future pharmacology

MDPI

Review

Why Do Glioblastoma Treatments Fail?

Alen Rončević [1,2,*], Nenad Koruga [1,2], Anamarija Soldo Koruga [2,3] and Robert Rončević [2,4]

[1] Department of Neurosurgery, University Hospital Center Osijek, 31000 Osijek, Croatia; nkoruga@gmail.com
[2] Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia; anamarijasoldo@gmail.com (A.S.K.); robert.roncevic27@gmail.com (R.R.)
[3] Department of Neurology, University Hospital Center Osijek, 31000 Osijek, Croatia
[4] Department of Diagnostic and Interventional Radiology, University Hospital Center Osijek, 31000 Osijek, Croatia
* Correspondence: aroncevic@mefos.hr; Tel.: +385-981698481

Abstract: Glioblastoma (GBM) is the most aggressive brain tumor, characterized by high recurrence rates and poor patient outcomes. Treatment failure is driven by multiple factors, including complex tumor heterogeneity, the presence of cancer stem cells, the immunosuppressive tumor microenvironment (TME), and many others. GBM's heterogeneity underlines its ability to resist therapies and adapt to the TME. The TME, which is highly immunosuppressive and shaped by hypoxia, impairs anti-tumor immunity and limits the efficacy of immunotherapy. The blood–brain barrier (BBB) remains a major obstacle to delivering sufficient drug concentrations to the tumor by restricting the penetration of therapeutic agents. Another problem is the lack of reliable biomarkers to perform better patient stratification or even guide personalized treatments, resulting in generalized therapeutic approaches that do not adequately address GBM complexities. This review highlights the multifactorial nature of GBM treatment failure and highlights the need for a paradigm shift and innovative, personalized strategies. A deeper understanding of tumor biology and advances in translational research will be crucial to developing effective therapies and improving patient outcomes in this devastating disease.

Keywords: glioblastoma; treatment resistance; tumor heterogeneity; cancer stem cells; blood–brain barrier; tumor microenvironment; biomarkers; personalized therapy

Academic Editor: Ali Zarrabi

Received: 18 December 2024
Revised: 20 January 2025
Accepted: 27 January 2025
Published: 1 February 2025

Citation: Rončević, A.; Koruga, N.; Soldo Koruga, A.; Rončević, R. Why Do Glioblastoma Treatments Fail? *Future Pharmacol.* **2025**, *5*, 7. https://doi.org/10.3390/futurepharmacol5010007

1. Introduction

Glioblastoma (GBM) is the most aggressive and common primary brain malignancy, representing a major treatment challenge due to its highly invasive and heterogeneous nature [1]. Even though we have significantly improved our understanding of the biology of GBM, which guided and further advanced multimodal treatment approaches, comprising maximally safe surgical resection, radiotherapy, and chemotherapy, the prognosis for patients diagnosed with GBM remains disappointing, with an average survival of 12 to 18 months [2]. These widely reported poor outcomes warrant further research into mechanisms related to treatment resistance and therapeutic failure in patients with GBM.

The limited effectiveness of the available treatments for GBM is caused by a variety of recognized and unrecognized factors. Treatment resistance is facilitated by intra-tumoral heterogeneity, which is characterized by diverse cellular subpopulations with unique genetic, epigenetic, and phenotypic traits [3]. Within the tumor tissue, glioma stem cells (GSCs) are recognized as the main drivers behind the tumor plasticity—they display migratory capabilities, promote tumor recurrence, and show increased resistance to standard treatments [4]. The effectiveness of treatment is further limited by the blood–brain barrier

(BBB), which prevents pharmacotherapeutics from accessing the tumor microenvironment (TME) [5]. This complex TME consists of hypoxic regions [6] and an immunosuppressive milieu [7], both of which play a significant role in promoting treatment resistance. Furthermore, successful treatment is complicated by genetic and molecular variables, including changes in pathways related to DNA repair, cell cycle regulation, and apoptosis, as well as mutations in genes including CHEK2, EGFR, and MGMT [8]. Developing novel approaches in order to overcome these and other obstacles and enhance patient outcomes requires a deep understanding of these complex mechanisms of resistance.

In this review, we will examine the current challenges in glioblastoma treatment, explore the biological underpinnings of therapeutic resistance, and discuss potential avenues for novel treatment strategies aimed at improving patient outcomes.

2. Infiltrative Growth

The first treatment option for GBM is surgical resection of the affected brain, which provides tissue for pathohistological confirmation of the GBM diagnosis [9]. If the affected brain tissue is "non-eloquent", then the resection can be performed more extensively. In these cases, a supramaximal (supramarginal) resection is performed—the extent of brain removal is beyond the contrast-enhancing region presented on T1-weighted magnetic resonance imaging (MRI) [10]. Furthermore, some studies have reported even more aggressive resections, with partial removal of the T2/FLAIR-hyperintense region [11]. Even with the implementation of modern tools, such as intraoperative imaging (neuronavigation, intraoperative MRI), and enhanced microscopic visualization of tumoral tissue with 5-aminolevulinic acid (5-ALA) or fluorescein, the complete surgical removal of tumor cells proved to be impossible [12]. The tools that are used intraoperatively to increase the extent of resection are presented in Table 1. Unfortunately, no matter how aggressive the resection is, the neurosurgical treatment did not prove to be curative [10]. In fact, it is somewhat unrealistic to expect a surgical resection of this tumor to be the definitive treatment option, as it would inevitably damage a healthy brain.

The main reason behind the failure of surgical resection to completely eliminate the GBM lies in its infiltrative growth pattern. Because of the aggressive nature of GBM cells, when the disease is first confirmed, the invasive glioma cells are already widely disseminated throughout the brain [13]. These cells can recolonize and result in a GBM recurrence even after the tumor has been removed since they are resistant to standard therapeutic methods [14]. Since these invasive cells have already spread to distant brain regions where they created potential tumor seeds, stopping their invasion alone might not completely eliminate these cells or significantly alter the patient's chances of survival [13].

Another problem that consequentially arises with this infiltrative growth pattern concerns the effectiveness of localized therapies. Most recurrences of GBM occur in the proximity of the resection cavity, often in the region that was exposed to the most radiation. By administering pharmacologic agents at or close to the tumor resection cavity, many novel therapies aim to prevent these local recurrences [15]. Although some of these new treatments have provided encouraging results in preclinical trials [15,16], these results rarely translate to patient outcomes. In order to maximize the effectiveness of local therapy for GBM, the development of potential anti-invasive therapies should be considered in conjunction with localized and other treatments in order to completely stop GBM [17].

Table 1. Tools for maximizing extent of resection for glioblastoma.

Tool	Benefits	Limitations	Impact
Neuronavigation	Improves tumor removal Reduces damage to healthy brain	Limited by preoperative imaging Brain shift reduces accuracy	Enhanced precision Cannot identify infiltrative tumor margins
Intraoperative MRI	Detects tumor residual intraoperatively Re-evaluates the resection	High cost Warrants specialized facilities	Increased extent of resection Allows intraoperative adjustments
5-Aminolevulinic Acid (5-ALA)	Highlights tumor boundaries Improves tumor margin visualization	Cannot detect all infiltrative cells High cost	Helps in achieving maximal resection of visible tumor tissue
Fluorescein	Cost-effective Better identification of tumor tissue	Cannot detect all infiltrative cells Limited penetration depth	Increased extent of resection Useful in low-resource settings
Ultrasound	Real-time imaging of brain and tumor Portable and cost-effective	Lower resolution Worse performance for deep lesions	Assists in resection guidance Useful in low-resource settings
Intraoperative Electrophysiology	Monitors brain function during surgery Helps in preserving eloquent areas	Time-consuming Needs a specialized team	Aids in intraoperative decision making Less postoperative neurological deficits

