 and CT2A cell lines can be used to study the effects of RT in immune-competent hosts, enabling evaluation of radiation-induced immune modulation. However, the relative immunogenicity of the GL261 line, leading to spontaneous immune rejection in a small portion of mice, does not recapitulate the immune-poor, myeloid-rich tumor environment of human GBM.[76] While CT2A tumors are comparatively less immunogenic and may better reflect the immunosuppressive microenvironment of human GBM, both syngeneic lines lack the genomic complexity of clinical disease; alternative platforms including F98 glioma cells in immunocompetent rats and humanized mouse models may more faithfully recapitulate the relevant immune context. PDXs preserve patient-specific DDR phenotypes and can model clinically relevant responses to TMZ/RT, yet they require immunodeficient mice and therefore cannot accurately model the interplay between RT and the adaptive immune system.

GEMMs offer the unique advantage of studying RT resistance in an autochthonous setting with intact vasculature, hypoxia, and immune surveillance, but the absence of defining genomic alterations in murine GBM, including extrachromosomal EGFR amplification and whole-chromosome aneuploidies that shape RT response limits their translational relevance. As a result, while each model contributes valuable mechanistic insight, the full complexity of radiation resistance emerges only through integrating observations across multiple systems.

Resistance to chemotherapy and targeted therapies

Chemotherapies

TMZ, an oral DNA-alkylating agent, is the standard chemotherapy for GBM. While TMZ provides a survival benefit, most GBMs either exhibit upfront resistance or eventually acquire resistance to TMZ, contributing to tumor recurrence. Adult isocitrate dehydrogenase (IDH)-wildtype GBMs often have molecular features that confer chemoresistance, including unmethylated O^6-methylguanine-DNA methyltransferase (MGMT) promoters and other DNA repair aberrations.

TMZ functions by the addition of methyl groups to DNA bases, including at the O^6 guanine position, creating an O^6-methylguanine. If this lesion is not repaired, mispairing with thymine during replication triggers mismatch repair and leads to lethal DNA strand breaks. MGMT is a major mediator of TMZ resistance in GBM, as it removes the methyl group from O^6-methylguanine, thereby preventing downstream DNA damage. In fact, *MGMT* expression level is inversely correlated with TMZ effectiveness in patients.[77] Approximately half of GBMs have methylation of the *MGMT* promoter, which epigenetically silences MGMT expression; these tumors respond better to TMZ. Conversely, GBMs that are MGMT-unmethylated (expressing the enzyme) are often refractory to TMZ.[77] Even in initially MGMT-methylated tumors, recurrence can involve MGMT upregulation through selection of MGMT-expressing clones or promoter demethylation.[78] One effort to overcome TMZ resistance in GBM exploits MGMT deficiency by using an agent that can generate reversible DNA lesions that, if unrepaired in the absence of MGMT, slowly convert into toxic interstrand cross-links, selectively killing tumor cells while limiting normal-cell toxicity and bypassing MMR-mediated resistance.[79] In the absence of MGMT-mediated removal, TMZ-induced O^6-methylguanine lesions activate the mismatch repair (MMR) system. MMR attempts to repair the methylation mispairing between O^6MeG and thymine, leading to repair cycles that ultimately trigger either A:T transition mutations or lead to apoptosis.[80] About 10% of GBMs may acquire resistance to TMZ by losing key MMR components at recurrence, including mutation or epigenetic silencing of MSH6, MLH1, MSH2, or PMS2.[81–84] These MMR-deficient tumors will no longer convert an O^6-methylguanine lesion into a lethal signal, and instead continue to proliferate while tolerating TMZ-induced damage. As a result, MMR deficiency confers strong resistance to TMZ and other alkylators.[84] These TMZ-induced hypermutated tumors have an average of >500 mutated genes per tumor, which may activate alternative oncogenic pathways and make the recurrent tumor highly genetically diverse, potentially complicating further treatment. Despite the likely increase in tumor neoantigen burden, chemotherapy-driven hypermutated gliomas have low response rates to PD-1 blockade.[85]

