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Review

Cellular Plasticity of Glioma Stem Cells—A Small Population That Poses a Major Therapeutic Challenge

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Highlights

What are the main findings?

- Glioma stem cells represent dynamic, interconverting states rather than fixed populations; their plasticity determines tumor aggressiveness and therapeutic resistance;
- In IDH-mutant gliomas, the stem cell compartment is compact and epigenetically ordered, whereas in IDH-wild-type glioblastomas it is plastic, multi-state, and prone to a mesenchymal drift that drives recurrence.

What are the implications of the main findings?

- Assessment of glioma stem cell composition should be incorporated into diagnosis and therapeutic decision-making, since current classifications do not adequately reflect the true complexity of the subtypes.

Abstract

Despite decades of intensive research, the prognosis for patients with high-grade gliomas, particularly glioblastoma, has improved only marginally. The exceptional malignancy of these tumors is attributable to glioma stem cells (GSCs)—a rare but highly adaptive pool of tumor cells implicated in tumor initiation, invasion, therapeutic resistance, and recurrence. However, GSCs do not constitute a single population: they exist as dynamically interconverting molecular, transcriptional, and metabolic states that differ in the balance between proliferative activity and quiescence. This review integrates current understanding of the uncertain cellular origin of high-grade gliomas with a multilayered analysis of GSC heterogeneity and plasticity. Particular attention is paid to the extreme plasticity of distinct GSC states and their mutual transitions under the influence of the microenvironment and therapeutic pressure. We also discuss therapeutic approaches targeting GSCs and analyze their prospects.

Keywords: glioma stem cells; glioblastoma; tumor heterogeneity; cellular plasticity; cell death; tumor recurrence; immunotherapy

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1. Introduction

Glioblastoma remains one of the most lethal solid tumors in adults, despite aggressive standard therapy. The median survival is approximately 14–16 months, and the incidence is 2–3 cases per 100,000 people. Recurrence is virtually inevitable, with long-term survival in

less than 5% of patients. The gap between the initial response to therapy and the inevitable fatal recurrence defines the central problem of this field of neuro-oncology.

Glioblastoma is one of the tumors classified as high-grade gliomas according to the World Health Organization 2021 classification (WHO 2021). This group includes glioblastoma (grade 4), astrocytoma (grades 3 and 4), and oligodendroglioma (grade 3). These histological tumor types differ substantially in their molecular genetic profiles. Oligodendrogliomas are characterized by mutations in the IDH1/2 genes in combination with complete codeletion of the 1p/19q chromosomal arms, which is often accompanied by mutations in the TERT gene promoter, as well as alterations in the CIC and FUBP1 genes. Astrocytomas also carry IDH1/2 mutations but lack 1p/19q codeletion; their key molecular markers are mutations in the TP53 gene and loss of ATRX expression. Glioblastomas, represented by tumors with wild-type IDH genes (IDHwt), possess the most aggressive molecular phenotype, which is characterized by TERT promoter mutations, EGFR gene amplification, PTEN deletion/inactivation, along with chromosomal aberrations (gain/disomy of chromosome 7 and deletion of chromosome 10). According to the current World Health Organization Classification of Tumors of the Central Nervous System (WHO CNS5), the term "IDH-mutant glioblastoma" has been completely excluded from medical nomenclature. Biologically, such tumors exist, but since 2021 they have been designated differently, and this diagnosis often results from confusion. If a patient is found to have a diffuse astrocytic glioma of grade 4 malignancy (with necrosis, microvascular proliferation, or homozygous CDKN2A/B deletion), but the tumor harbors an IDH1 or IDH2 mutation, it is now officially assigned the diagnosis "Astrocytoma, IDH-mutant, WHO CNS5 grade 4".

The development of therapy for high-grade gliomas has reached an impasse: chemotherapy directed at the proliferating tumor mass does not provide long-term control. Temozolomide (TMZ), the first-line chemotherapy agent, is administered as oral capsules that the patient can take at home. Temozolomide administration begins simultaneously with the first session of radiotherapy, usually 2–6 weeks after surgery, as a course lasting 8–9 months. The principal mechanism of the antitumor action of TMZ in glioblastomas is the induction of cytotoxic DNA damage through methylation of the O^6-position of guanine. The effectiveness of this process is limited by the activity of the repair enzyme MGMT, whose expression is variable within the structure of gliomas. Hypermethylation of the MGMT gene promoter leads to its epigenetic inactivation, which is associated with a reduction in the reparative potential of the tumor and a significant increase in its sensitivity to TMZ-based alkylating chemotherapy.

Unfortunately, more than 50% of patients have or develop resistance to treatment [1]. If the MGMT gene promoter is unmethylated, the tumor continuously synthesizes the enzyme of the same name. It instantly removes the methyl groups introduced by temozolomide, which substantially limits the therapeutic effect [2,3]. In addition to MGMT, the mismatch repair (MMR) system and the base excision repair (BER) system play an important role. Paradoxically, defects in the MMR system can cause the cell to become unresponsive to damage induced by temozolomide and to fail to undergo apoptosis (self-destruction), whereas the BER system successfully repairs other methylation sites (N3-adenine and N7-guanine) [4]. Third, efflux mediated by ABC transporters, chiefly ABCB1/P-glycoprotein and ABCG2/BCRP, may also contribute to TMZ resistance: both are highly expressed at the blood–brain and blood–tumor barriers and in glioblastoma cells, where they limit intratumoral drug exposure [5,6]. Their combined loss or inhibition markedly increases TMZ brain penetration and efficacy in orthotopic models [7], ABCB1 knockout restores chemosensitivity, and ABCG2 sustains glioma side populations [8,9]. The clinical relevance of this mechanism, however, remains incompletely defined and likely context-dependent. Fourth, the high heterogeneity of glioma cells means that only a portion of the cells can

"respond" to TMZ therapy. This leads to the question of cellular hierarchy within the tumor. Apparently, it is necessary to take into account the presence of a rare subpopulation of cells responsible for tumor initiation, maintenance of growth, and recurrence. According to current concepts, this population possesses stem cell characteristics and has innate resistance to chemotherapy. Glioma stem cells (GSCs) effectively cope with oxidative stress and alter energy metabolism, which allows them to survive the effects of temozolomide and repopulate the tumor.

Indeed, the cellular heterogeneity of glioblastoma is mainly driven by glioma stem cells, a unique population of cancer stem cells. The tumor is not maintained equally by all tumor cells, but by a distinct subpopulation possessing self-renewal capacity, multipotency, and high tumorigenicity. Although GSCs may initially originate from neural stem cells or progenitor cells acquiring oncogenic mutations, emerging evidence demonstrates that non-stem tumor cells can undergo dedifferentiation and acquire stem-like properties. This bidirectional plasticity—whereby differentiated cells can return to a stem-like state—further complicates the hierarchical model and underscores the dynamic nature of "stemness" in glioblastomas.

In this review, we examine the extent to which the GSC hypothesis explains glioma biology and, critically, what it offers for clinical practice. We discuss how current knowledge of the cellular composition of high-grade gliomas is being applied to diagnosis, patient stratification, and the development of personalized therapeutic strategies that go beyond the limitations of conventional cytotoxic approaches.

2. The Enigma of the Origin of High-Grade Gliomas

Glioblastoma is a grade 4 malignant brain tumor that differs from other cancers in that it almost never spreads beyond the brain, yet grows and invades surrounding tissues at an extreme rate. Rather than forming a well-defined solid tumor that is easy to remove, glioblastoma infiltrates deeply into healthy brain tissue, and for this reason it is practically impossible to remove it completely by surgery. It very rapidly creates its own blood vessels for nourishment and forms areas of necrosis within itself. Glioblastomas are also extremely heterogeneous and recur rapidly with the emergence of new tumor phenotypes.

The vast majority of glioblastomas (approximately 90% of cases) are primary glioblastomas, which develop rapidly de novo without radiological or histological evidence of a pre-existing less malignant precursor lesion [10–12]. About 10% of cases correspond to secondary glioblastomas, which progress from lower-grade gliomas. Although both types of glioblastoma are histologically indistinguishable, secondary glioblastomas are unequivocally characterized by the presence of mutations in the IDH1 gene (isocitrate dehydrogenase), in contrast to IDHwt glioblastomas. According to WHO 2021, all glioblastomas are IDHwt. Grade 3 and 4 astrocytomas and grade 3 oligodendrogliomas are high-grade gliomas with IDH mutations.

Defining the glioblastoma cell of origin is of key importance for understanding the etiology of the disease, as well as for developing early prognostic markers and preventive therapeutic approaches. The question of the cell of origin of wild-type IDH glioblastoma is particularly acute: this subtype develops spontaneously, without preceding pathological conditions, which gives rise to considerable disagreement in the scientific community. The cellular heterogeneity of glioblastomas is due, at least in part, to the existence of a unique population of stem cells known as glioma stem cells. Today it is believed that this distinctive subpopulation of cells makes a substantial contribution to the key aggressive characteristics of glioblastoma, such as angiogenesis and invasion, and is one of the key contributors to tumor recurrence and resistance to therapy [13,14]. In the study [15], the authors presented direct genetic evidence that glioblastoma develops from astrocyte-like neural stem cells in

the subventricular zone (SVZ). The authors confirmed this theory using deep sequencing of patient tissues, single-cell analysis, and modeling of mutations using the CRISPR/Cas9 system in mice. Apparently, under mutational burden, neuronal progenitors give rise to glioma stem cells, and these, already within the tumor focus, supply the tumor with new differentiated glioma cells [16].

The existence of cancer stem cells in gliomas has been demonstrated in adults [17–20] and in children [21,22]. Several characteristics of glioma stem cells support the hypothesis that these cells contribute to recurrence as well [23]. Two important lines of evidence support this. First, even a small population of GSCs is capable of reproducing the heterogeneous structure of the original glioblastoma, which directly models the process of recurrence after treatment [24]. Second, unlike rapidly dividing cells, which are targeted by most cytostatic agents and radiation, glioma stem cells are in a quiescent (slow-dividing) state. This makes them less accessible to agents that damage DNA during mitosis [14,25–28]. Recurrent tumors often differ genetically from the original tumor, having distinct drivers and sensitivity, which limits the informativeness of primary biopsies in the treatment of recurrent disease [29] and also underscores the rapid "intratumoral evolution" of glioma cell phenotypes. In addition, glioma stem cells stimulate angiogenesis, supporting the development of the tumor focus, which underscores the important contribution of this population to the malignancy of the disease [13].

Gimple et al. [23] propose the following definition of glioblastoma stem cells: glioma cells capable of self-renewal that initiate tumors upon serial transplantation and reproduce the heterogeneity of the tumor focus. The authors emphasize that difficulties exist in formulating the definition and that there are disagreements regarding the term. However, cancer stem cells can be definitively identified only after an experiment that alters the state of the original cell, which precludes prospective identification of GSCs using cell surface or other cellular markers.

However, it appears that different subtypes of high-grade gliomas do originate from different cells. Neural stem cells (NSCs) of the subventricular zone are the most convincingly demonstrated source for IDH wild-type glioblastoma grade 4 (glioma stem cells that form glioblastoma) [15]. For high-grade astrocytomas (grades 3, 4) and oligodendrogliomas (grade 3) or "secondary" (progressive, evolving) glioblastoma, which develop from a pre-existing low-grade astrocytoma or oligodendroglioma, the presumed source is more differentiated glial progenitors, namely oligodendrocyte progenitor cells (OPCs) or the glial progenitor cell (GPC) lineage [30]. The key event here is considered to be a mutation in the IDH1 gene, and the origin of these tumors is not associated with the subventricular zone.

Glioma stem cells represent the cells most likely to persist under standard treatment, and these cells appear to exhibit the greatest tumorigenic capacity. They are capable of generating multiple cell populations, with each clone primed for therapy resistance in various ways. Cells that survive therapy are "selected" (as in natural selection) as the most fit for tumor formation, survival in an aggressive environment, and retention of the most malignant characteristics. It is therefore not surprising that patients with tumors containing a large number of GSCs may have a worse prognosis.