3. Tumor Heterogeneity

A major obstacle in treating GBM is its inherent heterogeneity. The most recent World Health Organization (WHO) classification of the tumors of the central nervous system (CNS) recognized the complexity of brain tumors and classified GBM based on IDH status [18]. Therefore, GBM is by definition an IDH wild-type tumor, whereas IDH-mutated tumors are usually classified as astrocytoma or oligodendroglioma. The more precise classification of brain tumors underlines the differences in their biology and could have some implications for their treatment response. This heterogeneity actually pertains to various biological aspects of the tumoral tissue. It is well recognized that various genetic alterations can occur within a single GBM tumor. For instance, distinct cell groups within the same tumor could have mutations in genes such as EGFR, PTEN, TP53, and many others [3,19]. This is an important caveat that should always be kept in mind when obtaining tumor samples for pathohistological diagnosis during the surgical resection—numerous tissue samples should be obtained from different parts of the tumor to obtain more detailed insights into the molecular and genetic landscape of the tumor tissue within the same GBM [20]. Consequentially, simple biopsies may understate the actual genetic diversity in a tumor, as intra-tumoral heterogeneity is crucial for both molecular classification and identifying treatment targets for this disease. Because of this diversity, certain cell subpopulations are

resistant to specific treatments meant to address particular mutations. This phenomenon is very similar to the antibiotic resistance observed with some bacterial subpopulations [21]. During the treatment, some resistant clones may survive and repopulate the tumor after one population of cells is eliminated by the initiated treatment. Recent studies have started exploring the epigenetic diversity of GBM. In fact, Gempt et al. [22] examined the epigenomes of 56 treatment-naïve GBM patients and identified the epigenetic diversity of both DNA methylation patterns, as well as copy number variations (CNVs) in this population. Clinical trials combining personalized therapies with standard-of-care treatments for GBM have generally been concentrated on genetic hallmarks of the tumor [23]. However, recent trials have not targeted epigenetic-related targets, as has been performed with high-grade brain tumors in children [24–26]. The recognition of dysregulated epigenetic mechanisms actually had a notable effect on subgroup classification, prognosis, and response to therapy in these tumors and corresponding clinical trials [25]. Combining epigenetics with other study modalities can be utilized for improved tumor classification [27]. In order to obtain a more precise subclassification and to develop corresponding precision therapies, it will be crucial to include the significant role of epigenetic dysregulation to interpatient heterogeneity in GBM when characterizing the biomolecular landscape of this tumor.

The previously discussed heterogeneity within the single GBM is also evident in its spatial organization. Significant regional intra-tumoral differences are well described in the literature [28] and efforts are made to better delineate these structural layers. In their study, Greenwald et al. [29] set out to explore organizational patterns within GBM by combining spatial proteomics and transcriptomics with computational methods. Based on their findings, the authors defined five layers of GBM structure with hypoxia as the main driver of this structural organization. Despite being a significantly vascularized tumor, GBM has poor regional microcirculation, which results in certain regions of the tumor that are extremely hypoxic and necrotic [30]. It is well established that hypoxia induces the upregulation of hypoxia-inducible factor 1 (HIF-1), the key regulator that coordinates tissue adaptations to hypoxia [31]. Consequentially, the expression of angiogenic factors, such as VEGF, EPO, and PDGF, is also increased in hypoxic environments, such as those described within the GBM [32]. The traditional view of GBM metabolism highlighted the role of glucose metabolism for energy production, the so-called Warburg effect [33]. However, recent studies have challenged this classical thinking [34,35]. In fact, not all regions of the tumor exhibit low oxygen-related metabolic reprogramming—some subpopulations of the tumor cells depend on oxidative phosphorylation and lipid metabolism, whereas others rely more on glycolytic processes [36]. Additionally, GBM cells are able to metabolically adapt to their changing environment [37]. This also suggests that potential metabolic therapies should have multiple metabolic targets in order to be effective against this disease [38].

Nowadays, most treatment options aim to uniformly target the tumor tissue, and for them, this inherent tumoral heterogeneity presents a significant challenge. These therapeutic approaches might be helpful in one area of the tumor but ineffective in another [39]. Therefore, some subpopulations may be completely eliminated by therapies, while others remain intact and even develop further resistance, which could result in potential recurrence [40]. It would be indispensable to gain better insights into the intra-tumoral variabilities which are discussed here, as well as some potential unknown factors. For this particular task, imaging mass spectrometry could emerge as a leading tool which could better describe regional differences within a single GBM, as well as direct researchers toward potential targeted therapies [41]. There are plenty of available methods to analyze tumor heterogeneity and some of those are listed in Table 2.

Table 2. Advanced tools for investigating glioblastoma heterogeneity.

Tool	Function	Advantages	Limitations
Computational modeling	Uses computational tools to analyze data Integrates multi-omics data for analysis	Models can simulate tumor biology Reduces experimental costs	Limited by the quality of input data Hard to capture tumor complexity
Epigenomics	Identifies epigenetic signature of the tumor Reveals dysregulated epigenetic mechanisms	Identifies epigenetic biomarkers Can be combined with genetic data	Lack of spatial resolution Interpretation of data can be challenging
Functional imaging (PET, fMRI, and others)	Detects metabolic heterogeneity in vivo Helps in surgery planning	Non-invasive method to study tumor biology Evaluation of treatment response	High cost and limited accessibility Protocols are not standardized
Imaging mass spectrometry	Captures intra-tumoral heterogeneity Identifies potential biomarkers and therapeutic targets	Preserves the studied tissue (label-free) Multi-molecular analysis with spatial information	Limited quantitative accuracy High cost and needs technical expertise
Multiplex immunohistochemistry	Detects multiple markers Maps cell subpopulations	High sensitivity and specificity Preserves spatial information	Technically demanding Limited quantitative insights
Spatial proteomics	Identifies spatial distribution of proteins Defines tumor subregions	High-resolution data Reveals functional heterogeneity	Advanced computational analysis High cost and specialized equipment
Transcriptomics	Maps gene expression across tumor regions Identifies subregions of transcriptional changes	Gains insight into molecular pathways Supports in tumor classification	Lack of spatial resolution Does not capture post-translational modifications

4. Tumor Microenvironment

The TME of GBM is a very complex system that includes not only tumor cells but also numerous resident immune cells, native brain cells, and others [42]. GBM is located in a relatively immune-privileged organ due to the presence of the BBB and the lack of conventional lymphatic drainage [43]. While this immune privilege is not absolute, it limits the ability of the immune system and immune cells to effectively access the tumor and

induce appropriate responses. GBM exploits the surrounding environment by creating an immunosuppressive TME that protects it from immune-mediated destruction [44]. The adaptive nature of the TME is influenced by biochemical signals, such as the acidity and oxygen levels, changes in cellular composition, cell-to-cell interactions, and metabolic and other signals [45]. It is believed that GBM cells can also adapt the TME and take advantage of the dynamic surrounding environment in order to promote tumor growth and survival while also enhancing the resistance to conventional therapies [46]. The immunosuppressive TME of GBM represents a major obstacle to the development of effective treatment, particularly for immunotherapy approaches [44].

The recruitment of immunosuppressive cells, such as tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), is one of the key characteristics of the immunosuppressive TME [47]. According to published studies, up to 30% of the tumor mass in GBM is made up of tumor-associated microglia and TAMs [48,49]. It should be noted that most published research focus on the TAMs in the tumor core [48]. As we have previously discussed, GBM exhibits a significant degree of histological variability, displaying a variety of histological features, including tumor core, pseudopalisading cells, necrotic regions, neovascular regions, and tumor periphery [50]. Similarly, the presence of TAMs varies between the studied tumor regions; however, it is more common in the tumor core and surrounding hypoxic necrotic areas [51]. Moreover, there are notable differences regarding TAMs depending on these tumor regions. TAMs located in the tumor core are primarily macrophages with an M2-like, anti-inflammatory profile, whereas TAMs in the adjacent areas are generally microglia with an M1-like, pro-inflammatory profile [52]. The interaction between TAMs and GBM cells is influenced by various factors. The majority of these factors either polarize TAMs toward an M2-like phenotype, which supports tumor growth, or attract and recruit TAMs to the tumor. Finally, in order to facilitate its progression, the GBM alters and takes advantage of the TAMs [53].

In addition, GBM also suppresses adaptive immunity by impairing T-cell activity [54]. T cells that infiltrate the tumor often display a so-called exhausted phenotype, which is characterized by high expression of immune checkpoint molecules [55]. This T-cell exhaustion is defined by a steady decline in effector function, persistent expression of inhibitory receptors, metabolic dysfunction, and specific epigenetic and genetic adaptations which are driven by chronic exposure to tumor antigens and immunosuppressive cytokines in the TME [56]. Moreover, regulatory T cells (Tregs) are selectively recruited to the tumor region, further dampening the activity of effector T cells and natural killer (NK) cells [57]. Another important contributor to the immunosuppressive TME is the BBB, and the role of the BBB cannot be overstated. While the BBB is often disrupted in GBM, its partial integrity in other areas prevents appropriate immune reactions and limits the delivery of immunotherapeutic agents [58]. The role of the BBB will be further discussed in the upcoming paragraphs.

