









REVIEW ARTICLE

Instability as a source of diversity in glioblastoma

Isabele Pagani Pavan[†], Bianca Paulino Campanharo[†],
Felipe Ataides Mion[†], Flávio dos Santos Alvarenga[†],
Matheus Correia Casotti[†], Lorena Souza Castro Altoé[†],
Íuri Drumond Louro[†], and Débora Dummer Meira^{*}

Center for Human and Molecular Genetics, Federal University of Espírito Santo, Vitória, Espírito Santo, Brazil

Abstract

Glioblastoma (GB) is one of the most aggressive and prevalent primary brain tumors in adults, accounting for approximately 15% of all brain tumors and 78% of all malignant brain tumors. This article aims to review the current understanding of GB, with a particular focus on the role of tumor heterogeneity in treatment resistance, the underexplored role of ploidy variation in GB biology, and the emerging and individualized therapeutic strategies designed to enhance treatment efficacy. A narrative review of the literature was conducted, synthesizing recent findings on GB development, diagnosis, and therapeutic innovations. Special attention was given to studies that discuss: molecular and cellular characteristics of GB; novel therapeutic approaches, including targeted inhibitors, combination therapies, and virotherapy; the clinical significance of inter- and intratumoral heterogeneity. The results indicate that GB's resistance to therapy is closely linked to its pronounced heterogeneity, both at the inter- and intratumoral levels. Variability in ploidy among tumor cells has emerged as a potential contributor to treatment failure, yet it remains insufficiently investigated. Promising therapeutic developments include inhibitors targeting specific proteins or signaling pathways, synergistic combination treatments, and oncolytic virotherapy. Although these approaches show potential, most remain in early-stage research or clinical trials. The review underscores the importance of precision medicine in overcoming the barriers posed by GB's heterogeneity. While emerging therapies are promising, the development of individualized, patient-specific strategies remains essential.

Keywords: Cancer; Ploidy variation; Polyploidy; Multinucleation

[†]These authors contributed equally to this work.

***Corresponding author:**
Débora Dummer Meira
(debora.meira@ufes.br)

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1. Introduction

Glioblastoma (GB) is one of the most aggressive and common central nervous system tumors in adults, posing a significant health threat. This malignant brain neoplasm occurs most frequently in patients over 65 years of age and, unfortunately, has an average survival rate of 12–15 months after diagnosis. This tumor represents approximately 15% of all diagnosed brain tumors and a worrying 78% of all malignant tumors.¹

An important factor to be considered in the study of GB is the morphological multiplicity (also linked to the variation of ploidy), which refers to the presence of different subpopulations (such as cells with different numbers of chromosome sets) in

tumor cells. In the case of GB, it is common to observe a high variation of ploidy.² Such complexity of variation is closely related to tumor heterogeneity.²

This intratumoral diversity can manifest itself in significant differences in terms of genetics, phenotype, and biological behavior of cancer cells in the face of stresses, adding layer of challenges in the treatment of GB, since different parts of the tumor mass may respond differently to specific therapies.²

In view of such considerations, the present review aims to address some of the aspects that contemplate the impact of ploidy variation on the development and evolution of GB, from its initiation, through diagnosis, prognosis, and, finally, discussing the current therapeutic interventions, their obstacles, and prospects for new treatments (Figure 1).

2. The influence of polyploidy on the development and treatment of GB

Polyploidy, the result of the duplication of an organism's or a cell's genome, significantly impacts genes, genomes, cells, tissues, and even entire ecosystems. Cancer can be considered the result of an error in cellular development and growth, generating a mass that consumes nutrients from nearby tissues to the point of organ failure (Figure 2).

The same principle applies to GB; however, its treatment is particularly complex due to the tumor's location in a delicate region of the brain.² Furthermore, it adds to the

difficulty of an efficient diagnosis before it progresses to more harmful stages, resulting in the late identification of most cases. This means that late identification hampers diagnosis and adversely affects management and treatment,³ which is reflected in high mortality. Consequently, old age becomes one of the determining factors for survival in the face of a set of aggressive treatments and invasive surgeries.³

Polyploidy arises from an unrepaired error or cellular adaptation to multiple internal or external stresses. Typically, 10% of these cells may have subpopulations resulting from deletions that lead to genomic and chromosomal instability (CIN),⁴ including GB that arises from multipotent stem cells that are precursors of neoplastic glial cells, originating from astrocytes.⁵