The debate is far from settled [31], but recent data indicate that neural stem cells (and/or neural progenitors) are the main candidates for the role of the cell of origin of glioblastoma, whereas the oligodendrocyte progenitor cell gives rise to IDH-mutant forms of high-grade gliomas.

3. Decoding Glioma Stem Cells: Markers, Heterogeneity, and Functional Plasticity

3.1. Diversity of Glioma Stem-like Cell Populations

The concept of glioma stem cells quickly gained an important place in neuro-oncology, as it opened up possibilities for explaining the two most clinically significant aggressive properties of high-grade gliomas: the inevitability of recurrence and resistance to standard therapy [17,28,32]. Importantly, the original definition of GSCs is functional: they are cells capable of self-renewal, multilineage differentiation, and initiation of a phenocopy of the original tumor upon serial orthotopic xenotransplantation. Further stratification by molecular features is a description of this functional group. No generally accepted classification of GSCs exists to date; all existing literature on this topic represents research interpretations.

3.1.1. Profiling of Cell-Surface Markers

Initially, surface markers became the first tool for the isolation and functional characterization of glioma stem cells. Over the past two decades, a broad set of markers has been described, including CD133 [17,33], CD15/SSEA-1 [34], integrin α6 (CD49f) [35,36], CD44 [37,38], A2B5 [39], L1CAM [40], CD36 [41,42], CD109 [43], as well as EGFR/EGFRvIII [44,45] and PDGFRA [46,47] all of which simultaneously serve as both surface markers and oncogenic drivers. However, none of them is universal or strictly specific to glioma stem cells. Markers exhibit cross-expression, may label reversible cellular states rather than fixed subpopulations, and their interpretation requires mandatory functional validation. The most studied surface markers of GSCs are presented in Table 1.

Table 1. Key surface markers defining glioma stem-like cells.

Cell-Surface Marker	Molecular Characterization	Stemness Maintenance	Representative Articles
CD133	A pentaspan transmembrane glycoprotein of the prominin family contains glycosylated extracellular loops and is localized to lipid rafts and membrane protrusions	Enriches the self-renewing, tumor-initiating cell fraction; promotes neurosphere formation, clonogenicity, radioresistance, and tumorigenicity via PI3K/Akt/mTOR and MAPK signaling, and via Akt-dependent Wnt activation; expression is plastic and not an absolute marker of stemness	[48–51]
CD15	A carbohydrate epitope present on surface glycoproteins and glycolipids of glial progenitor cells is involved in cell adhesion, migration, and recognition during nervous system development	The evidence is largely correlative: SSEA-1-positive cells are enriched for tumorigenicity and co-express CD133, OLIG2, SOX2, BMI1, EZH2, and L1CAM; however, direct causal demonstration through knockdown or epitope restoration is lacking	[34,52–54]
CD44	A transmembrane glycoprotein and the canonical receptor for hyaluronic acid (HA); osteopontin (SPP1) is the second major ligand	Through interactions with hyaluronic acid and osteopontin, CD44 activates signaling pathways that reinforce the mesenchymal-associated stem-like phenotype and promote glioma stem cell survival. It also contributes to the maintenance of the perivascular niche and supports invasive growth and chemoresistance	[55–60]
Integrin α6/CD49f	A transmembrane receptor of the integrin family that, as part of the α6β1 or α6β4 heterodimers, binds laminin in the basement membrane; it mediates cell adhesion to the extracellular matrix and transduces microenvironmental signals into the cell	Through its interaction with laminin in the perivascular niche, integrin α6 supports self-renewal, proliferation, and tumor-initiating capacity. Its blockade reduces sphere formation and tumorigenicity and slows the growth of xenografts	[35,61–63]

Table 1. *Cont.*

Cell-Surface Marker	Molecular Characterization	Stemness Maintenance	Representative Articles
A2B5	A monoclonal antibody that recognizes the c-series gangliosides on the surface of glial progenitor cells	A2B5-positive glioma cells display self-renewal, multipotency, and tumorigenicity independently of CD133 status. Enzymatic removal of the epitope by sialidase or knockdown of ganglioside synthases reduces proliferation and migration, while the A2B5-positive fraction is enriched for tumor-initiating cells and is associated with invasiveness and disease progression	[39,64,65]
L1CAM (CD171)	A transmembrane glycoprotein of the immunoglobulin superfamily that functions as a cell adhesion and migration molecule and contributes to the differentiation and survival of neural cells	Its suppression disrupts neurosphere formation, reduces OLIG2 expression, increases p21 levels, and induces apoptosis. L1CAM inhibition also enhances radiosensitivity by impairing ATM/NBS1-dependent DNA repair	[40,66,67]
CD36	A class B scavenger receptor transmembrane glycoprotein (SCARB3) that binds and transports long-chain fatty acids, oxidized lipoproteins, and phospholipids. It is localized to lipid rafts and participates in lipid metabolism, cell adhesion, and the recognition of modified ligands	CD36 links stemness to lipid metabolism and stress survival. It is co-expressed with integrin α6 and CD133, and its knockdown reduces self-renewal and the proportion of cells with this phenotype, as well as impairing xenograft growth in vivo	[41,68]
CD109	A GPI-anchored membrane glycoprotein of the α2-macroglobulin family	CD109 physically interacts with glycoprotein 130 (GP130) and enhances IL-6/STAT3 signaling, which is required for glioma stem cell self-renewal, tumorigenicity, and chemoresistance. Its suppression reduces SOX2 and OLIG2 expression, disrupts neurosphere formation, shifts cells toward an astrocyte-like differentiated phenotype, and increases sensitivity to temozolomide; CD109 is induced by ionizing radiation, marks glioma stem cells at the invasive edge, and is associated with a mesenchymal shift	[43,69]
CXCR4 (CD184)	A seven-transmembrane G protein-coupled receptor (GPCR) coupled to Gi proteins; its ligand is the chemokine CXCL12; CXCR4 mediates chemotaxis, migration, survival, and proliferation	CXCR4 supports glioma stem cell self-renewal, sphere formation, and tumorigenicity through the autocrine/paracrine CXCL12–CXCR4 axis. Its activation promotes proliferation, homing to the perivascular niche, angiogenesis, and therapeutic resistance, whereas receptor blockade reduces Nestin, ABCG2, Msi1, Oct4, and Nanog expression, suppresses xenograft growth, and increases radiosensitivity	[70–73]
IL-6R (CD126)	A transmembrane glycoprotein that binds interleukin-6 and recruits the signaling co-receptor gp130 (CD130) to form the IL-6/IL-6R/gp130 hexameric complex. This complex activates JAK kinases and the transcription factor STAT3	IL-6R supports self-renewal, sphere formation, survival, and tumorigenicity through JAK/STAT3 signaling. Suppression of IL-6 or IL-6Rα reduces cell growth and neurosphere formation, increases apoptosis, and impairs xenograft growth in vivo	[74,75]
EGFR/EGFRvIII	EGFR: transmembrane receptor tyrosine kinase of the ErbB family; its ligand is epidermal growth factor; EGFRvIII: constitutively active mutant form with a deletion of exons 2–7 that is capable of ligand-independent dimerization and signaling	EGFR and EGFRvIII support glioma stem cell self-renewal, survival, and tumorigenicity through PI3K/Akt, Ras/MAPK, and JAK/STAT3 signaling. EGFRvIII-positive cells are enriched for CD133 and SOX2, display enhanced sphere-forming and tumor-initiating capacity, and signal paracrinally through IL-6/LIF to EGFRvIII-negative neighboring cells, thereby maintaining glioma heterogeneity and progression	[45,76–79]
PDGFRA	A class III receptor tyrosine kinase; its ligands are platelet-derived growth factors	Activation of PDGFRA by PDGF-A and PDGF-B maintains the undifferentiated state of oligodendrocyte progenitor-like cells. Inhibition of PDGF signaling arrests growth, depletes self-renewal capacity, and abolishes tumorigenicity in vivo	[80–82]

3.1.2. TCGA-Based Transcriptomic Classification of Gliomas

The most cited classification of GSCs is based on the transcriptomic stratification of glioblastoma proposed within The Cancer Genome Atlas (TCGA) project. Initially, the proneural (TCGA-PN GSC), neural (TCGA-N GSC), classical (TCGA-CL GSC), and mesenchymal (TCGA-MES GSC) subtypes were distinguished, each associated with a specific set of driver genetic events [83,84]. Independent studies have shown that GSC cultures derived from primary tumors stably reproduce at least the proneural and mesenchymal poles of this spectrum and retain their characteristics during prolonged culturing and xenotransplantation [85,86].

Proneural GSCs are GSCs whose transcriptional core is formed by neurodevelopmental factors—OLIG2, SOX2, SOX1, ASCL1, POU3F2, SALL2, PDGFRA, DLL3, and NCAM1 [87,88]. The combination of POU3F2, SOX2, SALL2, and OLIG2 has been experimentally defined as the minimal set of transcription factors sufficient to reprogram differentiated glioma cells back to a stem state [88]. The computational analysis by Fiscon et al. additionally identified switch genes associated with the stem-like identity of glioblastoma cells, including components of the regulatory networks maintaining this state [89]. Characteristic genetic features include PDGFRA amplification or mutation [83] and TP53 mutations [84]. In IDH-mutant gliomas, the TCGA-PN GSC profile is encountered almost invariably. Functionally, TCGA-PN GSCs are distinguished by high clonogenicity, pronounced capacity for neurosphere formation, active Notch signaling, and increased dependence on oxidative metabolism [85,88,90,91].

The classical GSC population is primarily defined by EGFR dependence, which implies EGFR amplification or expression of the oncogenic EGFRvIII variant [83,92]. The transcriptional profile of TCGA-CL GSCs is enriched in astrocytic and neural stem cell-associated genes, including NES, GFAP, AQP4, SOX9, NOTCH1, SMO, and GLI2, which corresponds to the AC-like cellular state [46].

Mesenchymal GSCs are characterized by the expression of CD44, CHI3L1 (YKL-40), VIM, MET, and ALDH1A3 [83,85,93,94], as well as components of the mesenchymal extracellular matrix (TNC, FN1) [95,96], and matrix metalloproteinases (MMP2, MMP9, MMP14) [97]. At the signaling level, this state is maintained by constitutive activation of the C/EBPβ–STAT3 axis [98] and NF-κB [86]; at the genomic level, the mesenchymal subtype is associated with loss of NF1 [83], and transcriptionally with a pronounced inflammatory signature. Functionally, TCGA-MES GSCs are distinguished by increased invasiveness, radioresistance [85,86], a metabolic shift toward glycolysis [99] and a hypoxia-dependent state in the single-cell landscape [46]. A fundamental feature of the TCGA-MES state is its predominantly non-genetic, inducible nature, since the transition to this state is triggered by tumor-associated macrophages via the OSM–OSMR/gp130–STAT3 axis [100], by hypoxia [46], as well as by radiation and drug therapy [86]. This is what distinguishes TCGA-MES GSCs from the TCGA-PN and TCGA-CL populations, which are determined by cell-autonomous genetic drivers (PDGFRA and EGFR, respectively).

An additional level of differences concerns paracrine communication: it has been shown that the molecular subtype and differentiation status of glioma stem cells determine the composition, protein profile, and proangiogenic activity of the extracellular vesicles they secrete, with TCGA-MES and TCGA-PN cultures forming qualitatively distinct vesicular repertoires [101]. Another level of differences between the TCGA-PN and TCGA-MES populations is associated with alternative splicing of genes involved in the cell cycle, DNA repair, and cytoskeletal organization, which may underlie their unequal proliferative and oncogenic activity [102].

In contemporary research, the neural subtype has been largely excluded from the classification of glioblastoma. Analysis of samples accounting for normal tissue admixture has shown that the corresponding signature largely reflects contamination of the tumor material with normal neurons and oligodendrocytes from the resection margin rather than an independent tumor state [103]. Therefore, with respect to GSCs, it is more appropriate to distinguish three transcriptional classes: TCGA-PN, TCGA-CL, and TCGA-MES.