In addition to previously described mechanisms, GBM also utilizes metabolic processes to induce immunosuppression. As we have already discussed, hypoxia is a hallmark of the GBM, but it also results in the production of metabolic byproducts which can be immunosuppressive, such as lactate that lowers the tissue pH levels [59]. The hypoxic environment also results in the accumulation of adenosine, which is speculated to be responsible for blocking anti-tumor immunity [60]. Adenosine also promotes immunosuppression by suppressing the effector functions of T cells, as well as affecting Tregs [61]. Furthermore, GBM cells upregulate the expression of indoleamine 2,3-dioxygenase (IDO), which depletes tryptophan and produces immunosuppressive kynurenine, effectively inhibiting T-cell proliferation and activation [62]. Overall, there are multiple components of the GBM TME which support the immunosuppressive environment, and most of these are listed in Table 3.

Table 3. Immunosuppressive components of glioblastoma tumor microenvironment.

Component	Immunosuppressive Effect
Tumor-associated macrophages (TAMs)	Promote M2 polarization
Myeloid-derived suppressor cells (MDSCs)	Suppress T cells' activity
Regulatory T cells (Tregs)	Inhibit effector T cells and NK cells
Exhausted T cells	Exhibit reduced effector function
Hypoxia	Metabolically immunosuppressive
Metabolic byproducts	Suppress T cells' activity
Blood–brain barrier (BBB)	Limits immune cell infiltration

The combination of all these factors, concerning the TME, creates a significant immunosuppressive barrier that limits the efficacy of existing immunotherapies such as immune checkpoint inhibitors, vaccines, and chimeric antigen receptor (CAR) T-cell therapies. While these treatments have shown promise in some tumors, their success in GBM treatment has been limited due to the immunosuppression within the TME [63]. Further studies are needed in order to reprogram the TME, including targeting TAMs, inhibiting immunosuppressive pathways like IDO, and combining immunotherapies with other treatment modalities to overcome all these challenges.

5. The Blood–Brain Barrier

The BBB plays a pivotal role in the failure of GBM treatments by limiting the delivery of therapeutic agents to the tumor tissue and contributing to the immune-privileged nature of the brain [5]. The BBB is a highly selective, semi-permeable barrier composed of endothelial cells joined by tight junctions, astrocytic end-feet, and pericytes. Its main function is to maintain CNS homeostasis by restricting the entry of potentially harmful substances, including toxins, pathogens, and peripheral immune cells, while allowing the passage of essential molecules [64]. While this physiological function is vital for protecting the brain, it poses a significant obstacle to treating GBM.

The BBB significantly restricts the penetration of most therapeutic agents and biological drugs such as monoclonal antibodies [65]. Selective permeability is determined by various factors: molecular size, lipophilicity, and the presence of specific transport systems. Many oncologic drugs are hydrophilic or large molecules, making them unable to cross the BBB. Even drugs that are capable of crossing, such as TMZ, often do so at subtherapeutic levels due to the active efflux mechanisms that pump drugs out of the CNS [66]. This inadequate drug delivery can result in incomplete tumor elimination and the survival of resistant cell subpopulations [21].

Although angiogenesis in GBM produces abnormal blood vessels, which frequently disrupts the BBB, this disruption varies greatly throughout different tumor regions [67]. In regions where the BBB is leaky, therapeutic agents may penetrate the BBB more readily, but these areas are often located in the tumor core, which is already necrotic or less metabolically active. On the other hand, the invasive GBM cells which are more frequently found at the periphery are hidden by an intact or partially intact BBB, rendering them underexposed to therapeutic agents. This is presented in Figure 1. Because of this heterogeneity, treatments may focus more on some parts of the tumor with leaky vasculature but not the invasive periphery, which could result in recurrence [68].

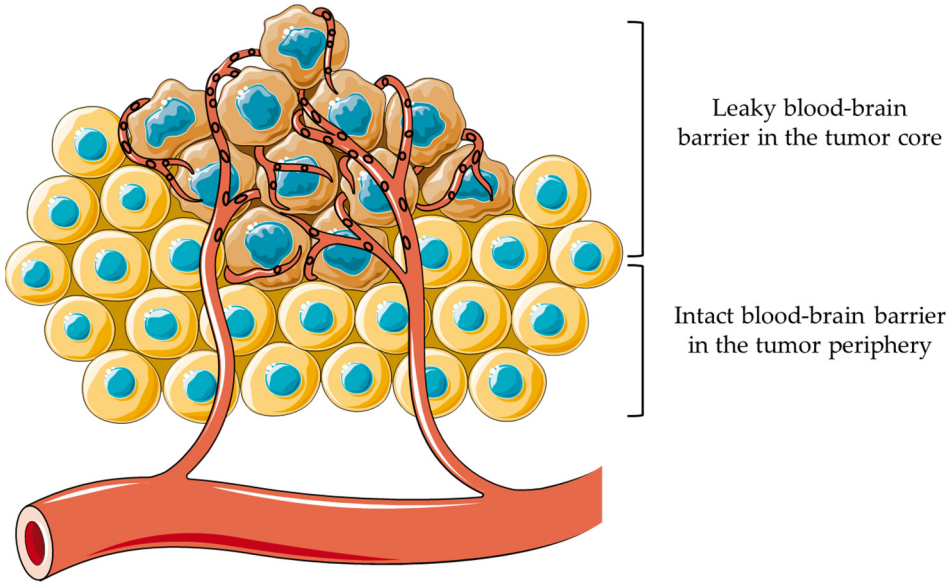

Figure 1. Regional heterogeneity of the blood–brain barrier in glioblastoma.

Significant efforts have been made to overcome the BBB; these include novel drug formulations, such as nanoparticle-based delivery systems, which can modulate the BBB permeability and improve drug penetration within the tumor [69]. Focused ultrasound is another novel approach: it temporarily disrupts the BBB to allow localized delivery of therapeutic agents [70]. Convection-enhanced delivery (CED) enables a direct delivery of therapeutics into the tumor and surrounding tissue, bypassing the BBB entirely while preventing systemic toxicity [71]. Despite notable advancements, the complexity and heterogeneity of the BBB in GBM remains a major challenge. In summary, the role of the BBB as both a physical and functional barrier limits the effectiveness of available GBM treatments by restricting drug delivery, reducing the immune response, and contributing to the ability of GBM to adapt and resist treatments. Addressing these challenges requires novel approaches to breach or bypass the BBB while minimizing damage to healthy adjacent brain tissue.

6. Treatment Resistance

As we have already discussed, treatment resistance is a defining characteristic of GBM and is a major reason why therapies, including radiotherapy, chemotherapy, and immunotherapy, fail. The standard treatment protocol, called the Stupp regimen, consists of concomitant radiotherapy and adjuvant maintenance chemotherapy with TMZ, followed by six cycles of adjuvant TMZ [72]. The resistance to this treatment protocol, and, consequentially, the failure of treatment, is a byproduct of a combination of intrinsic tumor properties and adaptive responses of GBM cells to treatment, which allows the tumor to avoid complete removal and promote recurrence [23]. Various factors are involved in GBM treatment failure, which is schematically depicted in Figure 2.

Radiotherapy, which is one of the crucial therapeutic approaches in the current GBM treatment protocol, is aimed at damaging tumor DNA and inducing tumor cell death [73]. However, GBM cells are protected from radiotherapy by various inherent mechanisms, as well as mechanisms related to the TME [74]. DNA repair machinery, as well as cell cycle arrest, the avoidance of programmed cell death, and many others, contribute to resistance to radiation [75]. These mechanisms enable GBM cells to efficiently repair radiation-induced DNA damage, limiting the effectiveness of radiotherapy, which leads to treatment failure and tumor recurrence [76]. Another important factor contributing to

radiation resistance is hypoxia in the TME [77]. In these hypoxic conditions, the production of reactive oxygen species (ROS) during radiation, which are critical for amplifying DNA damage, is markedly decreased [78]. In addition, hypoxia induces the expression of HIFs, which promote angiogenesis, tumor cell survival, and metabolic adaptations that enhance treatment resistance [79]. GSCs in GBM are particularly resistant to radiotherapy. These cells are often quiescent, making them even less vulnerable to radiotherapy, which primarily affects proliferating cells [80]. Moreover, GSCs display an improved radiation-induced DNA repair mechanism and antioxidant defense, allowing them to survive radiotherapy and drive tumor recurrence [80].