From this perspective, only polyploid cells that escape the chemical, physical, and biological barriers of the cell will be associated with cellular loss of control. These cell barriers refer to the mechanisms that normal cells use to protect themselves against aggression and treatments, such as the presence of proteins that neutralize drugs, structures that prevent the entry of harmful substances, and programmed cell death mechanisms that control growth⁶ as a possible culprit in the variation of ploidy and heterogeneity, due to the influence of GB cancer stem cells, with invasive characteristics, which can initiate the tumor in the cell subpopulation. Ploidy variations in GB occur in 1–5% of cases, mainly in children. However, more accurate diagnoses have been most frequently documented in adults.⁷ Mutations

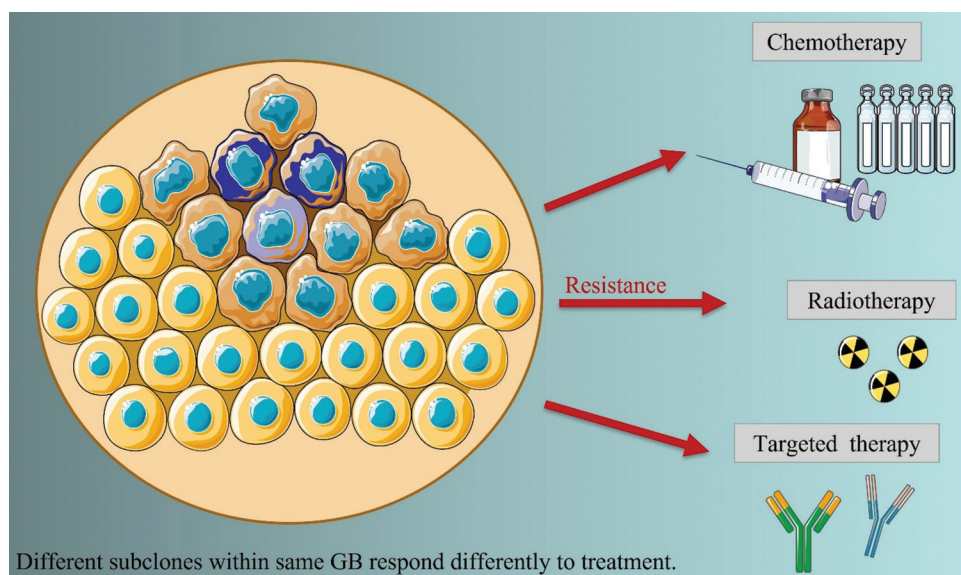


Figure 1. Intratumoral heterogeneity in glioblastoma (GB) and its impact on treatment response. Schematic representation of GB showing multiple cellular subclones with distinct molecular and functional profiles. These heterogeneous subpopulations respond differently to standard therapies such as chemotherapy, radiotherapy, and targeted therapy. Some subclones survive treatment, leading to therapeutic resistance and tumor recurrence. This diversity within the same tumor illustrates how genomic and cellular heterogeneity contributes to the poor clinical outcomes observed in GB patients. Adapted from illustrations by Servier Medical Art (<https://smart.servier.com>).