The main limitation of this classification, based on bulk sequencing, is that the tumor subtype is determined by averaging across a heterogeneous cell population, whereas cells of different states coexist within a single tumor [103]. Shifts in the balance of states at recurrence are discussed in Section 3.1.3.

3.1.3. A Cell-State Plasticity Model of Glioma

The transition to single-cell resolution in research has fundamentally changed the understanding of glioma heterogeneity. In a key study by Neftel et al. [46], which combined scRNA-seq data from more than 20 primary IDH-wt glioblastomas, it was shown that malignant cells are distributed across four reproducible cellular states—NPC-like, OPC-like, AC-like, and MES-like—each of which mimics a specific stage of normal neuroglial development or of the response to injury. These states are defined by gene-expression programs in malignant cells and are not synonymous with functionally defined glioma stem cells. Stem-like capacity (self-renewal, serial tumor initiation and reproduction of tumor heterogeneity) must be demonstrated functionally, and it can occur in more than one state or in cells that are not stem-like at a given moment. Therefore, the term "GSC states" is used here only to describe the stem-like fraction of these programs, whereas "malignant cell states" refers to the broader transcriptional classification.

Neural progenitor-like (NPC-like) cells express neural progenitor programs, including SOX4, SOX11, DCX, CD24, DLL3, STMN2, TUBB3, and ASCL1. This state is subdivided into NPC1, enriched in genes associated with oligodendrocyte progenitors (OLIG1/2, TNR, DLL3), and NPC2, characterized by neuronal lineage genes (STMN2, SOX11). CDK4 amplification is a genetic correlate of the NPC-like state and is associated with an increase in its proportion within the tumor [46]. NPC-like cells are characterized by high proliferative activity and are often localized in the invasive zone, at the site of contact with neurons and the normal neuronal network. Spatial and in vivo studies have shown that these cells can employ mechanisms similar to neural progenitor migration for invasion of the surrounding brain tissue [104].

Oligodendrocyte progenitor-like (OPC-like) cells carry the oligodendrocyte progenitor program, including PDGFRA, OLIG1/OLIG2, CSPG4/NG2, SOX10, APOD, DLL3, and ASCL1. Their genetic correlate is PDGFRA amplification, which is associated with an increase in the proportion of OPC-like cells within the tumor. Together with NPC-like cells, this state forms the proneural pole of the glioblastoma cellular landscape and collectively they determine the TCGA-PN subtype [46]. These cells also participate in neuron–tumor interactions and may be involved in tumor invasion along the white matter [104].

Astrocyte-like (AC-like) cells express the astroglial program, including GFAP, AQP4, S100B, MLC1, SLC1A3/GLAST, HEPACAM, APOE, CLU, and SPARCL1. The genetic correlate of this state is EGFR amplification, and tumors with a predominance of AC-like cells usually correspond to the TCGA-CL subtype [46]. Compared with OPC-like cells, the AC-like population is characterized by lower density and relatively low proliferative activity. In spatial studies, it is part of the structured regions of glioblastoma and may participate in the formation of the astrocytic tumor niche [105].

Mesenchymal-like (MES-like) cells express damage response, hypoxia, and inflammation programs, including those involving CD44, CHI3L1, VIM, ANXA1/ANXA2, LGALS1/LGALS3, TNC, HILPDA, ADM, VEGFA, and NDRG1. This state is subdivided into MES1, characterized by an inflammatory, hypoxia-independent program, and MES2, associated with hypoxia and glycolysis-dependent metabolism; the genetic correlate of the MES-like state is NF1 mutation or deletion [46]. MES-like cells are often enriched in perinecrotic hypoxic zones and in regions rich in tumor-associated macrophages [105]. It has been shown that macrophages secrete oncostatin M (OSM), which binds to OSMR or LIFR receptors in complex with GP130 on the surface of tumor cells and activates the STAT3 signaling pathway. As a result, cells predominantly from NPC- and OPC-like states transition to the MES-like state [100].

The key tenets of the Neftel model are as follows: (1) all four cellular states are present in every tumor, differing only in their relative proportions; (2) cells are capable of transitioning from one state to another, as confirmed by lineage-tracing experiments and xenotransplantation of sorted populations; (3) tumor-initiating potential is not confined to any single state but is distributed among them. Thus, the model replaces the classical hierarchical model based on a single stem population with the concept of multiple interconvertible cellular states [46].

The Neftel model has been reproduced and extended by independent studies across different cohorts and methods, thereby confirming the four-state scheme as a robust model of glioblastoma organization. Couturier et al. showed that glioblastoma reproduces a neuro-ontogenetic hierarchy organized around glial progenitor cells and including astrocytic, neuronal, and oligodendrocytic lineages; this organization partially corresponds to the NPC/OPC- and AC-like states of the Neftel model [106]. Bhaduri et al. identified several GSC subtypes within the tumor, including an invasive oRG-like population, and confirmed the coexistence of neuronal and glial programs [107]. Wang et al. described proliferating glioblastoma cells along a single "proneural–mesenchymal" axis [108]. Richards et al. identified a continuous gradient between states associated with neuro-ontogenetic programs (NPC/OPC/AC-like) and states of damage response and inflammation (MES-like) [109], while Garofano et al. proposed a classification of glioblastoma based on the activation of key signaling pathways, which distinguishes metabolically distinct tumor subgroups and complements the transcriptional model [99]. Johnson et al. and Chaligne et al. have shown that glioma cellular states are regulated at the epigenetic level [110,111]. Furthermore, glioblastoma tumor cells can acquire part of the expression program of myeloid cells (macrophages and microglia). Such reprogramming contributes to the formation of the MES-like state and helps the tumor evade immune system recognition. This process is called epigenetic immunoediting [112]. Data from the GLASS project in the study by Varn et al. demonstrated that, at recurrence, the set (composition) of tumor phenotypes is preserved, but their proportions shift, particularly toward the MES-like state [113]. Spatial studies by Greenwald, Mathur, and Hara have shown that different cellular states coexist within a single tumor and form reproducible spatial structures: for example, MES-like cells are predominantly located in hypoxic and perinecrotic zones, whereas NPC-/OPC-like cells are more frequently found in invasive and perivascular regions [105,114,115]. Thus, the Neftel classification reflects the fundamental organization of glioblastoma as a plastic system of interconvertible states controlled by genetics, the epigenome, and the microenvironment (Figure 1).

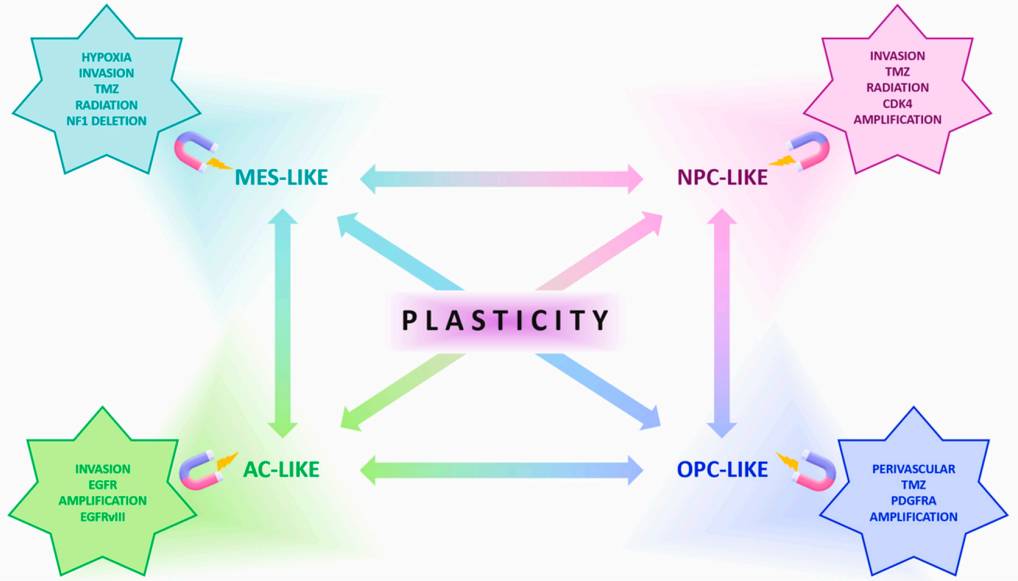

Figure 1. Plasticity of the glioma stem-like cell compartment: from state interconversion to therapy-driven selection. The scheme illustrates the interconvertibility of glioma stem-like cell (GSC) states—NPC-like, OPC-like, AC-like and MES-like—and the factors that bias this equilibrium. Arrows indicate potential transitions between GSC states, with bidirectional arrows denoting reversible interconversion. Oncogenic drivers (CDK4, PDGFRA, EGFR, NF1), microenvironmental cues (hypoxia, perivascular and invasive niches) and therapeutic pressure (radiotherapy, temozolomide) jointly shift the balance between states. This plasticity enables phenotypic compensation under selective pressure, driving therapy resistance, mesenchymal shift and tumor recurrence.

3.1.4. Proliferative and Quiescent Glioma Stem-like Cells

The functional "proliferative/quiescent GSC" axis is the most clinically significant, as it directly determines sensitivity to cytotoxic therapy. The rapidly dividing GSC fraction is characterized by increased expression of the proliferation marker Ki-67 [116], as well as activation of S- and G2/M-phase genes, including TOP2A, CCNB1, and MCM2 [117–120]. These cells efficiently form neurospheres in vitro [121]. Compared with quiescent GSCs, the rapidly dividing fraction demonstrates relatively greater susceptibility to alkylating agents and ionizing radiation, although its sensitivity to therapy remains limited [122,123]. Rapidly dividing GSCs are predominantly associated with the proneural pole and NPC-/OPC-like states, and are most often localized in the perivascular niche, where they receive trophic and Notch-dependent support from the endothelium [124,125].

The quiescent GSC fraction (quiescent, slow-cycling, dormant) is characterized by residence in the G0 phase [126], low Ki-67 levels, and activation of programs suppressing proliferation and differentiation: expression of the cell cycle inhibitors CDKN1A/p21 and CDKN1B/p27; the differentiation repressors HES1, ID1, and ID3 [127]. These cells also demonstrate increased expression of ABC transporters (drug resistance), antiapoptotic proteins, components of the DNA repair system (ATM/ATR, CHK1/2, RAD51), and enhanced antioxidant potential. Quiescent GSCs are predominantly localized in hypoxic and perinecrotic zones, where HIF-dependent programs maintain their stem state; phenotypically, this fraction tends toward the mesenchymal pole [128,129].

Important evidence for the significance of the functional classification has been obtained in genetic cell-labeling models. Quiescent Nestin^{+} cell populations survive a course of temozolomide and serve as a source of tumor repopulation, and their elimination in combination with chemotherapy markedly slows glioma growth [24]. In human glioma, the quiescent fraction is also responsible for initiation, expansion, and recurrence after

chemotherapy [130]. Clonal fate tracing has shown that the proliferative hierarchy is dynamic; that is, after therapy, repopulation is provided by previously quiescent cells [131]. A Notch- and KDM6/JMJD3-dependent mechanism of reversible drug tolerance in slowly dividing cells has also been described, which points to the epigenetic nature of this state [132].

Thus, the functional division of GSCs into actively proliferating and quiescent reflects not merely a difference in division rate, but two fundamentally different modes of tumor survival. Actively proliferating GSCs are more sensitive to cytotoxic therapy, whereas quiescent GSCs survive treatment and provide tumor repopulation and recurrence. Since these states dynamically interconvert, effective therapy must combine the elimination of dividing cells with the suppression of the quiescent fraction and its reactivation.