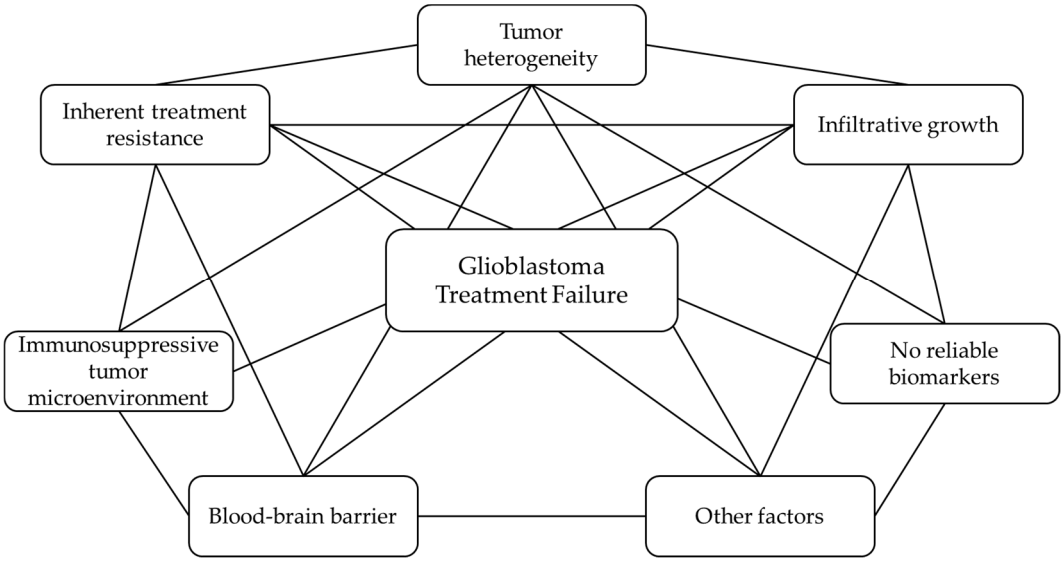

Figure 2. Glioblastoma treatment failure as a result of many interacting factors.

The best evidence of the chemotherapy resistance of GBM cells is the limited efficacy of TMZ, the first-choice chemotherapeutic agent for GBM [81]. TMZ is an alkylating agent that affects single strands of DNA and induces DNA damage [82]; however, its efficacy is highly dependent on the methylation status of the MGMT promoter [83]. A study by Leske et al. [84] corroborated the finding that the MGMT promoter is usually methylated in patients with longer overall survival. In tumors with an unmethylated MGMT promoter, the enzyme is actively expressed and repairs TMZ-induced DNA damage, making this treatment less effective. Another important resistance mechanism of GBM cells is the overexpression of efflux pumps. For example, ABC transporters, P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP) actively pump chemotherapeutic agents out of cells, reducing intracellular drug concentrations [85]. Additionally, as we have already discussed, metabolic adaptations can further protect GBM cells from the cytotoxic effects of chemotherapy [34,86]. The problem of tumor heterogeneity also plays a significant role in chemotherapy resistance. Different regions and cell subpopulations within a single GBM may exhibit varying sensitivities to treatment [3,19,36]. As a result, even if chemotherapy effectively targets one subpopulation, resistant clones can survive and repopulate the tumor.

Immunotherapy has revolutionized the treatments of many malignant tumors but has had limited success in GBM due to several reasons [87]. GBM creates an immunosuppressive microenvironment that limits the activation and function of immune cells [47]. TAMs and MDSCs within the TME suppress T-cell responses and promote GBM progression [48]. Checkpoint inhibitors showed promising therapeutic effects for some tumors [88], but so far, they have had limited efficacy in GBM treatment [89]. This is partly because GBM has a low level of tumor mutational burden, resulting in fewer neoantigens to stim-

ulate a strong immune response [90]. Even though some high-grade gliomas with high mutational burden have been identified, their response to immunotherapy still remains poor [91,92]. It is suggested that for GBM, a combination of neoantigens of high-quality, elevated T-lymphocyte-specific gene signature and CD8+ T-cell infiltration is needed for an improved response to immunotherapy [92]. Resistance to other immunotherapeutic approaches, such as CAR T-cell therapy, is linked to GBM's heterogeneity, limited number of GBM-specific antigens, and other immune-evasion strategies [93]. GBM cells can undergo immunoediting and change their antigen expression, rendering CAR T cells ineffective [94]. Furthermore, the immunosuppressive cytokines within the TME limit T-cell infiltration and function [95]. The role of B cells in GBM is less understood, but recent studies suggest that they may contribute to the TME [96]. B cells in the TME may exhibit both suppressive pro-inflammatory properties [97]. However, further research is needed to fully elucidate many presumed roles of B cells in GBM and their potential as therapeutic targets.

GBM cells are highly adaptive and capable of quickly developing cross-resistance to therapies [98]. The dynamic nature of GBM cells further complicates treatment. According to the study by Neftel et al. [99], the GBM cells can exhibit distinct cellular states and transition between those states. This plasticity enables GBM to evade targeted therapies and recur in a more aggressive and resistant form after treatment. Overcoming treatment resistance in GBM requires strategies that can overcome these obstacles. Combining radiotherapy and chemotherapy with some immunotherapeutic agents may enhance treatment efficacy. Targeting the TME could also improve immunotherapy efficacy. Additionally, the concept of personalized approaches that tailor treatments based on the molecular and genetic profile of individual tumors holds promise for overcoming resistance [23]. Taken together, the failure of current GBM treatments is driven by intrinsic resistance mechanisms, including efficient DNA repair, hypoxia, immune evasion, and tumor heterogeneity, as well as adaptive and dynamic responses to therapy. Addressing these challenges requires innovative, multimodal therapeutic strategies to effectively target GBM.

7. Lack of Biomarkers and Personalized Therapy

The lack of reliable biomarkers and personalized therapy options for GBM significantly contributes to treatment failure [23]. As many studies have already described [3,20,22,25,34,73], GBM is one of the most heterogeneous and aggressive malignant tumors, and current treatment protocols mostly rely on a "one-size-fits-all" strategy [100]. This contrast between the complexity of GBM and the available therapeutic approaches highlights the need for biomarker-guided and individualized treatment protocols.

Biomarkers are useful tools that can help in predicting treatment response, guiding potential drug selection, and monitoring disease progression. Unfortunately, for GBM, the identification of reliable biomarkers has been limited [101]. The most widely used biomarker, MGMT promoter methylation, predicts the response to TMZ [83]. Tumors with a methylated MGMT promoter are more likely to respond to TMZ because MGMT, which is a DNA repair enzyme, is silenced, thereby allowing chemotherapy-induced DNA damage to persist. Sadly, even in these cases, responses are not universal, underlining the need for more reliable biomarkers for improved patient stratification. The lack of reliable biomarkers also affects the outcomes of targeted therapies. Without consistent biomarkers to identify the patients most likely to benefit, the potential of these therapies remains unrealized.

The complex inter- and intra-tumoral heterogeneity further complicates biomarker discovery. Different regions within a single tumor can display different biomolecular profiles, including variations in genetic mutations, gene expression, and epigenetic modifications. As a result, a biomarker identified from a single biopsy may not be representative of the entire tumor, which could lead to ineffective treatment [6]. This heterogeneity also

contributes to treatment resistance, as some cell subpopulations may be resistant to targeted therapies or become resistant over time. Moreover, the dynamic nature of GBM also complicates biomarker discovery. GBM cells can undergo significant genetic, epigenetic, and metabolic adaptations, making previously reliable biomarkers obsolete. The lack of biomarkers also affects the success of immunotherapy in GBM. Reliable biomarkers for immunotherapy are either absent or poorly predictive for GBM [102], highlighting the need for novel biomarkers that reflect the unique biology of the tumor and the TME.

Despite many challenges, efforts to identify GBM biomarkers are ongoing. Advances in multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, are at the forefront for discovering biomarkers that can guide personalized therapy [103]. In addition, liquid biopsies are a promising non-invasive approach for biomarker discovery [104]. These, and many other, new approaches could accelerate the development of personalized therapies for GBM. The lack of validated biomarkers and personalized treatment options for GBM highlights the complexity of the disease and is a major reason for treatment failures. By developing biomarker-driven approaches for preclinical studies and clinical practice, it may be possible to develop more effective, personalized treatments for GBM.