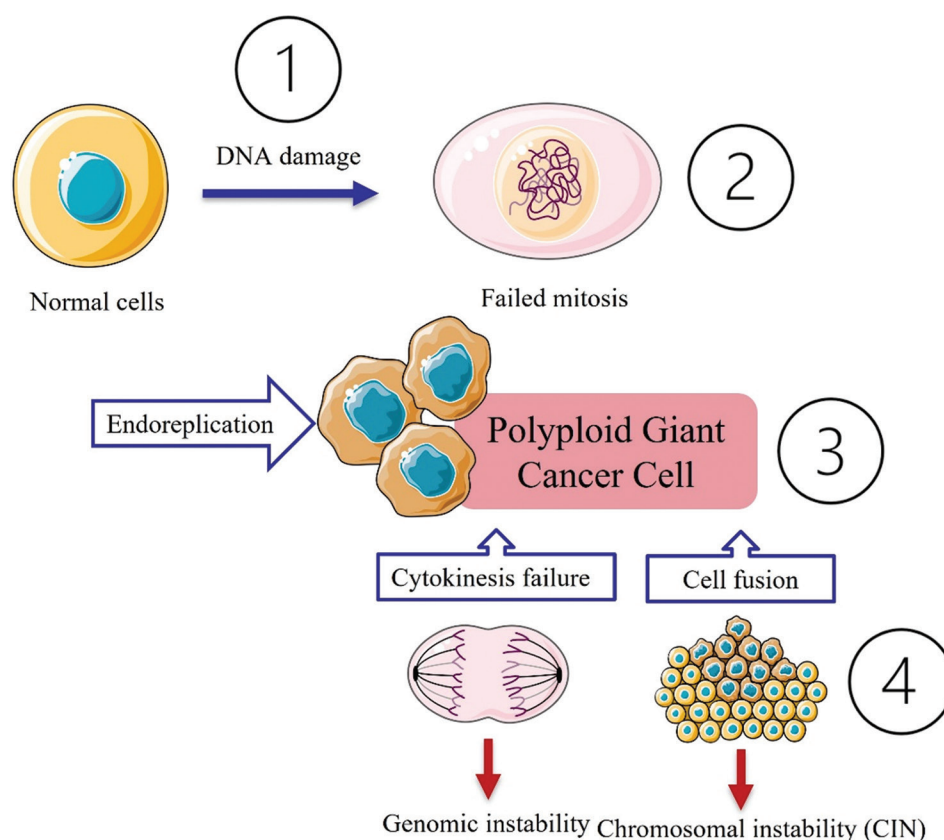


Figure 2. Mechanisms of polyploid giant cancer cell (PGCC) formation in glioblastoma. The main biological processes leading to the formation of PGCCs. (1) Normal glioblastoma cells exposed to genotoxic stress or DNA damage. (2) Failed mitosis generates multinucleated or enlarged cells. (3) PGCCs may arise through endoreplication, cytokinesis failure, or cell fusion. (4) These PGCCs contribute to genomic and chromosomal instability (CIN), promoting tumor heterogeneity and therapeutic resistance. Adapted from illustrations by Servier Medical Art (<https://smart.servier.com>).

involving giant cells in GB are rare and are characterized by the atypical presence of cells containing an abnormally high number of chromosome sets, and these giant cells can contribute to tumor aggressiveness and treatment difficulty, as they tend to resist therapies and favor the progression of the disease,⁸ exemplifying the complexity of tumor heterogeneity for diagnosis. In this line, the variation of ploidy provides a starting point for understanding the level of complexity of GB, at the pace of influencing positive outcomes of clinical studies for the development of early diagnosis and personalized and integrated treatment.

The generation of giant polyploid cells, which have a giant nucleus, multiple nuclei or micronuclei, can arise from mechanisms of endoreplication, mitotic slippage, failure of cytokinesis, cell fusion, or cell-in-cell structures (e.g., cell cannibalism), generating aggressive aneuploid daughter cells that have uncontrolled growth. These processes form benign or malignant tumors, causing deletions and mutations,⁹ and may have diverse precursors, both genetic or even as a result of therapeutic treatments that generate genotoxic stress.

Some studies observed how the response to X-ray treatments or the generation of new giant polyploid cells of this type in GB and in other tumor types, thus renewing tumorigenesis and benefiting cellular adaptation induced by stress.¹ Other tests with the combination of temozolomide (TMZ) and the gene mutation *Bloom* provided results correlating the presence of malignancy in GB, when generating deficiency of LN18 and LN229.¹⁰ In this way, the treatments themselves would be at the origin of the generation of polyploid cell giants, establishing a positive feedback process in the generation of genomic instability/resistance to treatments. Therefore, the resolution of this problem is still challenging. However, research continues to seek answers through the multidisciplinary union of different areas of knowledge.

2.1. Tumor aggression: Ploidy variation in GB, crosstalk, and heterogeneity

Aneuploidy, which manifests as the presence of an abnormal number of chromosomes in tumor cells, is a common occurrence in GB. These chromosomal alterations, with

chromosome gains or losses, contribute to the genomic heterogeneity of the tumor.¹¹ This phenomenon was evidenced in research conducted by Zhang *et al.*,¹² which explored distinct evolutionary patterns of astrocytoma and GB during recurrence. The study sequenced the exome of 65 paired primary and recurrent gliomas. The results revealed that recurrent lower-grade astrocytoma had a higher prevalence of aneuploidy alterations and acquired copy number variations compared to GB. Furthermore, patients who manifested acquired gains of specific genes during recurrence experienced shorter overall survival. These findings underscore the importance of chromosomal alterations in the evolutionary dynamics of GB, providing essential information on prognostic factors and potential therapeutic targets.

In parallel, polysomy, characterized by the presence of multiple copies of one or more chromosomes, is observed in specific GB cells, increasing genetic diversity and the tumor's ability to evade treatments.¹³ The precursor to genome duplication remains unknown. However, there are defense mechanisms that generate resistance in the event of DNA damage, resulting in the formation of cells with an abnormal number of chromosomes, such as apoptosis.¹⁴ One of these mechanisms is a kind of "confrontation" between the immune system and the cell undergoing these DNA changes. This battle between the immune system and the damaged cell helps maintain a certain stability in the cell's malformation. This may seem paradoxical, as the immune system is typically regarded as a defense mechanism that eliminates abnormal cells. However, in this case, the confrontation can balance the process of abnormal cell formation, providing a peculiar stability in the face of DNA errors.¹⁵ As a result of genome instability refers to a series of genetic alterations within a cell, encompassing modifications in the primary sequence of nucleic acids, such as mutations, insertions or deletions, as well as chromosomal rearrangements such as translocations, and aneuploidy, characterized by the loss or gain of single, multiple, or entire sets of chromosomes.¹ Thus, the term "genome instability" encompasses a broad spectrum of genetic aberrations, from subtle nucleotide changes to extreme genomic transformations.