It should be noted that any classification of glioma stem cells, regardless of the distinctions on which it is based, is not absolutely fixed. The groups thus defined rather reflect dynamic and interconvertible cell states influenced by microenvironmental signals and therapeutic pressure. A single cell may simultaneously possess a particular molecular profile, express a specific set of markers, occupy a corresponding niche, and reside in a quiescent or proliferative state. These characteristics reflect different but interrelated levels of GSC organization, and only their combined analysis allows the functional heterogeneity of glioma-initiating cells and their therapeutic vulnerability to be assessed most fully (Table 2).

Table 2. Integrated characterization of glioma stem-like cell states.

Feature	Proneural Axis	Classical Axis	Mesenchymal Axis
TCGA bulk subtype	Proneural (PN)	Classical (CL)	Mesenchymal (MES)
Single-cell states	OPC-like + NPC-like	AC-like	MES-like
Surface markers	$CD133^{high}$ CD15/SSEA-1$^+$ A2B5$^+$ CD24$^+$ PDGFRA$^+$ $CD44^{low}$	EGFR/$EGFRvIII^{high}$ intermediate CD44 expression	CD44$^+$ CD109$^+$ $CD133^{low}$
Transcriptional markers	OLIG2$^+$ $SOX2^{high}$	FAP AQP4 SLC1A3	CHI3L1$^+$ ALDH1A3$^+$ VIM$^+$
Key drivers	PDGFRA and CDK4 amplification; TP53 mutation; IDH1/2 mutation in IDH-mutant gliomas	EGFR amplification; CDKN2A/B deletion; PTEN loss	NF1 loss; constitutive NF-κB/STAT3 signaling; induction by macrophage-derived signals and hypoxia
Functional state	Predominantly proliferative state	Intermediate, with reduced proliferative activity	Enriched for quiescent, slow-cycling, and therapy-tolerant cells
Tumor niche	Perivascular, invasive, and neuron-associated	Perivascular and parenchymal niches	Hypoxic/perinecrotic and immune niches

Associations between TCGA subtypes, single-cell states, surface markers, niches, and functional states represent enrichment patterns observed in specific studies. They are not strict one-to-one relationships, and individual cells may display features of several columns.

3.2. Escaping Cytotoxic Therapy: Resistance and Cell Death Regulation in Glioma Stem-like Cells

Glioma stem cells not only possess high plasticity of cellular state and the capacity to restore the full diversity of tumor cells, but also have enhanced protection against the mechanisms that trigger or execute cell death programs after chemotherapy and/or radiotherapy. This cellular protection is implemented at several levels and leads to an elevated threshold for the activation of cell death processes.

One of the key mechanisms of such resistance is the attenuation of the apoptotic signal. Apoptosis is a caspase-dependent type of regulated cell death controlled by pro- and antiapoptotic proteins of the BCL-2 family. Studies on patient-derived GSCs have shown that GSCs may have a shifted balance of apoptosis regulation, manifested as a significant increase in the levels of the antiapoptotic proteins BCL-xL and MCL-1 with a relatively low level of free activator BH3 proteins. As a result, standard therapeutic stress

does not bring cells to the threshold at which mitochondrial membrane permeabilization occurs. Moreover, simultaneous inhibition of BCL-xL and MCL-1 led to the triggering of the apoptotic program, whereas inhibition of only one protein could be compensated by the activity of the second [133]. A similar principle of "double blockade" can be traced at the level of survival signaling pathways. In the study by Merati et al., the authors investigated how survival signaling pathways influence the susceptibility of GSCs to apoptosis. It was shown that inhibition of MEK1/2, which suppresses BIM, and of histone deacetylases (HDACs), which enhance the expression of antiapoptotic proteins, restores the proapoptotic signal of the BIM/BMF proteins. Moreover, the authors showed that the interaction between CD44, which is highly expressed on GSCs, and its ligand hyaluronic acid exerts an opposite antiapoptotic effect [134]. Certainly, the response differed between cell cultures from different patients, which once again underscores the intertumoral heterogeneity of gliomas. In addition to the intracellular control of mitochondrial apoptosis, GSCs can also evade death through attenuation of the receptor-mediated apoptotic pathway. In the study by Ding et al. [135], it was shown that, compared with monolayer cultures grown in serum-containing medium, neurospheres enriched in glioma stem cells express extremely low levels of the death receptors DR4 and DR5. Such a decrease in expression may explain the resistance of GSCs to TRAIL-mediated death. Moreover, in treatment-resistant cells, cFLIP is overexpressed, which prevents the cleavage and activation of caspase-8, thereby blocking signal transmission from death receptors to effector caspases.

The resistance of GSCs is not limited to apoptosis, since stem-like cells also possess specific features of iron metabolism and antioxidant defense that influence their sensitivity to ferroptosis. Ferroptosis is a type of regulated iron-dependent cell death characterized by uncontrolled peroxidation of polyunsaturated membrane phospholipids. The study by Schonberg et al. showed that GSCs, compared with more differentiated cells of the same tumor, more actively take up and accumulate iron: they express higher levels of the transferrin receptor and ferritin, and knockdown of ferritin/TfR selectively kills the stem population [136]. The expanded labile iron pool creates a potential substrate for the Fenton reaction and, consequently, increases the cell's vulnerability to lipid peroxidation. However, an increased iron burden per se does not mean inevitable death. Resistance also depends on the cell's ability to neutralize lipid peroxides. It has been shown that therapy-tolerant persister glioma cells acquire a critical dependence on GPX4 and, upon its inhibition, are more sensitive to ferroptosis [137,138]. This dependence is particularly relevant for quiescent astrocyte-like tumor cells. Such cells are characterized by impaired mitochondrial metabolism, elevated levels of reactive oxygen species, enhanced lipid peroxidation, and lower levels of reduced glutathione (GSH), an important component of the antioxidant system. Subsequent inhibition of GPX4 in patient-derived cultures and human tumor tissue slices led to a reduction in the astrocyte-like quiescent cell population due to the triggering of the ferroptosis program [139]. This vulnerability acquires particular significance in the context of standard therapy. Quiescent cells are more difficult to eliminate with temozolomide and radiotherapy, which most often induce cellular damage leading to apoptosis [25,140,141], since these treatments are considerably more effective against cells undergoing division or DNA replication. TMZ-induced DNA damage becomes lethal predominantly upon subsequent replication, when unrepaired O6-methylguanine is recognized by the mismatch repair system and leads to the formation of double-strand breaks [142]. Radiotherapy is also more effective against proliferating cells, whereas quiescent GSCs can survive treatment [143]. However, the resistance of quiescent GSCs cannot be explained solely by slowed cell cycle progression; it is additionally supported by enhanced DNA repair, checkpoint activation, antiapoptotic programs, and protective niches of the tumor microenvironment [4,144,145]. In a model of human glioblastoma recurrence,

quiescent stem-like cells were identified that retained the capacity for self-renewal and tumor initiation, and after chemotherapy the surviving GSC pool became activated, restored tumor heterogeneity, and induced tumor recurrence [130]. In this regard, a more promising strategy may be not attempting first to force all quiescent cells into the cell cycle, but rather exploiting their metabolic vulnerability, in particular their dependence on GPX4, to induce ferroptosis [146].

Finally, in addition to apoptosis and ferroptosis, the role of other forms of regulated cell death in the stem-like population of glioma remains insufficiently studied. Nevertheless, in the context of immunotherapeutic approaches directed against a broad spectrum of tumor cell states, considerable interest is focused not only on ferroptosis and apoptosis but also on cuproptosis, pyroptosis, and necroptosis, since these types of cell death possess high immunogenic potential, which makes them potentially useful not only for the direct destruction of tumor cells but also for the activation of the antitumor immune response [147–150].

4. From Initiation to Relapse: Glioma Stem-like Cells as Drivers of Malignant Progression

According to the current WHO 2021 classification, high-grade gliomas include IDH-mutant astrocytoma (grade 3 or 4), IDH-mutant oligodendroglioma with 1p/19q codeletion (grade 3), and IDH-wild-type glioblastoma (grade 4). The subtypes differ in their morphological and molecular genetic characteristics, sensitivity to therapy, pattern of growth and invasion, as well as in patient survival [151,152]. However, the spectrum of molecular, metabolic, and epigenetic features of gliomas is considerably broader: in 2021, many of them were still insufficiently studied to serve as the basis for a classification [153]. As the understanding of the cellular composition of gliomas, in particular of the role of stem cells, expands, the question arises as to whether there is a pattern in GSC content among astrocytomas, oligodendrogliomas, and glioblastomas. If such a pattern exists, it should manifest in two ways: as a quantitative gradient and as a qualitative difference in the organization of GSCs. In other words, what is likely at issue is not merely a cellular metric but a balance between self-renewal, differentiation, and adaptive plasticity that is specific to each subtype. Moreover, measurements of the stem-like compartment are determined primarily by the molecular class of the tumor, the grade of malignancy, the genetic background, and therapeutic pressure, rather than by the histological diagnosis as such. In addition, the results of such an analysis depend on the methodology used to identify the stem component (surface markers, transcriptomic analysis, functional roles, single-cell sequencing, etc.). Understanding GSC biology is an important step toward the development of new treatments for gliomas. However, no established classification of GSCs exists, and the classification of gliomas themselves is regularly revised; therefore, studies of the stem properties of glioma cells remain insufficiently systematic.

The first relationship to be discovered was a gradient in the number of stem-like cells depending on glioma grade. In the study by Singh et al. [17], $CD133^+$ tumor-initiating cells were isolated from human gliomas for the first time, with the proportion of $CD133^+$ cells varying from fractions of a percent to 25%, depending on the grade of malignancy. Later, clinical cases confirmed that the proportion of $CD133^+$ cells increases with grade and correlates with a worse prognosis and earlier recurrence [154,155]. Contemporary studies reproduce a similar trend. For example, in the study by Selvaraj [156], the proportion of CD133- and Nestin-expressing cells increased from 25.8% in WHO grade 2 gliomas to 58.6% in grade 3 and 73.8% in grade 4 glioblastoma. These studies, among other things, provided a major impetus for further research. To date, the analysis of stemness by the single marker CD133 is not exhaustive, since CD133- GSC populations were subsequently discovered [157].

The next stage was the comparison of glioma subtypes by the mutation status of the IDH enzyme. The bulk of cells in IDH-mutant glioma are characterized by a slower cell cycle and a relatively quiescent state. This was explained by analyzing the transcriptomic programs of such cells with respect to the astrocytic and oligodendrocytic branches of differentiation. It was shown that the majority of malignant cells in IDH-mutant gliomas are committed oligodendrocyte progenitors, whereas proliferative activity is concentrated in a small undifferentiated NPC-like pool constituting a few percent of tumor cells, and the differentiation programs are represented mainly by the oligodendrocytic and astrocytic branches [158,159].

Despite differences in histology, genetics, and microenvironment, astrocytoma and oligodendroglioma demonstrate a similar hierarchy. The differences between them concern mainly not the size of the stem pool, but rather the composition of differentiated cellular states and the microenvironment. Moreover, astrocytomas usually contain more tumor-associated macrophages and microglia, which may influence the maintenance of stem states and the response to therapy [160]. Importantly, IDH-mutant gliomas of both histological variants exhibit a common blockade of oligodendroglial differentiation: they are enriched in pre-OPC, OPC, and committed oligodendrocyte progenitor signals but depleted of myelination and mature oligodendrocyte programs. This allows the IDH mutation to be regarded not merely as a diagnostic marker but as a factor that sets a common ontogenetic "shift" of the tumor hierarchy [161].