8. Conclusions

GBM remains one of the most challenging malignancies to treat due to its aggressive biology, complex heterogeneity, and resistance to therapies. The tumor's genetic, cellular, and microenvironmental heterogeneity significantly contributes to therapeutic failure via the inherent treatment resistance, immune evasion, and adaptive survival mechanisms of GBM cells. Despite advances in therapeutic approaches, GBM cells have robust DNA repair systems, genetic, epigenetic, and metabolic adaptations, and resilience to cytotoxic damage. Additionally, the BBB presents a significant obstacle, limiting drug delivery to the tumor tissue. Immunotherapy, so far, has had no success, largely due to the immunosuppressive TME. Furthermore, the lack of reliable biomarkers and personalized therapeutic approaches is still evident. Addressing these challenges requires a paradigm shift in understanding the pathophysiology of GBM, developing new strategies such as biomarker-guided therapy, BBB-penetrating delivery methods, and immunomodulatory interventions. Given the biology of this tumor, it is improbable to single out a cause for treatment failure. It is likely that all of the factors we have presented, as well as other unknown or unrecognized factors, interact and contribute to treatment resistance. An interesting approach is the development of patient-derived GBM organoids for the development of patient-tailored therapies, as these organoids could reflect the biomolecular complexity of this malignancy [105]. Although these models are unable to capture all of the aforementioned barriers in GBM treatment (BBB, TME, etc.), they would be able to simulate inherent molecular and genetic heterogeneity, which could guide the development of personalized therapies. By leveraging multi-omics approaches, improving clinically relevant outcomes for GBM patients may become achievable. Future efforts must focus on developing patient-tailored therapies while at the same time overcoming the biological and technical obstacles that drive treatment failure and bridge the gap between preclinical studies and clinical outcomes.

Author Contributions: Conceptualization, A.R. and N.K.; methodology, A.S.K.; software, R.R.; validation, R.R.; formal analysis, A.R.; investigation, N.K. and A.S.K.; resources, N.K. and A.S.K.; data curation, A.R. and R.R.; writing—original draft preparation, A.R.; writing—review and editing, A.R., N.K., A.S.K. and R.R.; visualization, A.R.; supervision, N.K.; project administration, A.S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Figure 1 was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Grochans, S.; Cybulska, A.M.; Simińska, D.; Korbecki, J.; Kojder, K.; Chlubek, D.; Baranowska-Bosiacka, I. Epidemiology of Glioblastoma Multiforme-Literature Review. *Cancers* **2022**, *14*, 2412. [CrossRef]
2. Brown, N.F.; Ottaviani, D.; Tazare, J.; Gregson, J.; Kitchen, N.; Brandner, S.; Fersht, N.; Mulholland, P. Survival Outcomes and Prognostic Factors in Glioblastoma. *Cancers* **2022**, *14*, 3161. [CrossRef] [PubMed]
3. Eisenbarth, D.; Wang, Y.A. Glioblastoma heterogeneity at single cell resolution. *Oncogene* **2023**, *42*, 2155–2165. [CrossRef]
4. Pećina-Šlaus, N.; Hrašćan, R. Glioma Stem Cells-Features for New Therapy Design. *Cancers* **2024**, *16*, 1557. [CrossRef] [PubMed]
5. Ahmed, M.H.; Canney, M.; Carpentier, A.; Idbaih, A. Overcoming the blood brain barrier in glioblastoma: Status and future perspective. *Rev. Neurol.* **2023**, *179*, 430–436. [CrossRef]
6. Feldman, L. Hypoxia within the glioblastoma tumor microenvironment: A master saboteur of novel treatments. *Front. Immunol.* **2024**, *15*, 1384249. [CrossRef] [PubMed]
7. Lin, H.; Liu, C.; Hu, A.; Zhang, D.; Yang, H.; Mao, Y. Understanding the immunosuppressive microenvironment of glioma: Mechanistic insights and clinical perspectives. *J. Hematol. Oncol.* **2024**, *17*, 31. [CrossRef]
8. Huang, Y.-F.; Chiao, M.-T.; Hsiao, T.-H.; Zhan, Y.-X.; Chen, T.-Y.; Lee, C.-H.; Liu, S.-Y.; Liao, C.-H.; Cheng, W.-Y.; Yen, C.-M.; et al. Genetic mutation patterns among glioblastoma patients in the Taiwanese population—Insights from a single institution retrospective study. *Cancer Gene Ther.* **2024**, *31*, 894–903. [CrossRef]
9. Gerritsen, J.K.W.; Broekman, M.L.D.; De Vleeschouwer, S.; Schucht, P.; Nahed, B.V.; Berger, M.S.; Vincent, A.J.P.E. Safe surgery for glioblastoma: Recent advances and modern challenges. *Neuro-Oncol. Pract.* **2022**, *9*, 364–379. [CrossRef]
10. Dimou, J.; Beland, B.; Kelly, J. Supramaximal resection: A systematic review of its safety, efficacy and feasibility in glioblastoma. *J. Clin. Neurosci.* **2020**, *72*, 328–334. [CrossRef] [PubMed]
11. Pessina, F.; Navarria, P.; Cozzi, L.; Ascolese, A.M.; Simonelli, M.; Santoro, A.; Clerici, E.; Rossi, M.; Scorsetti, M.; Bello, L. Maximize surgical resection beyond contrast-enhancing boundaries in newly diagnosed glioblastoma multiforme: Is it useful and safe? A single institution retrospective experience. *J. Neuro-Oncol.* **2017**, *135*, 129–139. [CrossRef]
12. Karschnia, P.; Young, J.S.; Dono, A.; Häni, L.; Sciortino, T.; Bruno, F.; Juenger, S.T.; Teske, N.; Morshed, R.A.; Haddad, A.F.; et al. Prognostic validation of a new classification system for extent of resection in glioblastoma: A report of the RANO resect group. *Neuro-Oncol.* **2022**, *25*, 940–954. [CrossRef] [PubMed]
13. Seker-Polat, F.; Pinarbasi Degirmenci, N.; Solaroglu, I.; Bagci-Onder, T. Tumor Cell Infiltration into the Brain in Glioblastoma: From Mechanisms to Clinical Perspectives. *Cancers* **2022**, *14*, 443. [CrossRef]
14. Birzu, C.; French, P.; Caccese, M.; Cerretti, G.; Idbaih, A.; Zagonel, V.; Lombardi, G. Recurrent Glioblastoma: From Molecular Landscape to New Treatment Perspectives. *Cancers* **2020**, *13*, 47. [CrossRef]
15. van Solinge, T.S.; Nieland, L.; Chiocca, E.A.; Broekman, M.L.D. Advances in local therapy for glioblastoma—taking the fight to the tumour. *Nat. Rev. Neurol.* **2022**, *18*, 221–236. [CrossRef] [PubMed]
16. Cha, G.D.; Jung, S.; Choi, S.H.; Kim, D.-H. Local Drug Delivery Strategies for Glioblastoma Treatment. *Brain Tumor Res. Treat.* **2022**, *10*, 151–157. [CrossRef] [PubMed]
17. Hutóczki, G.; Virga, J.; Birkó, Z.; Klekner, A. Novel Concepts of Glioblastoma Therapy Concerning Its Heterogeneity. *Int. J. Mol. Sci.* **2021**, *22*, 10005. [CrossRef] [PubMed]
18. Louis, D.N.; Perry, A.; Wesseling, P.; Brat, D.J.; Cree, I.A.; Figarella-Branger, D.; Hawkins, C.; Ng, H.K.; Pfister, S.M.; Reifenberger, G.; et al. The 2021 WHO Classification of Tumors of the Central Nervous System: A summary. *Neuro Oncol.* **2021**, *23*, 1231–1251. [CrossRef] [PubMed]
19. Torrisi, F.; Alberghina, C.; D'Aprile, S.; Pavone, A.M.; Longhitano, L.; Giallongo, S.; Tibullo, D.; Di Rosa, M.; Zappalà, A.; Cammarata, F.P.; et al. The Hallmarks of Glioblastoma: Heterogeneity, Intercellular Crosstalk and Molecular Signature of Invasiveness and Progression. *Biomedicines* **2022**, *10*, 806. [CrossRef]
20. Lemée, J.-M.; Clavreul, A.; Menei, P. Intratumoral heterogeneity in glioblastoma: Don't forget the peritumoral brain zone. *Neuro-Oncol.* **2015**, *17*, 1322–1332. [CrossRef] [PubMed]
21. Oliver, L.; Lalier, L.; Salaud, C.; Heymann, D.; Cartron, P.F.; Vallette, F.M. Drug resistance in glioblastoma: Are persisters the key to therapy? *Cancer Drug Resist.* **2020**, *3*, 287–301. [CrossRef]