A subset of GBM cells expresses high levels of drug efflux transporters.[86] For example, GSCs and other GBM cells can upregulate ATP-binding cassette (ABC) transporters, which actively pump TMZ and other chemotherapeutics out of cancer cells.[87] In addition, some glioblastoma cells express aldehyde dehydrogenase (ALDH) and other enzymes that neutralize drug intermediates, and they can enter a reversible dormant state (cell-cycle arrest) during therapy, then re-proliferate afterward.[88] By evading the effects of chemotherapy, even a small number of resistant cells can lead to tumor recurrence.

Targeted therapies

Primary signaling pathways that are upregulated in GBM include amplification of the epidermal growth factor receptor (EGFR) and increased PI3K/Akt/mTOR pathway signaling. EGFR is amplified or mutated in between ⅓ and ½ of human GBMs, making it an appealing therapeutic target.[17] Although EGFR-inhibitors have been shown to inhibit proliferation of GBM cells in preclinical experiments, they have largely failed to improve outcomes for GBM patients.[89] This limited clinical efficacy is in part due to poor drug penetration through the blood-brain barrier, and in part due to the lack of a therapeutic

window. Unlike EGFR mutations in lung cancers, a common feature of EGFR mutations found in GBM is that the mutation occurs in the extracellular domain, rather than the intracellular kinase domain, making it challenging to target EGFR signaling within a tumor while preserving systemic EGFR signaling. Novel EGFR kinase inhibitors with BBB penetration and wider therapeutic windows are currently under investigation.[90,91]

The PI3K-Akt-mTOR signaling pathway is hyperactivated in 90% of GBMs due to PTEN loss or EGFR signaling, leading to increased cell proliferation and survival. Inhibitors targeting this pathway similarly demonstrated efficacy in preclinical models but have not yet shown promise in clinical trials.[92,93] Resistance to inhibition of the PI3K-Akt-mTOR signaling pathway occurs primarily via feedback activation of parallel pathways, genomic alterations that sustain downstream signaling regardless of inhibition, and challenges with drug delivery across the blood-brain barrier.[94,95] New approaches aim to target downstream "addictions" more specifically, or to use context-specific vulnerabilities. In summary, GBM's signaling redundancies and adaptability limit the impact of targeted therapies including EGFR and PI3K/Akt/mTOR inhibitors.

Preclinical modeling of resistance to chemotherapy and targeted therapy remains an ongoing challenge. MGMT promoter methylation is one of the strongest predictors of TMZ response in patients, yet this is challenging to study in GEMMs. Until the recent advances in CRISPRi technology, methylation of a single genomic locus could not be modeled.[96] While MGMT-methylated PDXs can model differential TMZ sensitivity, they can re-express MGMT or undergo selection for MGMT-positive clones during passaging, limiting long-term fidelity.[97] Hypermutation following TMZ exposure, a clinically important phenomenon associated with MMR loss at recurrence, is not easily reproduced in mouse models; MMR-deficient GEMMs tend to develop other spontaneous malignancies or embryonic lethality, precluding modeling of acquired MSH6/MLH1/PMS2 loss during therapy. These modeling gaps help explain why TMZ resistance mechanisms—particularly acquired hypermutation and transitions between MGMT expression states—remain poorly captured in preclinical systems.

Resistance to immunotherapies

Immunotherapy has revolutionized treatment for several cancers, but has failed to demonstrate any benefit for patients with glioblastoma.[98–100] A key reason lies in the unique immune environment of the central nervous system (CNS) and the concept of "immune privilege." Historically, the brain was considered an immune-privileged organ based on the observation that skin autografts, which could stimulate immune rejection in other parts of the body, failed to elicit immunity when transplanted to the brain.[101] The idea of immune privilege in the brain was further supported by publications describing the absence of a typical lymphatic system[102], limited responses to intracranial antigenic challenge[103], a paucity of dendritic cells in healthy brain tissue, and the BBB.