It was later shown that the hierarchy of IDH-mutant gliomas is epigenetically determined. Specifically, different transcriptional states of glioma are encoded by stable DNA methylation patterns. The variability of DNA methylation is directly linked to the cell's position on the "stemness–differentiation" axis. Stem and progenitor GSC states are characterized by the activity of SOX2, SOX8, and OLIG2, whereas more differentiated cells demonstrate enhanced programs of astrocytic differentiation and stress response, including the factors SOX9, JUND, and FOS. The increase in local DNA methylation disorder accompanies disease progression and the formation of a more aggressive phenotype. In IDH-mutant gliomas, the stem pool gives rise to relatively predictable branches of differentiation due to the global DNA hypermethylation characteristic of IDH-mutant tumors, since the product of the mutant enzyme, D-2-hydroxyglutarate, is a competitive inhibitor of a number of α-ketoglutarate-dependent dioxygenases (TET, KDM), which leads to DNA and histone hypermethylation. Hypermethylation decreases with progression or recurrence, accompanying the activation of genes associated with tumor progression, angiogenesis, and changes in the tumor microenvironment. By contrast, in IDH-wild-type glioblastoma, transcriptional states appear to be more dynamically interconvertible, and cell identity is generally less rigidly constrained, although the extent of this plasticity varies across tumors, niches, and experimental models [111].

Comprehensive single-cell analysis revealed mechanisms of plasticity and stress response regulation common to IDH-mutant and IDH-wild-type gliomas: under hypoxia and after irradiation, DNA methylation disturbances are enhanced, cellular states change, and pathways of response to hypoxia, oxidative stress, and DNA damage are activated. In stem-like cells, chromatin remodeling and DNA repair programs associated with EZH2 and BRCA1 were identified. In differentiated cells, stress and hypoxic programs predominated, including the activity of JUND, FOS, and HIF1A/ARNT [110].

In IDH–wild-type gliomas, lineage tracing experiments have revealed a stable proliferative hierarchy. Its foundation is a slowly cycling stem pool that survives therapy and subsequently re-establishes the full diversity of tumor cells [24,131]. The malignant cells themselves are distributed across four states reproducible from tumor to tumor—NPC-like, OPC-like, AC-like, and MES-like—and the equilibrium between them is shifted by onco-

genic drivers: CDK4 amplification tends toward the NPC-like program, PDGFRA toward the OPC-like, EGFR toward the AC-like, and NF1 deletion toward the MES-like [46]. The distribution of surface markers also proves to be non-random: CD24 predominantly marks NPC-like cells, CD133 marks OPC-like cells, EGFR marks AC-like cells, and CD44 marks the MES-like state [46]. That is precisely why no single marker is capable of adequately reflecting the "overall GSC content" in glioblastoma; it measures only one segment of the phenotypic spectrum.

A key property of these states is their mutual convertibility, demonstrated by cell labeling and lineage tracing, as well as by xenotransplantation of individual sorted fractions [46]. GSC cultures sorted by any of the markers restore the original marker diversity within several passages, and the microenvironment—primarily hypoxia and acidification—actively induces stem-like states de novo [162–164]. Plasticity also extends to already differentiated glioblastoma cells: they can be reprogrammed back to a stem-like state by a set of only four transcription factors—POU3F2, SOX2, SALL2, and OLIG2 [19]. The mesenchymal transition, in turn, is triggered predominantly by external stimuli—NF-κB and TNF signaling, irradiation, and, most importantly, interaction with tumor-associated macrophages and T cells [86,100].

The connection with the immune system is bidirectional: glioma stem cells not only respond to the immune environment but also shape it themselves, acquiring an immunosuppressive and immune-evasive phenotype through epigenetic switching of cellular states [112,165].

In addition, a clarification should be made regarding the most malignant subtypes of gliomas: grade 4 IDH-wt glioblastoma and grade 4 IDH-mut astrocytoma, the latter of which was classified until 2021 as a subtype—secondary grade 4 glioblastoma (a glioma that progressed from a less malignant stage) [151]. These are fundamentally different tumors in terms of origin, epigenetic landscape, and organization of the stem pool [166]. In grade 4 IDH-wt glioblastomas, the aggressive phenotype is usually present de novo and is maintained by a genetically heterogeneous, plastic stem landscape [167,168]. Grade 4 astrocytomas are closer in the architecture of their stem compartment to the less malignant astrocytomas from which they apparently originated; however, the process of malignant transformation occurs gradually, first through the epigenetic plasticity of OPC-like cells, then through their partial reprogramming into the NPC-like state, loss of hypermethylation-mediated control, and acquisition of genetic events, primarily CDKN2A/B deletion [169,170]. This factor, among others, explains why these subtypes of gliomas possess a different character and different mechanisms of resistance to therapy.

In primary IDH-wt glioblastoma, resistance to therapy is driven by the coexistence of several interconvertible stem states and their support from the tumor niche, which ensures phenotypic compensation under selective pressure [46,171]. Mesenchymal plasticity is particularly important in this context, since its activation is associated with radioresistance, invasion and recurrence [86]. The enhanced DNA damage response makes an additional contribution: CD133-positive GSCs display enhanced basal Chk1/Chk2 checkpoint activation and an elongated cell cycle, which may contribute to radioresistance, although increased DNA repair efficiency in these cells has not been universally observed [28].

Therefore, combined strategies that simultaneously suppress mesenchymal plasticity, DNA repair and key signaling pathways of GSC self-renewal appear preferable [169], IDH-dependent hypermethylation is lost, and epigenetic control of cell identity weakens [110,111], while after temozolomide therapy a mismatch repair deficiency accumulates, caused by mutations in MLH1, MSH2, MSH6 or PMS2. This forms a TMZ-induced hypermutated phenotype and secondary resistance [172,173]. Thus, in grade 4 IDH-mut tumors,

resistance reflects not an abrupt phenotypic switch but a sequential epigenetic and genetic remodeling of the stem pool during progression.

Recurrence of high-grade gliomas can arise through two non-exclusive mechanisms that should be distinguished. The first is clonal selection: therapy eliminates sensitive subclones and enriches pre-existing resistant genotypes or, in IDH-mutant astrocytomas, TMZ-induced mismatch repair deficiency generates new hypermutated subclones. The second is reversible non-genetic adaptation: transitions between GSC states and drug-tolerant quiescent or persister states allow the same genotype to re-establish a different phenotypic composition without new mutations. These mechanisms differ in stability and therapeutic implications: clonal resistance is heritable, in that it is fixed within the selected clone and requires targeting of the selected genotype, whereas non-genetic tolerance is dynamic and may be exploited through state-directed or sequential therapy. Recurrence is therefore formed by the selection and reprogramming of specific subpopulations with increased resistance to radiotherapy and chemotherapy, and the resistance of GSCs depends not only on their intrinsic properties but also on their localization in niches and their ability to switch between transcriptional states. Spatially, GSCs in the primary tumor are found in several niches, including the perivascular niche.

The perivascular niche comprises the vascular microenvironment, where the endothelium, via nitric oxide or membrane-bound Notch ligands (DLL1, DLL4, JAG1), activates Notch signaling in GSCs, thereby promoting the maintenance of the stem phenotype and tumorigenicity [93,174–176].

The hypoxic/necrotic niche supports GSCs predominantly through HIF-2α (EPAS1), which is selectively expressed in the stem fraction and is required for self-renewal, survival, and tumor-initiating potential [177–179]. HIF-1α also participates in maintaining stemness through activation of Notch signaling and by inducing dedifferentiation of more mature tumor cells into GSCs [180–183]. Current data indicate that hypoxia acts in a context-dependent manner: in some GSC lines it enhances invasiveness and MES-like programs, whereas in others it has no such effect [178,184,185].

The invasive niche forms at the border with normal parenchyma, in a neuron-rich zone where radial glia-like (RG-like) cells are enriched [186]. This niche is critical, since cells of the invasive margin generally remain beyond the resection boundaries and beyond the region of maximum radiation dose; their survival is ensured not only by anatomical inaccessibility but also by active interaction with neurons, vessels, immune cells, and the extracellular matrix [186–188]. For RG-like cells, the key survival mechanism in this niche is neuron-dependent invasive growth [107,186]. The key pattern is that standard therapy, including maximal resection, radiotherapy, and temozolomide, does not eliminate the stem fraction but enriches the residual tumor with it. This creates conditions under which part of the stem pool survives treatment even with effective elimination of the bulk of differentiated tumor cells.

Radiotherapy and temozolomide exert a dual effect on tumor tissue: overall tumor mass is reduced, but selective pressure favors cells with efficient DNA repair, quiescence capacity and phenotypic plasticity [189–191]. GSCs show more efficient activation of the ATM/CHK1/CHK2 checkpoints and better repair of radiation-induced damage than more differentiated cells [28,192]. High expression of MGMT and ALDH1A3 provides enzymatic protection against TMZ-induced damage and maintains the stem phenotype [172]. In GSC cultures, progenitor cells are less sensitive to TMZ than more differentiated tumor cells, confirming their role as a reservoir of resistance [172]. The entry of cells into a quiescent-like state under therapy and their re-entry into the cell cycle after treatment are described in Section 3.1.4.

Mesenchymal drift in IDH-wt glioblastoma is associated with loss of NF1 and an increase in the myeloid infiltrate [103,113], as well as with remodeling of the tumor and stromal compartments after therapy [193].

The proneural-to-mesenchymal transition (PMT) is a central example of reversible or partially reversible non-genetic plasticity in glioblastoma. Stable genetic alterations—including NF1 loss, CDK4 amplification, PDGFRA amplification, and EGFR amplification—provide a permissive background and bias the cellular-state composition of a tumor, but they do not rigidly determine the transcriptional state of individual cells. In contrast, PMT is a dynamic, non-genetic adaptation driven by convergent inflammatory, hypoxic, and neuronal-derived signals [194,195] that reprogram transcriptional and epigenetic networks in a relatively reversible manner. Thus, genetic alterations define the evolutionary context, whereas non-genetic mechanisms enable rapid and potentially reversible shifts in cellular identity [112,190,196,197].

The NF-κB pathway is a key mediator of this process. Inflammatory cytokines, particularly TNF-α, activate NF-κB signalling and promote a mesenchymal phenotype associated with CD44 enrichment and radioresistance [86]. IL-1β derived from myeloid cells can also activate NF-κB signalling in glioblastoma cells and promote inflammatory myeloid recruitment, although its direct role in PMT remains less well defined [198]. Multiple inputs may converge on this node, including paracrine myeloid-derived cytokines, cell-intrinsic alterations that may lower the threshold for NF-κB activation, and therapeutic stress [199,200]. In particular, irradiation can promote NF-κB-dependent mesenchymal differentiation and radioresistance [86,201], whereas temozolomide may contribute to therapy-associated plasticity, although its direct role in NF-κB-dependent PMT remains less well defined [200]. NF-κB therefore functions as an upstream coordinator that links microenvironmental inflammatory cues to mesenchymal transcriptional programmes [86,199,200]. Functionally, this may establish a feed-forward loop, as MES-like tumor regions are frequently enriched for myeloid cells and inflammatory signalling, which can reinforce an immunosuppressive microenvironment.

STAT3 and C/EBPβ are central transcriptional regulators of the mesenchymal programme and can promote a shift of proneural-like glioma stem cells (GSCs) toward a MES-like phenotype; their combined silencing collapses the mesenchymal signature and reduces tumor aggressiveness, whereas their co-expression is sufficient to reprogram neural stem cells towards a mesenchymal phenotype [98]. This core module is further supported by additional mesenchymal regulators, including RUNX1 and FOSL1 [201]. In addition, TAZ, whose promoter is hypermethylated in proneural and low-grade gliomas and derepressed in mesenchymal tumors, can reinforce mesenchymal identity through YAP/TAZ–TEAD-dependent transcriptional programmes [202]. This axis is further reinforced by the inflammatory microenvironment: macrophage-derived oncostatin M (OSM) activates STAT3 signalling through OSMR or LIFR in complex with GP130, thereby promoting MES-like conversion of glioblastoma cells [100,203]. A recent report suggests that neuronal activity can further promote PMT through neuron-derived exosomal miR-184-3p, which reduces m6A modification by inhibiting RBM15 and thereby activates STAT3 signalling in GSCs [204]. This arm of the circuitry is strongly shaped by extrinsic cues: genetically similar cells can acquire different states depending on the cytokine milieu. Thus, PMT is not exclusively a cell-intrinsic process but is actively shaped by macrophage- and neuron-rich niches [205].