In this scenario, CIN is a prominent feature of GB, resulting in frequent alterations in chromosome number and structure. This instability contributes to the tumor's genomic plasticity, influencing treatment response and disease progression.¹⁶ Although several anomalous pathways contribute to genome instability, such as microsatellite instability and CpG island methylator phenotype, it is undeniable that CIN or the continuous changes in chromosome complements is one of the most

prevalent, yet least understood, mechanisms.¹⁷ On the other hand, variation in gene expression resulting from ploidy variation in GB can lead to heterogeneity in the phenotypic characteristics of tumor cells, affecting tumor aggressiveness and its ability to resist therapies.¹⁶ GB heterogeneity participates in the induction of therapeutic resistance, through polyploid/multinucleated giant cancer cells polyploid giant cancer cells (PGCCs) and multinucleated giant cancer cells (MGCCs), tumor microtubules, functional syncytia, phenotypic plasticity and genomic instability, forming and enabling cellular phenotypes more adapted to the multiple stresses present in the tumor microenvironment, providing a "survival kit" to respond differently to numerous therapies.

Identifying heterogeneity in GB is crucial to understanding the complexity of these tumors. An effective approach for this characterization involves assessing glucose metabolic activity, since such analysis can be directly related to the different phases of tumor growth, as highlighted by Wu *et al.*¹⁸ Glucose plays a fundamental role as the brain's primary energy source, undergoing glycolysis to be converted to pyruvate. This pyruvate, in turn, is directed to the tricarboxylic acid (TCA) pathway, fueling the cycle and generating ATP through mitochondrial respiration. In GB cells, a striking characteristic is the preference for aerobic glycolysis, where most of the pyruvate is diverted, resulting in the production of lactate as an end product. This preference for aerobic glycolysis and the diversion of pyruvate to lactate production may be directly related to the reduced availability of glutamine in tumor cells.

Glutamine plays a crucial role in providing carbon and nitrogen for biosynthesis from glucose, both through various metabolic pathways and by entering the mitochondria to fuel TCA and generate ATP (Figure 3). GB cells are generally metabolically dependent on glutamine, and a shortage of this amino acid can induce a metabolic reprogramming that favors GB growth through glutaminogenesis,¹⁹ compromising the availability of metabolic precursors necessary for the synthesis of fundamental biomolecules. Furthermore, it is essential to investigate the metabolic pathways of GB, starting with genes related to glucose.

This cellular reprogramming caused by a lack of glutamine highlights its importance in GB biology and may have significant implications for the development of therapeutic strategies through multi-omics studies. Considering that the characteristic of polyploidy generates a conflict in the adoption of a single treatment. Later studies examined the role of polyploidy in the early stages of the tumor,²⁰ hypothesizing that polyploidy would generate