In recent years, this view of the brain as an immune-privileged organ has been revised. The meninges, which are the protective membranes that surround the brain and spinal cord, contribute to the integrity of the blood-brain barrier by regulating immune cell entry

into the CNS. The discovery in 2015 of functional lymphatic vessels in the meninges proved that the CNS has a direct connection to the peripheral immune system.[104] These meningeal lymphatics can drain cerebrospinal fluid and antigens from the brain to cervical lymph nodes, providing a route for immune cells to encounter brain-derived antigens. Therefore, the brain is now considered “immunologically distinct” rather than absolutely immune-privileged.

While the brain can mount immune responses, the baseline state of the CNS is tuned to prevent inflammation to protect vital neural tissue from immune-mediated damage. For example, a population of tissue-resident regulatory CD4 T cells is present in the meninges, and ablation of these cells permits CD8+ T cell infiltration into the brain parenchyma.[105] When T cells migrate into the CNS, they often interact with microglia, the resident macrophages of the brain and local antigen-presenting cells, which have a tolerogenic bias unless strongly activated.[106–108] Furthermore, the BBB, while not impenetrable, does restrict entry of many immune cells. In GBM, the BBB is partially disrupted (as evidenced by contrast enhancement on MRI indicating leakiness), which allows some immune cell extravasation. Importantly, when a tumor is present, immune cells can infiltrate glioblastoma; however, they often contribute to an immunosuppressive microenvironment, with myeloid cells alone comprising up to 50% of the tumor mass.[109]

GBMs establish an immunosuppressive tumor microenvironment. They release factors that recruit immunosuppressive cells and inhibit effector lymphocytes. For example, GBM cells secrete chemokines like CCL2 and CSF-1 that attract monocytes/macrophages and promote their differentiation into M2-polarized, tumor-supportive macrophages.[110,111] These tumor-associated macrophages (TAMs) and microglia produce cytokines (IL-10, TGF-β) and express checkpoint ligands such as PD-L1 that blunt T-cell activity.[112] GBM also upregulates enzymes like indoleamine 2,3-dioxygenase (IDO) that deplete tryptophan and create a poor metabolic environment for T cells, causing either dysfunction or exclusion to the tumor periphery.[113] GBM is infiltrated by low levels of active cytotoxic T cells, and high levels of suppressive immune cells (Tregs, TAMs, myeloid-derived suppressor cells), whose effects must be overcome to stimulate robust antitumor immunity.[114]

GBM has particularly low numbers of tumor-infiltrating lymphocytes and other immune cell effectors, as well as a low tumor mutational burden, both of which are correlated with decreased responses to immune-based therapies.[115,116] Despite this, neoantigen-targeting tumor vaccines can elicit CD4+ and CD8+ antigen-specific responses, although this approach has not demonstrated an improvement in OS thus far.[115] One major challenge is that dexamethasone, a highly potent corticosteroid that inhibits T cell proliferation, is often used to treat cerebral edema associated with GBM, but its use during immunotherapy can preclude the development of neoantigen-specific T cell responses.[115] However, activated T cells can effectively target tumor neoantigens, as was demonstrated in a study testing chimeric antigen receptor T cells targeting the EGFR variant III tumor-specific antigen that led to rapid and dramatic radiographic tumor regression.[117]

Normally, when foreign antigens are present in tissues, dendritic cells phagocytose these proteins, migrate to a tumor-draining lymph node, and present antigen in the context of