TGF-β signalling provides an additional route to mesenchymal conversion. TGF-β activates SMAD2-dependent transcription and induces a ZEB1-dependent mesenchymal phenotype associated with increased migration and invasion [206]. TGF-β can also maintain a stem-like compartment through LIF–JAK–STAT3 signalling [207] and may contribute

to local immunosuppression [208]. TGF-β-driven PMT is context-dependent and may converge with inflammatory and hypoxic cues rather than acting as an isolated pathway [206,209]. This may partly contribute, together with other mechanisms, to the limited efficacy of TGF-β pathway inhibition as monotherapy in clinical trials [210].

Hypoxia promotes a MES-like phenotype, particularly in hypoxic and perinecrotic regions. HIF-1α activates ZEB1 and promotes a mesenchymal, invasive phenotype [209], whereas HIF-2α promotes a stem-like phenotype and induces OCT4, NANOG, and c-MYC expression in non-stem glioma cells [163]. Single-cell analyses distinguish hypoxia-dependent (MES2) and hypoxia-independent (MES1) mesenchymal subpopulations [46]. Hypoxia therefore links tumor spatial organisation to malignant-cell transcriptional states and may contribute to radioresistance.

These pathways converge on epigenetic mechanisms that stabilise, but do not irreversibly fix, the mesenchymal phenotype. PMT is associated with enhancer reprogramming and altered H3K27ac deposition [211]. Proneural and mesenchymal GSCs show differential sensitivity to EZH2 and BMI1 inhibition, respectively [212]. G-CIMP erosion and global hypomethylation at recurrence correlate with mesenchymal shift without corresponding new genetic drivers [213,214]. Lineage-tracing and single-cell multi-omic studies show that transcriptional states are transmitted to daughter cells with incomplete fidelity [111].

Reversibility is supported by experimental and clinical evidence. Sorted GSC subpopulations regenerate the full phenotypic repertoire within days [46,162]. Drug-tolerant persister cells revert to a sensitive phenotype after drug withdrawal [132]. In paired primary–recurrent cohorts, mesenchymal shift at recurrence frequently occurs without new mesenchymal driver mutations, while clonal composition may remain largely stable despite marked phenotypic change [103,215].

Collectively, stable genetic alterations provide a permissive context and shape the evolutionary constraints of a tumor, whereas inflammatory, hypoxic, and neuronal signals, together with epigenetic regulation, drive PMT as a reversible or partially reversible non-genetic adaptation under therapeutic pressure. This distinction is important: genetic alterations are heritable and relatively stable, whereas non-genetic plasticity is dynamic and may be reversed or remodelled when the initiating cues change. Because these pathways are interconnected, targeting a single node is unlikely to be sufficient. The reverse transition from MES-like to proneural-like states is less well characterized, and much of the evidence for reversibility derives from in vitro systems. These limitations should be considered when interpreting the therapeutic implications of PMT.

Recurrence is the result of coevolution of GSCs and the niche: therapy selects resistant subpopulations and restructures immune and vascular support. Consequently, the recurrent tumor may acquire phenotypic features distinct from the primary tumor not through the emergence of a new cell population, but through selection of a pre-existing plastic pool under therapeutic pressure and stabilization of the MES-like state by a therapy-remodeled microenvironment.

This shows that a regularity in the content and organization of glioma stem cells does exist, but it is determined not by the histological diagnosis, but by the molecular class, the malignancy grade, the genetic background, and the therapeutic pressure. Across malignancy grades, a quantitative gradient can be traced, yet its meaning is not reducible to a simple increase in the GSC fraction [154–156]. In IDH-mutant gliomas, the stem-like compartment is generally more restricted and epigenetically ordered than in IDH-wild-type glioblastoma, although its size, composition, and degree of epigenetic constraint may vary between tumors and disease stages [158,161], whereas in IDH-wt glioblastoma, stemness is distributed among four interconvertible states whose balance is shifted by oncogenic drivers [46]. Epigenetic variability acts as an intermediate level between the

genetic subclone and the adaptive phenotype, explaining why similar genotypes give rise to different cellular landscapes [110,111]. IDH-mut astrocytoma and oligodendroglioma differ not in the size of the stem pool but in the composition of differentiated states and the microenvironment [158,159,161]. The transition from less malignant forms of glioma to glioblastoma implies a change in the type of organization, including a shift from a rigid hierarchy with a narrow pool to a plastic multipolar landscape [46,167]. Therefore, IDH-wt glioblastoma and grade 4 IDH-mut astrocytoma are biologically distinct entities in terms of origin, epigenome, and stem fraction architecture [166,169,170]. The dominant mechanisms of resistance also differ. IDH-wild-type glioblastoma is commonly associated with state plasticity and mesenchymal shift [86,103]. IDH-mutant astrocytoma more frequently follows a stepwise evolutionary trajectory involving OPC-like to NPC-like reprogramming, erosion of IDH-associated hypermethylation, and acquisition of MMR deficiency after temozolomide exposure [169,172,173]. A feature common to all classes is that standard therapy does not eliminate the stem fraction but enriches the residual tumor with it [24,131,216]. Moreover, recurrence is the result of coevolution of GSCs and the niche, with mesenchymal shift as the most reproducible event of progression [193,217,218]. The practical implication for therapeutic strategies in IDH-mut gliomas involves targeting the compact undifferentiated pool and IDH-dependent epigenetics. In contrast, IDH-wild-type glioblastomas are characterized by greater heterogeneity and plasticity; therefore, their treatment requires a more comprehensive approach: simultaneous suppression of mesenchymal plasticity, DNA repair, and stem cell signaling pathways, along with the elimination of resistant stem cells, since targeting only a single cell state is compensated by the activation of alternative states [28,46,171].

5. From Resistance to Vulnerability: Glioma Stem Cells as a Target for Personalized Therapy

Glioma stem cells are regarded as the central mechanism of resistance and recurrence of high-grade gliomas [12,23]. Possessing self-renewal capacity and their own resistance to temozolomide and ionizing radiation [9], they maintain tumor heterogeneity [195,196]. Standard therapy, directed primarily at differentiated, rapidly proliferating cells, leaves the relatively quiescent GSC pool largely intact (Section 3.1.4). The surviving cells invade the surrounding brain tissue, which is inaccessible to local control, and repopulate the tumor, which clinically manifests as inevitable recurrence.

GSCs are not merely one of the targets but a key condition for long-term disease control: without elimination of this pool, any therapy directed at the tumor mass remains palliative. This substantiates the need to incorporate GSC-specific strategies into the personalized treatment plan as a means of preventing recurrence, and not only as an option upon progression.

5.1. Therapy Directed at a Defined Molecular Target

In preclinical studies, a number of inhibitors of transcription factors or of indirect regulatory molecules targeting glioma stem cells are being investigated. There are several targets that are being studied as potentially therapeutically beneficial.

SOX2 is a key transcription factor for maintaining GSC stemness. Recent studies have shown that the Par6 protein maintains GSC stemness by interacting with SOX2 and enhancing the transcriptional activation of EGFR. Par6i-P1 is a specific inhibitory peptide that selectively disrupts the interaction between the Par6 and SOX2 proteins [219]. Blockade of this complex suppresses the self-renewal capacity of GSCs and reduces their stemness markers (Nestin, CD133, OCT4). The use of Par6i-P1 in combination with temozolomide

helps overcome tumor resistance to chemotherapy and effectively suppresses tumor growth in vitro and in vivo.

The non-receptor tyrosine kinase BMX is actively expressed in glioblastoma stem cells, whereas its level is minimal in neural progenitors and astrocytes. BMX activates the STAT3 signaling pathway to maintain the self-renewal and tumorigenic potential of GSCs. It has been shown that suppression or knockdown of BMX impairs GSC proliferation and neurosphere formation, reduces tumor growth in vivo, and increases sensitivity to radiotherapy [220,221]. In preclinical models, the small-molecule inhibitor ibrutinib is capable of irreversibly blocking BMX, suppressing the BMX–STAT3 signaling pathway, reducing tumor growth, and overcoming GSC radioresistance with minimal toxic effects on normal neural progenitor cells [221].

Bmi-1 (B-cell-specific Moloney murine leukemia virus insertion site-1) belongs to the highly conserved polycomb group (PcG) protein family and functions as a transcriptional repressor of various genes, including those regulating cell proliferation and differentiation. Studies in glioblastoma have shown that Bmi-1 is overexpressed and can serve as a prognostic biomarker. Bmi-1 plays a critical role in modulating the tumor microenvironment and maintaining the plasticity of glioma stem cells [222]. High expression levels of BMI1 are characteristic mainly of CD133-positive cell fractions. This protein coordinates gene networks that maintain the cell in a state of stemness and prevent its differentiation. Artificial knockdown of BMI1 initiates the differentiation process. The use of small molecules (e.g., PTC-209 or PTC596) leads to a decrease in BMI1 levels. In vitro and in vivo experiments show that this arrests neurosphere growth, suppresses metastasis and cell migration capacity, and also induces massive apoptosis within the tumor [223].

Since both EZH2-dependent (proneural) and BMI1-dependent (mesenchymal) stem cells coexist within the tumor, blocking only one pathway leads to tumor adaptation. Simultaneous suppression of EZH2 and BMI1 showed a synergistic effect, eliminating both populations of glioma stem cells and substantially prolonging survival in animal models [212].

Inhibition of cyclin-dependent kinase 9 (CDK9) is regarded as one of the most promising therapeutic strategies for eliminating glioma stem cells. CDK9 inhibition leads to a sharp decrease in the expression of oncogenes and stemness markers such as SOX2, SOX9, EGFR, and PDGFR-A [224]. Experiments on orthotopic xenografts show that systemic administration of CDK9 inhibitors significantly suppresses glioma growth and prolongs survival without pronounced neurotoxicity [225]. The CDK9 inhibitor zotiraciclib showed excellent results at preclinical stages (elimination of glioma stem cells, reduction of the levels of the oncoproteins c-Myc and Mcl-1), and it was advanced to the stage of active clinical trials [226].

Although these findings provide a strong preclinical rationale for targeting GSC-associated pathways, their clinical translation remains limited. Early-phase clinical studies have therefore been initiated to evaluate the safety, tolerability, dosing, and preliminary antitumor activity of the most advanced candidates. A completed phase Ib/II trial (NCT02942264) evaluated the safety, tolerability, and dosing of zotiraciclib in combination with TMZ chemotherapy in adult patients with recurrent anaplastic astrocytoma (grade 3) and glioblastoma (grade 4) [226]. A Phase Ib/II study in elderly patients with newly diagnosed glioblastoma, as well as in patients with recurrent disease [227], showed low clinical activity as monotherapy in recurrent glioblastoma and significant toxicity in combination regimens. An ongoing phase I/II clinical trial (NCT05588141) is evaluating the safety and efficacy of zotiraciclib monotherapy in patients with recurrent IDH1- or IDH2-mutant gliomas. This is an open-label, phase I/II study that investigates the efficacy and safety of zotiraciclib as monotherapy for patients with recurrent gliomas (grade 2–4) carrying muta-

tions in the IDH1 or IDH2 genes. As monotherapy, zotiraciclib demonstrated an excellent safety profile, and the study is continuing at the Phase II stage.

Ibrutinib, initially approved for the treatment of hematologic malignancies (such as lymphoma and chronic lymphocytic leukemia), has successfully progressed from preclinical testing in glioblastoma to clinical trials in humans. The EQUILIBRIUM study (Phase I, NCT03535350) demonstrated the safety and tolerability of ibrutinib in combination with TMZ, and the median overall survival for all participants was 21.9 months [228]. Although pharmacological inhibition of BMX effectively targets the GSC pool, the clinical use of ibrutinib is limited by its toxic side effects. Its administration may cause atrial fibrillation, bleeding, and infectious complications. Therefore, the focus of researchers is now shifting toward identifying the optimal therapeutic window and developing more selective inhibitors.