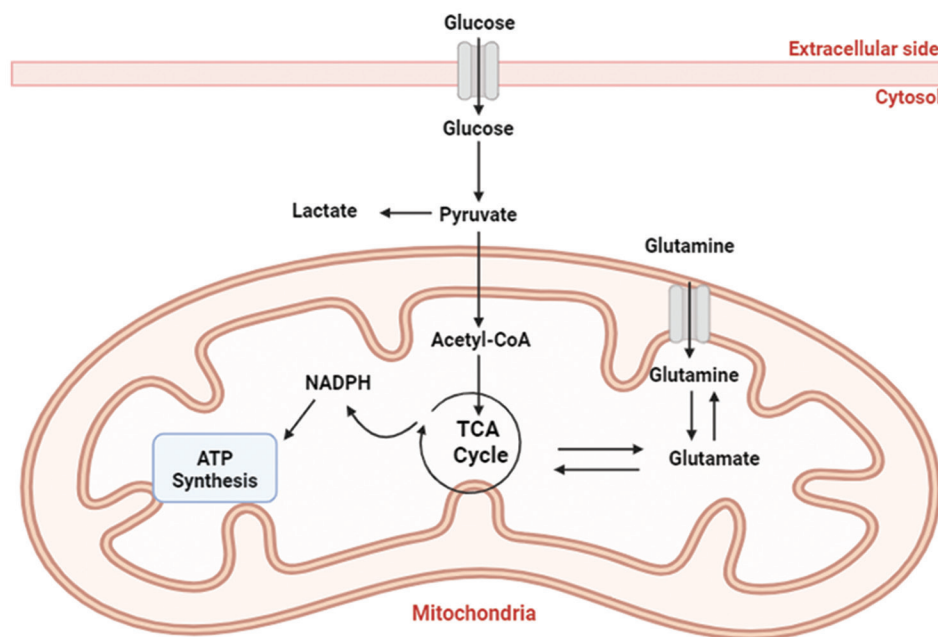


Figure 3. The relationship between glucose metabolism and glioblastoma (GB) growth. Adapted from Zarzuela *et al.*¹⁹

phenotypic jumps in response to tumor treatment, that is, cancer cells would create resistance, preventing their eradication during therapy, on the contrary to other healthy cells in the body.

The *crosstalk*, cross-cellular communication (or interconnections) between the different subpopulations of tumor and stromal cells in the surrounding environment, plays a key role in the aggressiveness of GB.²¹ A study on *crosstalk* between these cells has already demonstrated that this is driven by paracrine signals and disseminated by factors secreted²² that act through a membrane, cytoplasmic, or nuclear receptor. In particular, the role of microglial cells and astrocytes in the progression and aggressiveness of GB was studied and reviewed, confirming the existence of paracrine interactions between GB cells and the heterogeneous tumor microenvironment.²¹ This interplay between aneuploidy and metabolic reprogramming underscores the adaptive nature of GBM and helps explain its notorious resistance profile.

2.2. Clinical approach to ploidy variation in GB

Alterations that result in polyploid tumor cells lead to numerous clinical outcomes in GB. This type of tumor has an incidence of 1 in every 33,000 people, accounting for 2% of deaths/year. Studies indicate that aurora kinase genes, whose expression is altered in several types of cancer, are among those that promote cell proliferation and polyploidy in GB, in addition to acting as oncogenes in malignant gliomas. The aurora kinase (*AURK*) gene

family comprises three variants: *AURKA*, *AURKB*, and *AURKC*, all of which are serine/threonine kinases that regulate cell division. Among them, *AURKB* exhibits the highest therapeutic potential in GB, as its inhibition results in stronger suppression of GB cell proliferation and mitotic progression compared to *AURKA* and *AURKC*.²³

According to the World Health Organization, there are three subtypes of GB: gliosarcoma, conventional GB, and giant cell GB. They are highly aggressive due to their ability to undergo vasculogenic mimicry, which consists of a greater formation of blood channels by the cell mass, which, when perfused and associated with the matrix, contribute to a greater complexity in the prognosis of the disease. Such vascular channels strongly express the *Flk1* gene, which encodes the vascular endothelial growth factor receptor. Furthermore, each subtype of GB presents its genetic variants, alterations, polyploids, and numerous molecular characteristics, which influence disease progression and make clinical practice complex.⁶ Studies using Western blotting, sequencing, immunofluorescence, and flow cytometry indicate that the enzyme BLM helicase interferes with DNA repair pathways, sustaining cell proliferation even in the presence of chemotherapy.¹⁰ Furthermore, there are cases of homotypic fusion, which corresponds to the fusion of two cells generating MGCC, which secretes pro-survival factors such as p-AKT, BIRC3, and BCL-XL. MGCC survives due to inhibition of the pathway involving p-Cdk1 (Y15), which under normal conditions prevents cell cycle progression and the activation of protein kinase

B (p-AKT). In turn, this inhibits TNF(IAP)2 (BIRC3) and B-cell lymphoma 2 (Bcl-xL) protein, which is responsible for regulating apoptosis.⁶

Current treatment for GB consists of a combination of surgery, chemotherapy, and radiotherapy; however, these approaches are insufficient for complete tumor removal. Polyploid cells may exhibit resistance during chemotherapy—some enter a dormant state, while others undergo depolyploidization, express self-renewal genes, or exchange genetic material with neighboring cells. These cells are generally large and characterized by multiple nuclei and micronuclei. Their excessive genetic content, chromosomal abnormalities, and aneuploidy increase their potential for metastasis and activate multiple molecular mechanisms that enable them to evade genotoxic, non-genotoxic, and hypoxic therapies.²⁴

Conventional clinical approaches often do not recognize polyploid cells as senescent, dormant, or dying. However, specific biomarkers can be used to detect them, such as β -galactosidase associated with senescence (SA- β -gal), which identifies stress-induced premature senescent cells.¹² Furthermore, subpopulations of PGCCs/MGCCs exhibiting a wild-type TP53 phenotype and mutated PTEN, in conjunction with activation of the p53/p63/p73 pathways, can develop resistance to conventional therapies.^{1,25}