MHC to activate T cells.[118] In GBM, there is evidence that some antigen presentation occurs within the brain itself by microglia or macrophages, but this may lead to T cell tolerance rather than activation.[108] Instead, effective anti-tumor T cell priming likely requires tumor antigens to reach cervical lymph nodes via the meningeal lymphatics, where professional antigen-presenting cells present them to T cells.[119] However, GBM can impede this process by suppressing dendritic cell maturation and reducing expression of co-stimulatory molecules.[120] GL261 mouse model studies demonstrated that the tumor's immunogenicity is crucial – GL261 (which expresses more MHC I and is immunogenic) sometimes spontaneously provokes an immune response leading to tumor rejection, which is rarely the case for human GBM.[121,122] Human GBM actively downregulates MHC I expression, leading immune evasion from CD8 T cell killing.[123] This tumor cell immune evasion explains some of the failures of immunotherapies that rely on CD8+ T cell killing, including vaccines and immune checkpoint inhibitors. T cells activated in the periphery may struggle to find and attack tumor cells that present very few identifiable antigens and reside in a suppressive CNS milieu.

When immunotherapies are employed in GBM, even initially responding tumors can adapt and become resistant rapidly. One striking example is with CAR T-cell therapies targeting tumor-specific antigens like EGFRvIII or IL13Rα2. While some initial responses were seen, relapses occurred via antigen loss or immune modulation. In trials of EGFRvIII-directed CAR T cells, post-treatment tumors showed loss of the EGFRvIII mutation in many cells (selection for antigen-negative clones) and a compensatory influx of immunosuppressive cells (Tregs, M2 macrophages) along with elevated levels of PD-L1, IDO1, and immunosuppressive cytokines.[124] Similarly, IL13Rα2-targeted CAR T therapy led to outgrowth of IL13Rα2-negative tumor cells, demonstrating that antigen drift can allow the tumor to evade single-antigen targeting therapies.[125]

Even with checkpoint blockade, adaptive resistance is evident: e.g., anti-PD-1 therapy can lead to upregulation of alternative inhibitory ligands or recruitment of macrophages that mediate T-cell suppression despite PD-1 blockade.[126] Intratumoral heterogeneity in GBM describes not only tumor cells, but also heterogeneity in immune susceptibility; some tumor clones may lack targetable antigens or have innate expression of checkpoints. Moreover, the use of corticosteroids to reduce cerebral edema in GBM patients limits immunotherapy efficacy by blunting lymphocyte activity and antigen presentation during anti-PD1 therapy.[127] Thus, several emerging immunotherapeutic treatment modalities are under study in GBM. Although the trial's external control design and mid-study endpoint modification have drawn methodological scrutiny, a dendritic cell vaccine (DCVax-L) represents the first phase 3 systemic treatment to demonstrate a survival benefit in newly diagnosed GBM in nearly two decades.[128] Neoantigen-targeted mRNA vaccines are also under active investigation, though GBM's characteristically low tumor mutational burden limits the pool of immunogenic neoantigens and complicates personalized vaccine design. Oncolytic virotherapy has shown renewed promise: intratumoral administration of G47Δ, a third-generation oncolytic HSV-1, achieved a 1-year survival rate of 84.2% in a phase 2 trial of residual or recurrent GBM, with biopsies confirming elevated CD4+/CD8+ tumor-infiltrating lymphocytes, supporting its regulatory approval in Japan as the first licensed oncolytic virus for GBM.[129] Similarly, a phase I trial of CAN-3110,

an oncolytic HSV retaining the ICP34.5 neurovirulence gene under a nestin promoter, reported no dose-limiting toxicities in 41 recurrent GBM patients, with HSV1 seropositivity significantly associated with both improved survival and viral clearance from injected tumors, suggesting that pre-existing antiviral immunity may potentiate rather than attenuate therapeutic responses.[130] Across immunotherapeutic modalities, a unifying challenge is the GBM immunosuppressive microenvironment, which blunts both innate priming and effector responses, and rational combinations, including with radiation therapy, are therefore increasingly prioritized as the field moves beyond single-agent paradigms.