22. Gempt, J.; Withake, F.; Aftahy, A.K.; Meyer, H.S.; Barz, M.; Delbridge, C.; Liesche-Starnecker, F.; Prokop, G.; Pfarr, N.; Schlegel, J.; et al. Methylation subgroup and molecular heterogeneity is a hallmark of glioblastoma: Implications for biopsy targeting, classification and therapy. *ESMO Open* **2022**, *7*, 100566. [CrossRef]
23. Rončević, A.; Koruga, N.; Soldo Koruga, A.; Rončević, R.; Rotim, T.; Šimundić, T.; Kretić, D.; Perić, M.; Turk, T.; Štimac, D. Personalized Treatment of Glioblastoma: Current State and Future Perspective. *Biomedicines* **2023**, *11*, 1579. [CrossRef] [PubMed]
24. Badodi, S.; Marino, S. Epigenetic mechanisms in paediatric brain tumours: Regulators lose control. *Biochem. Soc. Trans.* **2022**, *50*, 167–185. [CrossRef] [PubMed]
25. Lucchini, S.; Constantinou, M.; Marino, S. Unravelling the mosaic: Epigenetic diversity in glioblastoma. *Mol. Oncol.* **2024**, *18*, 2871–2889. [CrossRef]
26. Suter, R.K.; Rodriguez-Blanco, J.; Ayad, N.G. Epigenetic pathways and plasticity in brain tumors. *Neurobiol. Dis.* **2020**, *145*, 105060. [CrossRef] [PubMed]
27. Klughammer, J.; Kiesel, B.; Roetzer, T.; Fortelny, N.; Nemc, A.; Nenning, K.-H.; Furtner, J.; Sheffield, N.C.; Datlinger, P.; Peter, N.; et al. The DNA methylation landscape of glioblastoma disease progression shows extensive heterogeneity in time and space. *Nat. Med.* **2018**, *24*, 1611–1624. [CrossRef]
28. Waqar, M.; Van Houdt, P.J.; Hessen, E.; Li, K.-L.; Zhu, X.; Jackson, A.; Iqbal, M.; O'Connor, J.; Djoukhadar, I.; van der Heide, U.A.; et al. Visualising spatial heterogeneity in glioblastoma using imaging habitats. *Front. Oncol.* **2022**, *12*, 1037896. [CrossRef] [PubMed]
29. Greenwald, A.C.; Darnell, N.G.; Hoefflin, R.; Simkin, D.; Mount, C.W.; Gonzalez Castro, L.N.; Harnik, Y.; Dumont, S.; Hirsch, D.; Nomura, M.; et al. Integrative spatial analysis reveals a multi-layered organization of glioblastoma. *Cell* **2024**, *187*, 2485–2501.e2426. [CrossRef] [PubMed]
30. Mosteiro, A.; Pedrosa, L.; Ferrés, A.; Diao, D.; Sierra, À.; González, J.J. The Vascular Microenvironment in Glioblastoma: A Comprehensive Review. *Biomedicines* **2022**, *10*, 1285. [CrossRef] [PubMed]
31. Ziello, J.E.; Jovin, I.S.; Huang, Y. Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J. Biol. Med.* **2007**, *80*, 51–60. [PubMed]
32. Domènech, M.; Hernández, A.; Plaja, A.; Martínez-Balibrea, E.; Balañà, C. Hypoxia: The Cornerstone of Glioblastoma. *Int. J. Mol. Sci.* **2021**, *22*, 12608. [CrossRef] [PubMed]
33. Warburg, O. The metabolism of carcinoma cells. *J. Cancer Res.* **1925**, *9*, 148–163. [CrossRef]
34. Badr, C.E.; Silver, D.J.; Siebzehnrubl, F.A.; Deleyrolle, L.P. Metabolic heterogeneity and adaptability in brain tumors. *Cell. Mol. Life Sci.* **2020**, *77*, 5101–5119. [CrossRef]
35. Bi, J.; Chowdhry, S.; Wu, S.; Zhang, W.; Masui, K.; Mischel, P.S. Altered cellular metabolism in gliomas—An emerging landscape of actionable co-dependency targets. *Nat. Rev. Cancer* **2020**, *20*, 57–70. [CrossRef]
36. Hoang-Minh, L.B.; Siebzehnrubl, F.A.; Yang, C.; Suzuki-Hatano, S.; Dajac, K.; Loche, T.; Andrews, N.; Schmoll Massari, M.; Patel, J.; Amin, K.; et al. Infiltrative and drug-resistant slow-cycling cells support metabolic heterogeneity in glioblastoma. *EMBO J.* **2018**, *37*, e98772. [CrossRef]
37. Strickland, M.; Stoll, E.A. Metabolic Reprogramming in Glioma. *Front. Cell Dev. Biol.* **2017**, *5*, 43. [CrossRef] [PubMed]
38. Bernhard, C.; Reita, D.; Martin, S.; Entz-Werle, N.; Dontenwill, M. Glioblastoma Metabolism: Insights and Therapeutic Strategies. *Int. J. Mol. Sci.* **2023**, *24*, 9137. [CrossRef]
39. Kang, W.; Mo, Z.; Li, W.; Ma, H.; Zhang, Q. Heterogeneity and individualized treatment of microenvironment in glioblastoma (Review). *Oncol. Rep.* **2023**, *50*, 217. [CrossRef]
40. Xie, X.P.; Ganbold, M.; Li, J.; Lien, M.; Chipman, M.E.; Wang, T.; Jayewickreme, C.D.; Pedraza, A.M.; Bale, T.; Tabar, V.; et al. Glioblastoma functional heterogeneity and enrichment of cancer stem cells with tumor recurrence. *Neuron* **2024**, *112*, 4017–4032.e4016. [CrossRef] [PubMed]
41. Rončević, A.; Koruga, N.; Soldo Koruga, A.; Debeljak, Ž.; Rončević, R.; Turk, T.; Kretić, D.; Rotim, T.; Krivdić Dupan, Z.; Troha, D.; et al. MALDI Imaging Mass Spectrometry of High-Grade Gliomas: A Review of Recent Progress and Future Perspective. *Curr. Issues Mol. Biol.* **2023**, *45*, 838–851. [CrossRef]
42. Martinez-Lage, M.; Lynch, T.M.; Bi, Y.; Cocito, C.; Way, G.P.; Pal, S.; Haller, J.; Yan, R.E.; Ziober, A.; Nguyen, A.; et al. Immune landscapes associated with different glioblastoma molecular subtypes. *Acta Neuropathol. Commun.* **2019**, *7*, 203. [CrossRef] [PubMed]
43. Proulx, S.T.; Engelhardt, B. Central nervous system zoning: How brain barriers establish subdivisions for CNS immune privilege and immune surveillance. *J. Intern. Med.* **2022**, *292*, 47–67. [CrossRef] [PubMed]
44. Pombo Antunes, A.R.; Scheyltjens, I.; Duerinck, J.; Neyns, B.; Movahedi, K.; Van Ginderachter, J.A. Understanding the glioblastoma immune microenvironment as basis for the development of new immunotherapeutic strategies. *eLife* **2020**, *9*, e52176. [CrossRef]
45. Sharma, P.; Aaroe, A.; Liang, J.; Puduvalli, V.K. Tumor microenvironment in glioblastoma: Current and emerging concepts. *Neuro-Oncol. Adv.* **2023**, *5*, vdad009. [CrossRef]