The molecule PTC-209 showed excellent results in vitro but proved unstable in vivo and had poor bioavailability, which makes it difficult to use in its classical form in vivo [223]. The molecule PTC596 demonstrates increased stability, easily crosses the blood–brain barrier, and has advanced to a phase I clinical trial in pediatric high-grade gliomas (NCT03605550). Nevertheless, independent molecular studies of glioblastoma have revealed a previously unrecognized mechanism of action for PTC596: it functions as an inhibitor of microtubule assembly (by binding to tubulin) and causes mitotic arrest. At certain concentrations, this may inadvertently push the remaining cells toward the epithelial–mesenchymal transition (EMT), which requires strict dose calibration. Despite the biological activity of the drug and the completion of all phase Ib objectives (safety, pharmacokinetics), the developing company shifted its focus of attention.

5.2. Cell Therapy Against Glioma Stem Cells

Cell therapy approaches targeting glioma stem cells are currently under intensive investigation, primarily through harnessing the capabilities of innate immunity or genetically modified immune cells, thereby enabling the overcoming of immune tolerance and resistance maintained by GSCs.

5.2.1. CAR-T Cell Therapy: Targeting Surface Antigens of GSCs

Chimeric antigen receptor T-cell (CAR-T) therapy targeting GSCs is currently transitioning from preclinical studies to first-in-human clinical trials. The main strategy consists of addressing recurrence and resistance driven by GSC heterogeneity through dual targeting (e.g., CD44/CD133), while simultaneously reducing toxicity via local administration.

Studies indicate that in GSCs, the expression of CD44 and CD133 demonstrates an inverse correlation: CD44 is relatively highly expressed in the mesenchymal subtype, whereas CD133 is relatively highly expressed in the proneural subtype. This implies that CAR-T cells targeting a single antigen may miss the other GSC subtype, which prompted the development of a dual-targeting strategy. Both antigens are highly enriched in glioblastoma specimens and exhibit limited expression in normal tissues. The antitumor efficacy of CAR-T cells was evaluated in patient-derived GSCs, intracranial xenograft models, patient-derived xenograft models, and glioblastoma organoids. Locoregionally administered CAR-T cells induced long-term tumor regression with the desired safety outcomes. Patient-derived autologous CAR-T cells showed robust antitumor activity against organoids [229].

This research has evolved into clinical trials that are currently in phase I/II (NCT07318818). A phase I study (NCT05577091) targeting GSCs for the treatment of recurrent grade 4 glioma demonstrated safety and selective efficacy [230,231]. The current stage focuses on determining the maximum tolerated dose and the recommended phase II dose, as well as on characterizing the dynamics of CAR-T cells in the cerebrospinal fluid.

The AC133 epitope of the CD133 protein serves as a marker of cancer stem cells in glioblastoma. A chimeric antigen receptor (CAR) was generated against it, and T cells bearing this receptor eliminated glioblastoma stem cells in vitro and in vivo [232]. When such CAR-T cells came into direct contact with patient-derived glioblastoma stem cells, their expression of the CD57 molecule—a marker of mature T cells—increased. It turned out that CD57 appeared on activated T cells upon contact with CD57-bearing glioblastoma stem cells, but not upon contact with conventional glioma lines lacking CD57. However, it was shown that after differentiation of glioblastoma stem cells, CD57 did not disappear from them. Moreover, even differentiated glioblastoma cells still induced the appearance of CD57 on CAR-T cells and other activated T cells. Apparently, CD57 may appear on activated human T cells simply upon contact with target cells bearing CD57 and is not a specific marker of stem cells. Nevertheless, it may serve as a target for therapy against $CD57^+$ GSCs.

A major limitation of CAR-T therapy targeting GSC-associated antigens is antigen heterogeneity and the potential for antigen escape. Because GSCs can switch between transcriptional states, CAR-T cells targeting a single antigen may allow antigen-low or antigen-negative cells to persist [109,233]. Antigen escape may also result from antigen downregulation, masking, shedding, or selection of antigen-negative subclones; dual-antigen targeting, such as CD44/CD133, may partially reduce but not eliminate this risk [234,235]. Off-tumor toxicity is an additional concern, as candidate antigens including CD133, EGFR and IL13Rα1 may be expressed at low levels in normal tissues [236,237]. Locoregional delivery may reduce systemic exposure but does not completely eliminate on-target/off-tumor effects [238]. Finally, the immunosuppressive tumor microenvironment and the blood–brain barrier can impair CAR-T-cell expansion, persistence, cytotoxic function, and trafficking [237,239]. Therefore, the clinical benefit of GSC-directed CAR-T therapy will depend not only on antigen selection but also on strategies that improve trafficking, persistence, and function within the immunosuppressive tumor microenvironment [233,234].

5.2.2. NK Cell Therapy

Evidence suggests that NK cells preferentially attack stem-like cancer cells rather than differentiated ones [240], including in glioblastoma [241].

Unlike CAR-T, NK cells offer the advantage of an off-the-shelf allogeneic product and a lower risk of cytokine storm, but face challenges of persistence in the body and the immunosuppressive tumor microenvironment. NK cell therapy targeting glioma stem cells is currently at the preclinical stage, but cell product candidates (e.g., FT538) have already emerged, demonstrating preliminary efficacy in GSC models and preparing for clinical translation. FT538 is an NK cell product derived from induced pluripotent stem cells, developed by Fate Therapeutics, with three key modifications: a high-affinity non-cleavable CD16 Fc receptor, a membrane-bound IL-15/IL-15Rα fusion protein (enhancing persistence), and CD38 knockout (enhancing metabolic fitness). In vitro and in vivo testing on patient-derived GSC lines showed selective efficacy.

Using the same technology as for T cells, NK cells are genetically modified to target specific antigens expressed by tumor stem cells, such as EGFR, EGFRvIII, HER2, GD2, IL13Rα2, and CD133. Unlike T cells, CAR-NK cells retain their natural receptors (e.g., NKG2D, NKp30, NKp46). This enables them to kill tumor cells even when the glioma "hides" the primary antigen to which the CAR receptor is directed. The modified killers induce direct lysis (destruction) of glioma stem cells through the release of perforin and granzymes, as well as through the activation of death receptors (e.g., TRAIL). In preclinical models, CAR-NK therapy demonstrates high primary cytotoxicity and the ability to selectively eliminate the most resistant pools of glioma stem cells [242].

5.2.3. Dendritic Cell Vaccines: Active Immunization Targeting GSC Antigens

Dendritic cell vaccines targeting GSCs represent a strategy of active specific immunotherapy, the key idea of which is to "educate" the patient's immune system to recognize and eliminate precisely the subpopulation of tumor cells (or at least to engage this subpopulation) responsible for therapy resistance and recurrence. Dendritic cells (DCs) are the most effective professional antigen-presenting cells in the body, capable of triggering the activation of naive T cells [243,244], and this is precisely why their potential is particularly great with respect to glioblastoma [245–247], where existing therapeutic strategies remain extremely limited. First of all, dendritic cells accomplish this task by virtue of their professional specialization: antigen capture, processing, and presentation are their innate function rather than an incidental property. Moreover, antigen loading can occur through different pathways—via efferocytosis and phagocytosis—while evolutionarily refined mechanisms of intracellular processing ensure rapid antigen presentation on MHC molecules. It is also significant that dendritic cells express a broad array of costimulatory molecules, and these signals are duplicated, which increases the probability of full-fledged activation of the T-cell arm against specific antigens and reduces the risk of signal loss. Finally, dendritic cells possess the ability to rapidly proliferate in the body and to migrate readily throughout it, acting literally as scouts and detectives that promptly deliver information to the lymph nodes, while dendritic cell vaccine therapy itself is characterized by a favorable safety profile—virtually without adverse effects.

The development of therapeutic dendritic cell vaccines involves the stages of collecting biomaterial from the patient: collecting blood components to obtain dendritic cells and collecting a tumor sample to educate the immune system. The targets of such vaccines may be individual GSC antigens or the full spectrum of glioma stem cell antigens.

GSC antigen-based vaccines.

Unlike whole-lysate vaccines, the single-antigen approach aims to educate the immune system to recognize specific distinctive proteins.

One of the most extensively studied GSC antigens is CD133, a surface protein associated with the self-renewal and radioresistance of glioma stem cells. In an experimental study by Do et al. [248], mRNA technology was used to create a vaccine, in which dendritic cells were transfected with modified CD133 mRNA. The distinctive feature of this approach is that the antigen is synthesized within the dendritic cell itself, which ensures efficient presentation of its fragments via both MHC I and MHC II. This resulted in strong simultaneous activation of both $CD8^+$ and $CD4^+$ T cells, providing a potent and durable immune response that suppressed the spread of CD133-positive GSCs and tumor growth in humanized mice. In human glioma cultures, targeting against CD133 also demonstrated efficacy in preclinical models [249].

Interestingly, Hua et al. [250] demonstrated that a CD133-enriched antigen from glioma stem cells derived from irradiated tumor cells elicited significantly more pronounced immune responses. It is a known fact that irradiation can induce immunogenic cell death (ICD) or modify the antigenic profile, making the antigen more effective for DC activation [251,252]. We believe that in this study, it was precisely this effect that led to a more immunogenic response. As a result, such dendritic cells exhibited higher cytotoxicity compared with those loaded with antigens from non-irradiated cells.

When developing vaccines based on individual antigens, it is important to consider the limitations of such systems. Although CD133 is an attractive target, its expression on normal hematopoietic stem cells requires caution. At the same time, early clinical studies have reported preliminary safety and immunogenicity data [253].

Whole GSC lysate-based vaccines.

The whole glioma stem cell lysate approach differs from single-antigen-based strategies in that dendritic cells are loaded with the entire spectrum of proteins present in tumor stem cells. This enables the immune system to recognize multiple targets simultaneously, reducing the risk of tumor "escape" upon loss of a single antigen. In experimental in vivo models, this approach demonstrates high efficacy [254]. Furthermore, upon induction of ICD in glioma stem cells and the subsequent creation of a vaccine based on this lysate, the stem cell fraction acquires immunologically significant antigens that distinguish it from more differentiated tumor cells, which enhances the effect of the vaccine in vivo [255].

Furthermore, in a preclinical translational study by Zhou and colleagues, the induction of GSC apoptosis was used as a strategy to generate a whole-antigen lysate [256]. Induction of apoptosis could also trigger ICD in glioma stem cells, which increased immunogenicity. This study demonstrated that GSC-DCs enhanced IFN-γ production by T cells. The authors showed that the dendritic cell vaccine could promote the inhibition of glioma stem cell proliferation by T cells in vitro and suppress tumor growth in vivo.

Despite its theoretical appeal, the approach under consideration retains serious barriers that must be taken into account when interpreting the available data and planning further research. Glioblastomas create a potent immunosuppressive environment that suppresses the effector T cells induced by the vaccine and is capable of suppressing part of the achieved immune response. To this is added immune tolerance: most GSC antigens are "self", and the body possesses mechanisms for suppressing a strong response to them, which limits the strength of the developing cytotoxic arm. A substantial problem is represented by tumor plasticity, since GSCs can change their antigenic profile. At the time of recurrence, the tumor may differ genetically from the original tumor from which the lysate for the vaccine was obtained, which creates a risk of antigenic escape. Finally, one cannot ignore manufacturing complexities: the creation of autologous vaccines from a patient's cells is an objectively expensive, labor-intensive, and personalized process requiring GMP conditions and appropriate infrastructure. Thus, dendritic cell vaccines against GSCs remain a strategy with real but not unconditional potential: their clinical efficacy will be determined not only by the strength of the induced immune response but also by the ability to overcome the suppressive microenvironment, antigenic tolerance, and tumor plasticity, as well as the economic and technological feasibility of personalized manufacturing.