The microtubules of cells/polyploids are also extremely important for the survival and resistance of GB. They are responsible for cellular communication and the production of connective networks through cell migration. *In vitro* and *in vivo* studies, based on data obtained by RNA sequencing and proteomics, demonstrated that microtubules are produced from expression of *TGFB* (transforming growth factor beta), which is essential for the formation of microtubules in resistant cells. One way to mitigate its production is to regulate the inhibition of TGF- β 1/thrombospondin 1/suppressor of mothers against decapentaplegic (TGF- β 1/TSP1/SMAD), since TGF- β 1 stimulates TSP1, which in turn stimulates the SMAD.²⁶ However, it is necessary to consider the phenotypic complexity of the polyploid cells that influence therapeutic mechanisms.²⁷

Furthermore, it is necessary to understand the tumor microenvironment. Current clinical practice only considers the tumor itself, but not the surrounding cells. These cells interact with the GB, engendering tumor resistance to therapies by influencing pH variations, development, and tumoral composition. The tumor microenvironment provides critical insights into the relationship between oxidative phosphorylation and metabolic heterogeneity, as well as the impact of the distance between the tumor

and blood vessels on nutrient availability. Indeed, the biochemical gradient dependent on distances between tumor/vessels may influence the tumoral evolution.²¹

Despite the challenges and the need for possible paths that spawn improvements in the clinical approach, numerous current practices contribute positively to the treatment of GB, such as tumor detection techniques (e.g., histochemistry and micrometer measurement) that allow cells to be differentiated from polyploid normal cells, based on the size of the nucleus.

Regarding the diagnosis, squash cytology and immunohistochemistry are capable of distinguishing giant-cell GB from conventional GB.⁸ Next-generation sequencing, omics sciences, magnetic resonance imaging, and computed tomography can also aid in the identification and characterization of giant-cell GB, thanks to the distinctive aberrant number of chromosomes. Such preoperative analyses are crucial for the subsequent choice of chemotherapy regimen and other therapeutic actions.^{19,28} Clinically, recognizing polyploid subpopulations may improve prognostic accuracy and guide therapeutic decisions.

Understanding the molecular processes that lead to ploidy variation and genomic instability offers a theoretical foundation for therapeutic development. The same mechanisms that promote heterogeneity and resistance also reveal new molecular targets for intervention. As a result, therapeutic approaches aimed at modulating polyploidy, controlling CIN, or selectively eliminating polyploid subpopulations have emerged as promising strategies for improving the treatment of GB.

3. Therapeutic approaches to ploidy variations

Building on the molecular understanding of ploidy and genomic instability discussed in the previous section, this part explores how these biological insights translate into therapeutic strategies. GB is among the most treatment-resistant cancers, partly because of its significant genomic diversity and the presence of persistent polyploid cell subpopulations. Identifying these mechanisms has informed the creation of therapeutic strategies that either directly target polyploid cells, adjust CIN, or increase the sensitivity of resistant subclones to standard treatment.

Given what has been discussed so far, GB presents significant challenges in the field of oncology due to its high genetic heterogeneity, including variations in ploidy, and there is still no 100% effective treatment. However, several studies are being carried out to increase the survival of these patients.

In this section, we will examine the various therapeutic approaches that have been investigated to address GB, encompassing both innovative and conventional approaches (Table 1).

3.1. Therapeutic combinations

Therapeutic combinations have become a key approach to combating the most complex diseases. Pharmacological combinations exemplify new avenues for personalized, more effective treatments (Table 2).

As shown in Table 2, combination strategies targeting polyploid cells appear more promising, yet their clinical translation remains limited.

3.1.1. Adaptive therapy

Traditionally, when chemotherapy is initiated, a fixed maximum dosage is established. This has proven effective

for relatively homogeneous tumors in the short and long term, controlling the proliferation of cells adapted to the tumor microenvironment.¹² However, in many cases, such as GB, cancerous masses are heterogeneous. By eliminating cells that are not resistant to chemotherapy, an opening is created for the development of more aggressive cellular phenotypes. These phenotypes, by requiring more space to grow, escape competition from other tumor cells that are less adaptable to environmental changes induced by external stressors, such as chemotherapy.