46. Bikfalvi, A.; da Costa, C.A.; Avril, T.; Barnier, J.-V.; Bauchet, L.; Brisson, L.; Cartron, P.F.; Castel, H.; Chevet, E.; Chneiweiss, H.; et al. Challenges in glioblastoma research: Focus on the tumor microenvironment. *Trends Cancer* **2023**, *9*, 9–27. [CrossRef]
47. Himes, B.T.; Geiger, P.A.; Ayasoufi, K.; Bhargav, A.G.; Brown, D.A.; Parney, I.F. Immunosuppression in Glioblastoma: Current Understanding and Therapeutic Implications. *Front. Oncol.* **2021**, *11*, 770561. [CrossRef] [PubMed]
48. Andersen, R.S.; Anand, A.; Harwood, D.S.L.; Kristensen, B.W. Tumor-Associated Microglia and Macrophages in the Glioblastoma Microenvironment and Their Implications for Therapy. *Cancers* **2021**, *13*, 4255. [CrossRef] [PubMed]
49. Charles, N.A.; Holland, E.C.; Gilbertson, R.; Glass, R.; Kettenmann, H. The brain tumor microenvironment. *Glia* **2011**, *59*, 1169–1180. [CrossRef] [PubMed]
50. Puchalski, R.B.; Shah, N.; Miller, J.; Dalley, R.; Nomura, S.R.; Yoon, J.-G.; Smith, K.A.; Lankerovich, M.; Bertagnolli, D.; Bickley, K.; et al. An anatomic transcriptional atlas of human glioblastoma. *Science* **2018**, *360*, 660–663. [CrossRef] [PubMed]
51. Tamura, R.; Ohara, K.; Sasaki, H.; Morimoto, Y.; Kosugi, K.; Yoshida, K.; Toda, M. Difference in immunosuppressive cells between peritumoral area and tumor core in glioblastoma. *World Neurosurg.* **2018**, *120*, e601–e610. [CrossRef]
52. Darmanis, S.; Sloan, S.A.; Croote, D.; Mignardi, M.; Chernikova, S.; Samghababi, P.; Zhang, Y.; Neff, N.; Kowarsky, M.; Caneda, C.; et al. Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front of Human Glioblastoma. *Cell Rep.* **2017**, *21*, 1399–1410. [CrossRef] [PubMed]
53. Khan, F.; Pang, L.; Dunterman, M.; Lesniak, M.S.; Heimberger, A.B.; Chen, P. Macrophages and microglia in glioblastoma: Heterogeneity, plasticity, and therapy. *J. Clin. Investig.* **2023**, *133*, e163446. [CrossRef]
54. Ravi, V.M.; Neidert, N.; Will, P.; Joseph, K.; Maier, J.P.; Kückelhaus, J.; Vollmer, L.; Goeldner, J.M.; Behringer, S.P.; Scherer, F.; et al. T-cell dysfunction in the glioblastoma microenvironment is mediated by myeloid cells releasing interleukin-10. *Nat. Commun.* **2022**, *13*, 925. [CrossRef]
55. Losurdo, A.; Di Muzio, A.; Cianciotti, B.C.; Dipasquale, A.; Persico, P.; Barigazzi, C.; Bono, B.; Feno, S.; Pessina, F.; Santoro, A.; et al. T Cell Features in Glioblastoma May Guide Therapeutic Strategies to Overcome Microenvironment Immunosuppression. *Cancers* **2024**, *16*, 603. [CrossRef] [PubMed]
56. Watowich, M.B.; Gilbert, M.R.; Larion, M. T cell exhaustion in malignant gliomas. *Trends Cancer* **2023**, *9*, 270–292. [CrossRef]
57. Sonabend, A.M.; Rolle, C.E.; Lesniak, M.S. The Role of Regulatory T Cells in Malignant Glioma. *Anticancer Res.* **2008**, *28*, 1143–1150. [PubMed]
58. Guan, Z.; Lan, H.; Cai, X.; Zhang, Y.; Liang, A.; Li, J. Blood-Brain Barrier, Cell Junctions, and Tumor Microenvironment in Brain Metastases, the Biological Prospects and Dilemma in Therapies. *Front. Cell Dev. Biol.* **2021**, *9*, 722917. [CrossRef] [PubMed]
59. Park, J.H.; Lee, H.K. Current Understanding of Hypoxia in Glioblastoma Multiforme and Its Response to Immunotherapy. *Cancers* **2022**, *14*, 1176. [CrossRef]
60. Bova, V.; Filippone, A.; Casili, G.; Lanza, M.; Campolo, M.; Capra, A.P.; Repici, A.; Crupi, L.; Motta, G.; Colarossi, C.; et al. Adenosine Targeting as a New Strategy to Decrease Glioblastoma Aggressiveness. *Cancers* **2022**, *14*, 4032. [CrossRef] [PubMed]
61. Churov, A.; Zhulai, G. Targeting adenosine and regulatory T cells in cancer immunotherapy. *Hum. Immunol.* **2021**, *82*, 270–278. [CrossRef]
62. Miyazaki, T.; Moritake, K.; Yamada, K.; Hara, N.; Osago, H.; Shibata, T.; Akiyama, Y.; Tsuchiya, M. Indoleamine 2,3-dioxygenase as a new target for malignant glioma therapy. *J. Neurosurg.* **2009**, *111*, 230–237. [CrossRef]
63. Rocha Pinheiro, S.L.; Lemos, F.F.B.; Marques, H.S.; Silva Luz, M.; de Oliveira Silva, L.G.; Faria Souza Mendes Dos Santos, C.; da Costa Evangelista, K.; Calmon, M.S.; Sande Loureiro, M.; Freire de Melo, F. Immunotherapy in glioblastoma treatment: Current state and future prospects. *World J. Clin. Oncol.* **2023**, *14*, 138–159. [CrossRef]
64. Abbott, N.J.; Patabendige, A.A.K.; Dolman, D.E.M.; Yusof, S.R.; Begley, D.J. Structure and function of the blood–brain barrier. *Neurobiol. Dis.* **2010**, *37*, 13–25. [CrossRef]
65. Arvanitis, C.D.; Ferraro, G.B.; Jain, R.K. The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. *Nat. Rev. Cancer* **2020**, *20*, 26–41. [CrossRef] [PubMed]
66. Ostermann, S.; Csajka, C.; Buclin, T.; Leyvraz, S.; Lejeune, F.; Decosterd, L.A.; Stupp, R. Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients. *Clin. Cancer Res.* **2004**, *10*, 3728–3736. [CrossRef]
67. Wolburg, H.; Noell, S.; Fallier-Becker, P.; Mack, A.F.; Wolburg-Buchholz, K. The disturbed blood–brain barrier in human glioblastoma. *Mol. Asp. Med.* **2012**, *33*, 579–589. [CrossRef]
68. Hempel, C.; Johnsen, K.B.; Kostrikov, S.; Hamerlik, P.; Andresen, T.L. Brain tumor vessels—A barrier for drug delivery. *Cancer Metastasis Rev.* **2020**, *39*, 959–968. [CrossRef] [PubMed]
69. Luo, H.; Shusta, E.V. Blood–Brain Barrier Modulation to Improve Glioma Drug Delivery. *Pharmaceutics* **2020**, *12*, 1085. [CrossRef] [PubMed]
70. Brighi, C.; Salimova, E.; de Veer, M.; Puttick, S.; Egan, G. Translation of focused ultrasound for blood-brain barrier opening in glioma. *J. Control. Release* **2022**, *345*, 443–463. [CrossRef]
71. Rolfe, N.W.; Dadario, N.B.; Canoll, P.; Bruce, J.N. A Review of Therapeutic Agents Given by Convection-Enhanced Delivery for Adult Glioblastoma. *Pharmaceuticals* **2024**, *17*, 973. [CrossRef] [PubMed]