Traditional dendritic cell vaccination strategies face a fundamental limitation: exogenous DCs poorly penetrate the blood–brain barrier, and the immunosuppressive microenvironment of glioblastoma rapidly inactivates their function. A fundamentally different approach consists in not administering exogenous DCs, but rather directly reprogramming glioma stem cells within the tumor into cells possessing DC function—iDC-APCs. The key advantage of this strategy is that the tumor cells themselves carry the entire spectrum of tumor antigens, including private neoantigens. In immunocompetent mice, intratumoral administration of iDC-APCs significantly suppressed tumor growth and prolonged survival, whereas in immunodeficient mice the effect was absent, which proves dependence on the adaptive immune response. Analysis of the mechanisms showed that after iDC-APC treatment, the infiltration of $CD4^+$ T cells and activated $CD8^+$ CTLs in the tumor microenvironment significantly increases; that is, a "cold tumor" is converted into a "hot" one [257].

5.3. Limitations and Challenges

Despite the key role of glioma stem cells in tumor initiation, resistance, and recurrence, therapeutic strategies targeting this pool face a number of fundamental limitations. Stemness in glioma is not a fixed property of a single subpopulation but a dynamic, reversible state dependent on genetic background, epigenetic status, and the microenvironment. It should

be noted that the limitations considered below are intrinsic to the concept of targeting the stem-like compartment itself. There are several serious limitations to the anti-GSC approach.

Molecular evolution and target reversibility. Under therapeutic pressure, GSCs switch between transcriptional states, and suppression of one pathway is compensated by activation of parallel molecular cascades. Such redundancy of signaling pathways and their crosstalk limits the efficacy of monotherapy: an intervention directed at a single marker or pathway rarely provides a durable effect, since alternative stem-like subclones persist or re-emerge [121,187].

Quiescent stem cells as a pharmacological problem. A significant proportion of GSCs resides in a quiescent or slowly cycling state, which makes them less sensitive to agents acting on proliferating cells. After standard treatment, it is precisely the "dormant" GSCs in hypoxic and perivascular niches that may reactivate and repopulate the tumor. This creates a dilemma: agents against dividing cells do not affect the quiescent fraction, whereas agents that "awaken" GSCs may enhance their proliferation and invasiveness [258].

Dedifferentiation capacity of glioma tumor cells. Phenotypic plasticity enables cells under therapeutic pressure to reacquire stem-like properties, including the expression of CD133, SOX2, and Nestin, even if they did not initially express them. The elimination of a single GSC subpopulation thus does not guarantee tumor eradication: compensatory mechanisms rapidly restore the stem cell pool [259].

Therapeutic window: shared targets of GSCs and normal neural stem cells. The most dangerous problem, in our opinion, is severe neurotoxicity. Most of the markers and molecular pathways used for targeting GSCs are simultaneously inherent in and critical for normal neural stem and progenitor cells, which substantially limits the selectivity of therapy [259]. Systemic suppression of these pathways and targeting of the stem component may prospectively lead to neurotoxicity, cognitive impairment, and dysfunction of the normal brain and other organs with a rapidly renewing cell pool. The therapeutic window for most GSC-directed strategies is thus extremely narrow, as confirmed by the clinical failures of γ-secretase inhibitors due to dose-limiting toxicity [260]. However, it is unknown how delayed the neurotoxicity effect will be; possibly, in the context of the median survival of patients with high-grade gliomas, the potential benefit of GSC elimination may outweigh the associated adverse effects, especially if drug delivery can be localized or temporal windows of exposure can be exploited.

Limited ability to identify and quantify GSCs. A further limitation is the difficulty of identifying and quantifying GSCs within the bulk tumor. In the absence of a universal marker, and given the dynamic and reversible nature of the stem-like state, GSCs cannot be reliably defined based on the expression of individual molecules alone. Their characterization requires a combination of functional assays, molecular profiling, and analysis of transcriptional states, which limits the accurate assessment of both the proportion of GSCs and their functional status in clinical samples. These limitations are compounded by constraints of experimental models: single-cell transcriptomics captures only a snapshot of RNA expression and may be affected by dissociation-induced stress, batch effects, and incomplete recovery of rare or niche-associated cells; GSC cultures and organoids do not fully reproduce tumor diversity and spatial organization; xenografts simplify the human immune, vascular, and stromal microenvironment; and surgical specimens represent only part of the tumor and may be influenced by treatment, hypoxia, and regional heterogeneity. Therefore, findings from individual models should be interpreted as complementary rather than definitive evidence of GSC biology in patients.

Thus, the limitations listed above, together with the biological complexity, multifactorial nature, and high aggressiveness of gliomas, no longer allow the contribution of GSCs to disease progression and therapy resistance to be ignored. This requires the development of a multilevel and dynamically adaptable therapeutic strategy.

6. Conclusions

We argue that GSCs should be regarded not as a static subpopulation but as an adaptive core of malignant progression and therapeutic resistance. This plasticity simultaneously represents the principal barrier to durable disease control and the most promising source of therapeutic vulnerabilities. The future of high-grade glioma treatment will depend on whether clinical practice can become as dynamic, multi-layered, and adaptive as the tumor itself.

It can also be anticipated that the WHO classification of central nervous system tumors will be revised in the future. It may incorporate new data on distinct glioblastoma subtypes, taking into account the stem cell component. It is becoming increasingly evident that these subtypes represent distinct diagnoses requiring different therapeutic approaches. However, implementing this approach requires the development of standardized and clinically validated methods for the diagnosis and study of this population. Such methods must accurately reflect the cellular composition and molecular markers of the GSC population.

Existing methods for identifying this complex population provide only a single "snapshot" of the tumor and do not capture its spatial and temporal dynamics. A static description is fundamentally incapable of capturing the plasticity of the GSC population, which is likely to determine the clinical behavior of the tumor. A solution may lie in combining several approaches—spatial transcriptomics and single-cell multi-omics together with liquid biopsy—which enables longitudinal, non-invasive profiling over time. However, at present these approaches remain expensive and unavailable in routine clinical practice. Refinement of these methods will make their implementation in clinical practice possible and will allow the therapeutic strategy to be adapted to the current composition of the tumor at each stage of the disease, thereby substantially improving the quality of medical care.

Data accumulated in recent years also dictate the need to revise therapeutic strategies. Therapy, and immunotherapy in particular, must begin to take into account a deeper subclassification of high-grade gliomas. This requires the joint efforts of clinicians, molecular oncologists, and biotechnologists: only such a transition—to an adaptive strategy that takes into account the state of GSCs—will make it possible to move closer to durable disease control and to turn personalized therapy into a clinical reality.

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Abbreviations

The following abbreviations are used in this manuscript:

A2B5	A2B5 ganglioside epitope
ADM	Adrenomedullin
ALDH1A3	Aldehyde Dehydrogenase 1 Family Member A3
ANXA1/ANXA2	Annexin A1/Annexin A2
APOD	Apolipoprotein D
APOE	Apolipoprotein E
AQP4	Aquaporin 4
ASCL1	Achaete-Scute Family BHLH Transcription Factor 1
ATM/ATR	Ataxia Telangiectasia Mutated/ATM and Rad3-Related
ATRX	ATRX Chromatin Remodeler
BCL-xL	B-cell Lymphoma-extra large
BER	Base Excision Repair
BIM	BCL2 Like 11
BMF	BCL2 Modifying Factor
BMX	BMX Non-Receptor Tyrosine Kinase
BRCA1	BRCA1 DNA Repair Associated
CCNB1	Cyclin B1
CD109	CD109 Molecule
CD133	Prominin 1 (CD133)
CD15/SSEA-1	CD15 Molecule/Stage-Specific Embryonic Antigen 1
CD24	CD24 Molecule
CD36	CD36 Molecule
CD44	CD44 Molecule
CD49f	Integrin Subunit Alpha 6
CDK4	Cyclin-Dependent Kinase 4
CDKN2A/B	Cyclin-Dependent Kinase Inhibitor 2A/2B
CHI3L1 (YKL-40)	Chitinase 3 Like 1 (YKL-40)
CHK1/2	Checkpoint Kinase 1/2
CIC	Capicua Transcriptional Repressor
CLU	Clusterin
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9
CSPG4/NG2	Chondroitin Sulfate Proteoglycan 4/Neuron-Glial Antigen 2
DCX	Doublecortin
DLL3	Delta Like Canonical Notch Ligand 3
DNA	Deoxyribonucleic Acid
DR4/DR5	Death Receptor 4/Death Receptor 5
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor variant III
EZH2	Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit
FN1	Fibronectin 1
FOS	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit
FUBP1	Far Upstream Element Binding Protein 1
GFAP	Glial Fibrillary Acidic Protein
GLI2	GLI Family Zinc Finger 2
GPC	Glial Progenitor Cell
GPX4	Glutathione Peroxidase 4
GSC	Glioma Stem Cell
HDAC	Histone Deacetylase
HEPACAM	Hepatocyte Cell Adhesion Molecule
HES1	Hes Family BHLH Transcription Factor 1
HIF	Hypoxia-Inducible Factor

HILPDA	Hypoxia-Inducible Lipid Droplet Associated
ICD	Immunogenic Cell Death
ID1	Inhibitor of DNA Binding 1
ID3	Inhibitor of DNA Binding 3
IDH1/2	Isocitrate Dehydrogenase 1/2
IDHwt	IDH-wildtype
JUND	JunD Proto-Oncogene, AP-1 Transcription Factor Subunit
KDM	Lysine Demethylase
L1CAM	L1 Cell Adhesion Molecule
LGALS1/LGALS3	Galectin 1/Galectin 3
MCL-1	Myeloid Cell Leukemia 1
MCM2	Minichromosome Maintenance Complex Component 2
MET	MET Proto-Oncogene, Receptor Tyrosine Kinase
MGMT	O-6-Methylguanine-DNA Methyltransferase
MLC1	Modulator of VRAC Current 1
MMP14	Matrix Metallopeptidase 14
MMP2	Matrix Metallopeptidase 2
MMP9	Matrix Metallopeptidase 9
MMR	Mismatch Repair
NCAM1	Neural Cell Adhesion Molecule 1
NDRG1	N-Myc Downstream Regulated 1
NES	Nestin
NF1	Neurofibromin 1
NKG2D	Natural Killer Group 2 Member D
NKp30	Natural Cytotoxicity Receptor NKp30 (NCR3)
NKp46	Natural Cytotoxicity Receptor NKp46 (NCR1)
NOTCH1	Notch Receptor 1
NSC	Neural Stem Cell
OLIG1	Oligodendrocyte Transcription Factor 1
OLIG2	Oligodendrocyte Transcription Factor 2
OPC	Oligodendrocyte Progenitor Cell
Par6	Partitioning defective 6 homolog
PDGFRA	Platelet Derived Growth Factor Receptor Alpha
POU3F2	POU Class 3 Homeobox 2
PTEN	Phosphatase and Tensin Homolog
RAD51	RAD51 Recombinase
S100B	S100 Calcium Binding Protein B
SALL2	Spalt Like Transcription Factor 2
scRNA-seq	Single-cell RNA sequencing
SLC1A3/GLAST	Solute Carrier Family 1 Member 3/Glutamate Aspartate Transporter
SMO	Smoothened, Frizzled Class Receptor
SOX1	SRY-Box Transcription Factor 1
SOX10	SRY-Box Transcription Factor 10
SOX11	SRY-Box Transcription Factor 11
SOX2	SRY-Box Transcription Factor 2
SOX4	SRY-Box Transcription Factor 4
SOX9	SRY-Box Transcription Factor 9
SPARCL1	SPARC Like 1
STMN2	Stathmin 2
SVZ	Subventricular Zone
TCGA	The Cancer Genome Atlas
TERT	Telomerase Reverse Transcriptase
TET	Ten-Eleven Translocation methylcytosine dioxygenase
TMZ	Temozolomide
TNC	Tenascin C

TOP2A	DNA Topoisomerase II Alpha
TP53	Tumor Protein P53
TUBB3	Tubulin Beta 3 Class III
VEGFA	Vascular Endothelial Growth Factor A
VIM	Vimentin
WHO2021	WHO Classification of Tumors of the Central Nervous System, 5th edition (2021)

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