In summary, the traditional view of the brain as an immune-privileged site has been revised; while the central nervous system is capable of immune surveillance, glioblastoma takes advantage of the brain's inherently low immunogenicity to establish a deeply immunosuppressive microenvironment. This environment includes a partially isolated tumor site (due to the BBB), predominantly anti-inflammatory immune cells, lack of robust antigen presentation, and numerous inhibitory signals. These factors collectively blunt the efficacy of immunotherapies in GBM. Effective treatment will likely require strategies that overcome the tumor's immunosuppressive effects by boosting antigen presentation, enhancing T cell trafficking, reactivating microglia/macrophages to an anti-tumor state, and blocking or reprogramming multiple immunosuppressive pathways. Ongoing research and clinical trials are actively pursuing these goals, informed by the growing understanding of CNS immunology and lessons from preclinical models and patient studies.

Immunotherapy resistance is particularly difficult to model because each preclinical system captures only a fraction of the human immune landscape. Xenografts and PDXs must be grown in immunodeficient mice, precluding evaluation of antigen presentation, T-cell exhaustion, or myeloid-mediated immunosuppression. Humanized mouse models partially restore human immune function but remain limited by incomplete lymphoid reconstitution, high variability, and cost, and they still lack key CNS-specific immunologic features. Syngeneic models including the GL261 and CT2A cell lines allow investigation of immune–tumor interactions and checkpoint blockade, yet these tumors are far more immunogenic than human GBMs, often overstating responses to immunotherapies.[76] GEMMs offer insight into immune editing and antigen presentation in an intact microenvironment, but many GEMMs fail to model MGMT- or therapy-driven hypermutation or the profound T-cell scarcity characteristic of patient tumors. No single model accurately mirrors the immunologic suppression, antigen paucity, and myeloid dominance that define human GBM.

Conclusion and Future Directions

Therapeutic resistance in adult IDH-wildtype glioblastoma is driven by diverse molecular mechanisms that allow tumor cells to survive virtually all treatment modalities. These include upregulating DNA repair and checkpoint activation, a profoundly immunosuppressive microenvironment, immune evasion, and mechanisms for tolerating or even repairing DNA damage. Many of these resistance pathways overlap across treatment types—for example, glioma stem cells contribute to resistance to chemotherapy,

radiotherapy, and immunotherapy, while hypoxic tumors promote both immune resistance (via myeloid cell recruitment) and radioresistance (via HIF-1–mediated DNA repair).

Over the past decade, molecular and single-cell profiling has deepened our understanding of how tumors evolve over time and under the selection pressures imposed by therapy. Furthermore, advances in mouse modeling and genome editing have improved our ability to model and study features of GBM *in vivo*. Overcoming resistance will likely require multi-faceted strategies that target both tumor-intrinsic pathways and the surrounding microenvironment to prevent adaptive escape. Adaptive trial designs offer one promising strategy, enabling therapy to be adjusted in real time based on early biomarkers of resistance —such as adding a second agent in response to rising MGMT activity or alternate pathway activation. Liquid biopsy approaches, including detection of tumor DNA or RNA in cerebrospinal fluid, may enable early identification of resistance mechanisms. Furthermore, functional synaptic connections between GBM cells and neurons have recently been identified, and their impact on tumor progression and therapeutic resistance is just beginning to be understood.[131–133]

Continued research and integration of these approaches are essential. By simultaneously targeting multiple resistance pathways, we may be able to prevent tumor adaptation, extend disease control, and move closer to durable cures. Future therapies informed by these insights hold real promise for improving outcomes for this devastating disease.

Funding:

HT is supported by **1U54CA283114 (awarded to FMW)**.