In response to this complex scenario, adaptive therapy has emerged, an innovative approach that seeks to effectively monitor the dynamics of heterogeneous tumors (Figure 4). These tumors, characterized by a dynamic tumor microenvironment, develop resistance strategies that allow them to survive even high doses of conventional chemotherapy.¹² The effectiveness of adaptive therapy

Table 1. Innovative and conventional therapeutic approaches for glioblastoma

Therapy	Mechanism (ploidy-related)	Limitations	References
Conventional – Radiotherapy	Induction of PGCCs/MGCCs cells; senescence/ quiescence; formation of radiation-resistant cells	Intrinsic radioresistance of tumor cells; formation of resistant cells and PGCCs, making complete eradication difficult	1
Conventional – Chemotherapy	Mitotic and cytokinetic dysfunction; increased ploidy; formation of multinucleated cells	M2 macrophages induce resistance and invasion; mitotic errors promote polyploidy and cellular heterogeneity	29
Conventional – Chemotherapy with aurora/ tyrosine kinase inhibitors	Mitotic spindle disorganization; polyploidy; genomic instability	Inhibition of kinases can affect normal cells; adaptive resistance and off-target effects in non-tumor tissues	30
Innovative – Cdk1 and cytokinesis inhibitors	Prevention of proliferation of radiation-resistant cells; induction of apoptosis by blocking homotypic fusions	Difficulty in selectively delivering inhibitors to radiation-resistant cells; risk of toxicity and ineffective apoptosis in normal cells	6
Innovative – Targeted therapy for PGCCs	Study of PGCCs as therapeutic targets; differentiated sensitivity; adaptive resistance	PGCCs are still poorly characterized <i>in vivo</i> ; functional heterogeneity and limitations of preclinical models	31
Innovative – Senescence inhibition	Temozolomide-induced senescence generates polyploid cells; CDK4 inhibitor (palbociclib) blocks PGCC formation	Side effects of CDK4 inhibition in normal tissues; tumor plasticity can generate compensatory pathways	15

Abbreviations: MGCC: Multinucleated giant cancer cells; PGCC: Polyploid giant cancer cells.

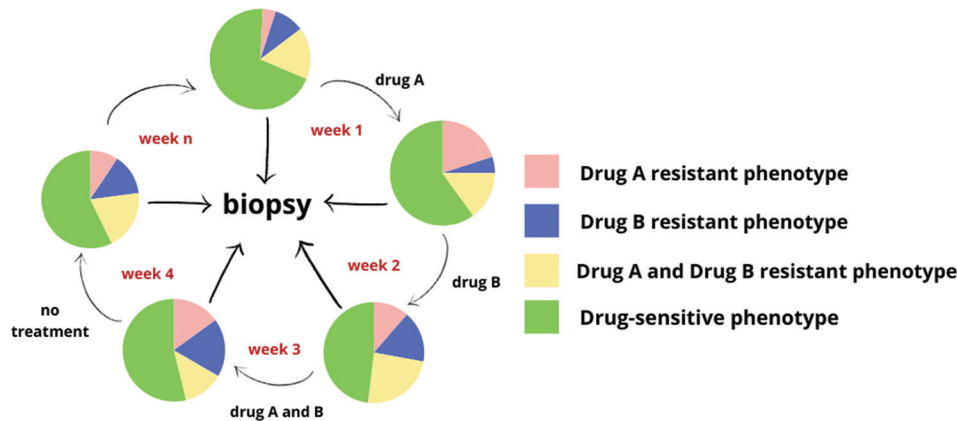


Figure 4. Tumor cycle in an adaptive therapy approach. Adapted with modifications from Zhang *et al.*¹²