72. Stupp, R.; Mason, W.P.; van den Bent, M.J.; Weller, M.; Fisher, B.; Taphoorn, M.J.; Belanger, K.; Brandes, A.A.; Marosi, C.; Bogdahn, U.; et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **2005**, *352*, 987–996. [CrossRef] [PubMed]
73. Cantidio, F.S.; Gil, G.O.B.; Queiroz, I.N.; Regalin, M. Glioblastoma—Treatment and obstacles. *Rep. Pract. Oncol. Radiother.* **2022**, *27*, 744–753. [CrossRef] [PubMed]
74. Li, R.; Wang, H.; Liang, Q.; Chen, L.; Ren, J. Radiotherapy for glioblastoma: Clinical issues and nanotechnology strategies. *Biomater. Sci.* **2022**, *10*, 892–908. [CrossRef] [PubMed]
75. Wu, Y.; Song, Y.; Wang, R.; Wang, T. Molecular mechanisms of tumor resistance to radiotherapy. *Mol. Cancer* **2023**, *22*, 96. [CrossRef]
76. Meng, W.; Palmer, J.D.; Siedow, M.; Haque, S.J.; Chakravarti, A. Overcoming Radiation Resistance in Gliomas by Targeting Metabolism and DNA Repair Pathways. *Int. J. Mol. Sci.* **2022**, *23*, 2246. [CrossRef] [PubMed]
77. Chédeville, A.L.; Madureira, P.A. The Role of Hypoxia in Glioblastoma Radiotherapy Resistance. *Cancers* **2021**, *13*, 542. [CrossRef] [PubMed]
78. Shen, H.; Cook, K.; Gee, H.E.; Hau, E. Hypoxia, metabolism, and the circadian clock: New links to overcome radiation resistance in high-grade gliomas. *J. Exp. Clin. Cancer Res.* **2020**, *39*, 129. [CrossRef]
79. Kabakov, A.E.; Yakimova, A.O. Hypoxia-Induced Cancer Cell Responses Driving Radioresistance of Hypoxic Tumors: Approaches to Targeting and Radiosensitizing. *Cancers* **2021**, *13*, 1102. [CrossRef] [PubMed]
80. Arnold, C.R.; Mangesius, J.; Skvortsova, I.-I.; Ganswindt, U. The Role of Cancer Stem Cells in Radiation Resistance. *Front. Oncol.* **2020**, *10*, 164. [CrossRef]
81. Singh, N.; Miner, A.; Hennis, L.; Mittal, S. Mechanisms of temozolomide resistance in glioblastoma—A comprehensive review. *Cancer Drug Resist.* **2021**, *4*, 17–43. [CrossRef] [PubMed]
82. Friedman, H.S.; Kerby, T.; Calvert, H. Temozolomide and treatment of malignant glioma. *Clin. Cancer Res.* **2000**, *6*, 2585–2597.
83. Hegi, M.E.; Diserens, A.C.; Gorlia, T.; Hamou, M.F.; de Tribolet, N.; Weller, M.; Kros, J.M.; Hainfellner, J.A.; Mason, W.; Mariani, L.; et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* **2005**, *352*, 997–1003. [CrossRef] [PubMed]
84. Leske, H.; Camenisch Gross, U.; Hofer, S.; Neidert, M.C.; Leske, S.; Weller, M.; Lehnick, D.; Rushing, E.J. MGMT methylation pattern of long-term and short-term survivors of glioblastoma reveals CpGs of the enhancer region to be of high prognostic value. *Acta Neuropathol. Commun.* **2023**, *11*, 139. [CrossRef] [PubMed]
85. Mason, W.P. Blood-brain barrier-associated efflux transporters: A significant but underappreciated obstacle to drug development in glioblastoma. *Neuro-Oncol.* **2015**, *17*, 1181–1182. [CrossRef]
86. Bailleul, J.; Vlashi, E. Glioblastomas: Hijacking Metabolism to Build a Flexible Shield for Therapy Resistance. *Antioxid. Redox Signal.* **2023**, *39*, 957–979. [CrossRef] [PubMed]
87. Liu, Y.; Zhou, F.; Ali, H.; Lathia, J.D.; Chen, P. Immunotherapy for glioblastoma: Current state, challenges, and future perspectives. *Cell. Mol. Immunol.* **2024**, *21*, 1354–1375. [CrossRef] [PubMed]
88. Xin Yu, J.; Hubbard-Lucey, V.M.; Tang, J. Immuno-oncology drug development goes global. *Nat. Rev. Drug Discov.* **2019**, *18*, 899–900. [CrossRef]
89. Ser, M.H.; Webb, M.J.; Sener, U.; Campian, J.L. Immune Checkpoint Inhibitors and Glioblastoma: A Review on Current State and Future Directions. *J. Immunother. Precis. Oncol.* **2024**, *7*, 97–110. [CrossRef] [PubMed]
90. Hodges, T.R.; Ott, M.; Xiu, J.; Gatalica, Z.; Swensen, J.; Zhou, S.; Huse, J.T.; de Groot, J.; Li, S.; Overwijk, W.W.; et al. Mutational burden, immune checkpoint expression, and mismatch repair in glioma: Implications for immune checkpoint immunotherapy. *Neuro Oncol.* **2017**, *19*, 1047–1057. [CrossRef]
91. Samstein, R.M.; Lee, C.H.; Shoushtari, A.N.; Hellmann, M.D.; Shen, R.; Janjigian, Y.Y.; Barron, D.A.; Zehir, A.; Jordan, E.J.; Omuro, A.; et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat. Genet.* **2019**, *51*, 202–206. [CrossRef] [PubMed]
92. Zhang, J.; Caruso, F.P.; Sa, J.K.; Justesen, S.; Nam, D.H.; Sims, P.; Ceccarelli, M.; Lasorella, A.; Iavarone, A. The combination of neoantigen quality and T lymphocyte infiltrates identifies glioblastomas with the longest survival. *Commun. Biol.* **2019**, *2*, 135. [CrossRef]
93. Luksik, A.S.; Yazigi, E.; Shah, P.; Jackson, C.M. CAR T Cell Therapy in Glioblastoma: Overcoming Challenges Related to Antigen Expression. *Cancers* **2023**, *15*, 1414. [CrossRef] [PubMed]
94. Barish, M.E.; Weng, L.; Awabdeh, D.; Zhai, Y.; Starr, R.; D'Apuzzo, M.; Rockne, R.C.; Li, H.; Badie, B.; Forman, S.J.; et al. Spatial organization of heterogeneous immunotherapy target antigen expression in high-grade glioma. *Neoplasia* **2022**, *30*, 100801. [CrossRef] [PubMed]
95. Yeo, E.C.F.; Brown, M.P.; Gargett, T.; Ebert, L.M. The Role of Cytokines and Chemokines in Shaping the Immune Microenvironment of Glioblastoma: Implications for Immunotherapy. *Cells* **2021**, *10*, 607. [CrossRef]

96. Hou, D.; Castro, B.; Dapash, M.; Zolp, A.; Katz, J.; Arrieta, V.; Biermann, J.; Melms, J.; Kueckelhaus, J.; Benotmane, J.; et al. B-cells Drive Response to PD-1 Blockade in Glioblastoma Upon Neutralization of TGFβ-mediated Immunosuppression. *Res. Sq.* **2023**. [CrossRef]
97. Hou, D.; Wan, H.; Katz, J.L.; Wang, S.; Castro, B.A.; Vazquez-Cervantes, G.I.; Arrieta, V.A.; Dhiantravan, S.; Najem, H.; Rashidi, A.; et al. Antigen-presenting B cells promote TCF-1(+) PD1(−) stem-like CD8(+) T-cell proliferation in glioblastoma. *Front. Immunol.* **2023**, *14*, 1295218. [CrossRef] [PubMed]
98. Motaln, H.; Koren, A.; Gruden, K.; Ramšak, Ž.; Schichor, C.; Lah, T.T. Heterogeneous glioblastoma cell cross-talk promotes phenotype alterations and enhanced drug resistance. *Oncotarget* **2015**, *6*, 40998–41017. [CrossRef]
99. Neftel, C.; Laffy, J.; Filbin, M.G.; Hara, T.; Shore, M.E.; Rahme, G.J.; Richman, A.R.; Silverbush, D.; Shaw, M.L.; Hebert, C.M.; et al. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. *Cell* **2019**, *178*, 835–849.e821. [CrossRef] [PubMed]
100. Janjua, T.I.; Rewatkar, P.; Ahmed-Cox, A.; Saeed, I.; Mansfeld, F.M.; Kulshreshtha, R.; Kumeria, T.; Ziegler, D.S.; Kavallaris, M.; Mazzieri, R.; et al. Frontiers in the treatment of glioblastoma: Past, present and emerging. *Adv. Drug Deliv. Rev.* **2021**, *171*, 108–138. [CrossRef] [PubMed]
101. Tataranu, L.G.; Turliuc, S.; Rizea, R.E.; Dricu, A.; Alexandru, O.; Staicu, G.-A.; Kamel, A. A Synopsis of Biomarkers in Glioblastoma: Past and Present. *Curr. Issues Mol. Biol.* **2024**, *46*, 6903–6939. [CrossRef] [PubMed]
102. Lynes, J.P.; Nwankwo, A.K.; Sur, H.P.; Sanchez, V.E.; Sarpong, K.A.; Ariyo, O.I.; Dominah, G.A.; Nduom, E.K. Biomarkers for immunotherapy for treatment of glioblastoma. *J. Immunother. Cancer* **2020**, *8*, e000348. [CrossRef] [PubMed]
103. Koh, L.; Novera, W.; Lim, S.W.; Chong, Y.K.; Pang, Q.Y.; Low, D.; Ang, B.T.; Tang, C. Integrative multi-omics approach to targeted therapy for glioblastoma. *Pharmacol. Res.* **2022**, *182*, 106308. [CrossRef] [PubMed]
104. Ronvaux, L.; Riva, M.; Coosemans, A.; Herzog, M.; Rommelaere, G.; Donis, N.; D'Hondt, L.; Douxfils, J. Liquid Biopsy in Glioblastoma. *Cancers* **2022**, *14*, 3394. [CrossRef]
105. Xu, C.; Yuan, X.; Hou, P.; Li, Z.; Wang, C.; Fang, C.; Tan, Y. Development of glioblastoma organoids and their applications in personalized therapy. *Cancer Biol. Med.* **2023**, *20*, 353–368. [CrossRef] [PubMed]