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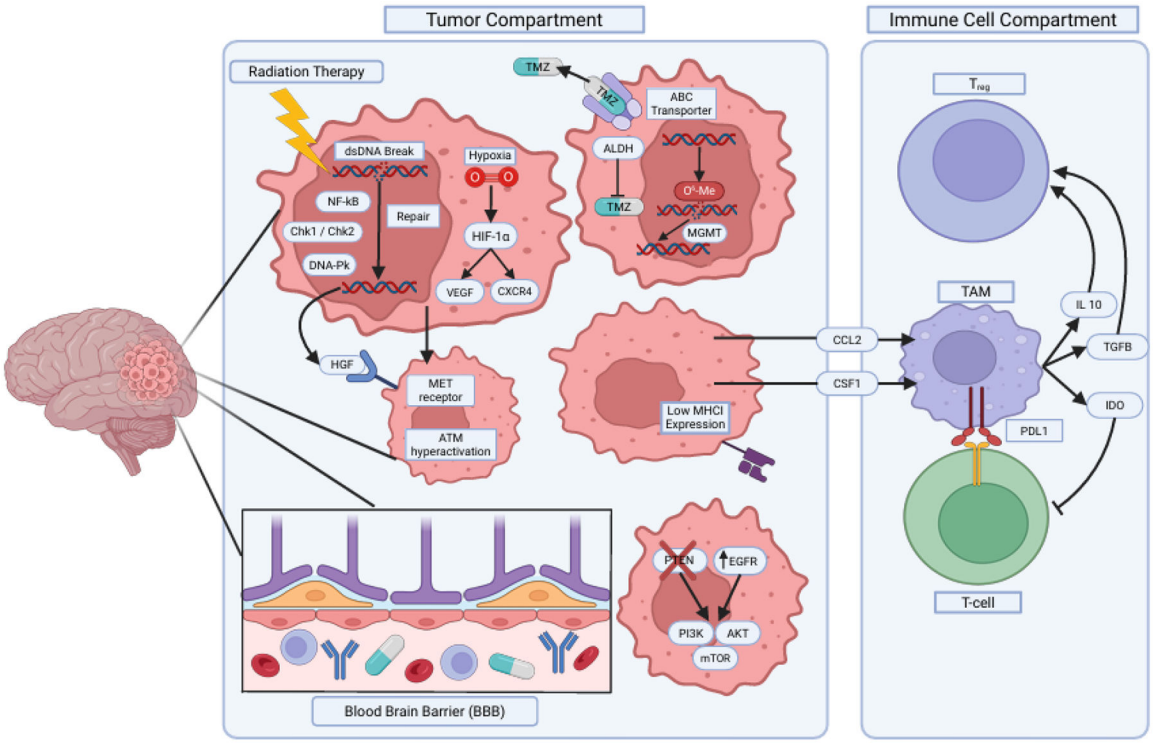

Figure 1. Diverse mechanisms of therapeutic resistance in glioblastoma integrate tumor-intrinsic pathways, metabolic adaptations, and microenvironmental barriers.

Glioblastoma cells withstand radiation therapy by activating DNA damage response molecules such as Chk1/Chk2, NF-kB, and DNA-PK, while MET-driven ATM signaling and HIF-1α stabilization in hypoxic niches promote invasion and survival after irradiation. The efficacy of temozolomide (TMZ) is limited by MGMT, which removes cytotoxic O^6-methylguanine lesions. Resistance is also amplified by loss of mismatch repair function, expression of aldehyde dehydrogenase (ALDH) which neutralizes drug intermediates, and expression of ABC transporters that promote detoxification and efflux of TMZ. Hyperactivation of the PI3K/Akt/mTOR pathway, often resulting from increased EGFR signaling or PTEN loss, further drives tumor proliferation. The blood–brain barrier restricts drug and immune cell entry, resulting in limited therapeutic access. GBMs shape an immunosuppressive microenvironment by secreting CCL2 and CSF1 to recruit tumor-associated macrophages (TAMs), which secrete IL-10, TGF-β, and IDO and express PD-L1 to suppress effector T-cell activity. Regulatory T cells reinforce this suppression and weaken effective antitumor immune responses. Tumor cells also evade immune recognition by downregulating expression of MHC-I, allowing them to evade elimination by cytotoxic T-cells.