Table 2. Pharmacological combination approaches for the treatment of GB

Approach	Results	References
Inhibition of the phosphatidylinositol pathway 3-kinase (PI3K) through the pan class I PI3K inhibitor, NVP-BKM120, in GB cells	<ul style="list-style-type: none"> • Potent inhibition of PI3K pathway activity independent of PTEN status • Growth inhibition, with greater sensitivity in cells with wild-type p53 • Increased survival in an intracranial glioma xenograft model 	32
Combined inhibition of the PI3K pathway and Hsp90 protein with BKM120 and HSP990, respectively	<ul style="list-style-type: none"> • Greater induction of apoptosis and reduction of cell viability compared to isolated drugs • Downregulation of AKT and BAD proteins in the PI3K-AKT pathway • Cell cycle arrest in G2/M phase • Radiosensitization and decreased tumor growth in xenograft models <i>in vivo</i> 	33
Inhibition of MERTK protein with UNC2025 in GB cells	<ul style="list-style-type: none"> • Reduction of clonal expansion, colony formation, and cell proliferation • Induction of polyploidy, senescence, and cell death 	34
Inhibition and knockdown of survivin in GB cells and glioma	<ul style="list-style-type: none"> • Cell cycle arrest in G1 phase in wild-type U87-MG cells mediated by p21 waf/cip • Increased expression of hypoxia-inducible factor 1α (HIF-1α) and the death receptor TRAIL R2, which made GB cells more susceptible to lysis by activated natural killer cells • Diminished tumor growth and longer survival of mice, with tumors up to three times smaller 	35,36
BLM deficiency in cells of GB, generated by knockout of BLM using a custom CRISPRCLEAR™ Transfection Ready kit, and the influence on the combination treatment of temozolomide and olaparib	<ul style="list-style-type: none"> • Reduction of cell proliferation • Positive expression of genes related to cell cycle regulation, DNA repair, and cell cycle arrest in the G2/M phase, in addition to senescence in BLM-deficient cells (BLM knockout) 	10
Inhibition of Myc, a transcription factor b HLHZip (basic helix-loop-helix leucine zipper transcription factors), through Omomyc, a dominant negative mutant of the Myc dimerization domain	<ul style="list-style-type: none"> • Growth limitation of transformed neuroprogenitors • Reduced cell proliferation and increased cell death • Reduction of the mitotic index and limitation of growth of patient-derived GB neurospheres from surgically resected human GB 	37
Silencing of plexin-A2 in U87MG cells	<ul style="list-style-type: none"> • Inhibition of cell proliferation and tumor formation • Cell cycle arrest, cell flattening, reduced proliferative rate of senescent cells • Cell proliferation is restored through re-expression of plexin-A2 cDNA 	38
Use of the toxin cytotoxic necrotizing factor 1 (CNF1) produced by <i>Escherichia coli</i> in GB cells	<ul style="list-style-type: none"> • Dose-dependent inhibition of cell proliferation • Reduced migration capacity, formation of multinucleated cells, activation of the senescence pathway, and prolonged cytotoxic effect • 57% increase in survival of mice with GB 	39
Mathematical modeling and application of adaptive therapy in heterogeneous tumors	Stabilization of tumor burden with better prognosis through adaptive therapy	12
Combined use of three oncolytic viruses (NDV, PV, and VV) in patients with GB	Disease stabilization or complete response with survival between 4 and 14 years	40
Investigation of the tumor microenvironment and cellular interactions in GB, with a focus on microglial cells	Identification of therapeutic resistance and tumor progression factors mediated by interactions with microglia and extracellular matrix	21
Analysis of metabolic reprogramming and crosstalk between signaling pathways in GB	<ul style="list-style-type: none"> • Relationship between glycolytic metabolism, glutamine depletion, and therapeutic resistance • Implications for new therapeutic approaches 	19

Abbreviation: GB: Glioblastoma.

is supported by mathematical models, such as Lotka–Volterra model, which are commonly used to simulate treatment dynamics in adaptive therapy. These simulations can reveal insights not easily observable in experiments, taking into account factors such as drug type, dosage, and timing of administration. Such modeling is crucial for controlling more aggressive tumor phenotypes. This finding has been verified in both mathematical models and preclinical experiments in ovarian, breast, skin, and colorectal cancers.¹² In this way, adaptive therapy provides

tumor stability, resulting in a significant improvement in the patient's prognosis.

Integrated with evolutionary principles, adaptive therapy emerges as a promising approach to cancer treatment, yielding encouraging results in preclinical models and experiments; however, the current challenge lies in the practical application of these concepts in clinical settings, considering the nuances of tumor heterogeneity and the limitations of existing monitoring methods. However, with advances in imaging diagnostics, which

eliminate the need for surgical interventions to monitor tumor progression, adaptive therapy is expected to be part of the novel methods for treating GB.

4. Conclusion

This article emphasizes the critical role of ploidy variation and tumor heterogeneity in GB resistance and recurrence—areas still underexplored in the current landscape of therapeutic strategies. By integrating emerging approaches such as targeted therapies, virotherapy, and multi-omics research, this review highlights the urgency of individualized treatments. It frames cellular instability not as a limitation but as a potential therapeutic opportunity, proposing that embracing the biological complexity of GB may unlock more effective and personalized interventions. By viewing genomic instability not solely as a limitation but as a therapeutic opportunity, this review proposes a conceptual shift in GB research and treatment.

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Author contributions

Conceptualization: Isabele Pagani Pavan, Bianca Paulino Campanharo

Visualization: Débora Dummer Meira, Iúri Drumond Louro

Writing—original draft: Isabele Pagani Pavan, Bianca Paulino Campanharo, Felipe Ataides Mion, Flávio Alvarenga, Matheus Correia Casotti, Lorena Souza Castro Altoé

Writing—review & editing: Felipe Ataides Mion, Flávio dos Santos Alvarenga, Matheus Correia Casotti, Lorena Souza Castro Altoé

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