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REVIEW

Marizomib in the therapy of brain tumors—how far did we go and where do we stand?

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Abstract
Out of several types of tumors of the central nervous system (CNS), glioblastoma (GBM) represents one of the most frequent and malignant forms of brain neoplasms. To date, GBM holds very limited therapeutic options leaving patients with poor prognosis of survival. As such, novel treatment approaches are constantly quested. One of these strategies is based on the utilization of proteasome inhibitors (PIs). However, although several PIs have been approved as therapy for patients with hematological malignancies, these treatment benefits cannot not be easily extrapolated to brain tumors. This is mostly due to the blood-brain barrier (BBB) impermeability of the majority of PIs, which is then followed by their low brain bioavailability. Marizomib (MZB) is a unique, irreversible, second-generation proteasome inhibitor, which unlike other PIs can penetrate through the BBB, making it a promising therapeutic tool in brain tumors. Despite an indisputable therapeutic potential of MZB, it has yet failed to be successfully introduced to the clinics as a ready-to-use chemotherapy for GBM-suffering patients. Therefore, in this work we describe the potential of PIs as candidates for neuro-oncological drugs, present results of preclinical and clinical investigations concerning MZB in brain tumors, discuss possible reasons of failure of MZB-based therapies and delineate future directions of MZB-related studies.

Keywords Apoptosis · ER stress · Glioblastoma · Marizomib · Proteasome inhibitors

Abbreviations

Atf4	Activating transcription factor 4
Atf6α	Activating transcription factor 6α
Atg5	Autophagy protein 5
BBB	The blood–brain barrier
Bcl2	B–cell leukemia/lymphoma 2 protein
CHOP	C/EBP homologus protein
C-L	caspase–like
cl-Casp3	cleaved caspase 3
cl-PARP	cleaved PARP
CNS	The central nervous system
CT-L	chymotrypsin–like
Cyt C	Cytochrome c
DR5	Death receptor 5
FDA	Food and Drug Administration
GBM	Glioblastoma
GRP78	Glucose regulated protein 78
GSCs	Glioma stem-like cells
IC_{50}	Half–maximal inhibitory concentration
IRE1α	Inositol–requiring transmembrane kinase endoribonuclease–1α
MCL	Mantle cell lymphoma
MGMT	O^6–Methylguanine–DNA–methyltransferase
MZB	Marizomib
NAC	N–acetyl cysteine
NAD+	Nicotinamide adenine dinucleotide
PAN	Panobinostat
p-EIF2α	phosphorylated eukaryotic translation initiation factor 2α
PERK	Protein kinase RNA–like ER kinase
PI	Proteasome inhibitor
PIs	Proteasome inhibitors
PLGA	Polylactic–co–glycolic acid
p-SAPK/JNK	phosphorylatedstress–activated protein kinase
ROS	Reactive oxygen species

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RT	Radiotherapy
T-L	trypsin–like
TMZ	Temozolomide
UPR	Unfolded protein response
UPS	Ubiquitin–proteasome system
γH2AX	phosphorylated histone H2AX

Introduction

Brain cancers, also referred to as central nervous system (CNS) cancers, are a group of several types of tumors originating mostly from glial and neuronal precursor cells [1]. The most common histological types of primary CNS tumors in adults are gliomas. Gliomas constitute a group of malignant brain tumors, including low-grade gliomas (astrocytoma, oligodendroglioma) and high-grade glioma or glioblastoma (GBM) [1]. GBM is considered one of the most frequent and aggressive type of brain neoplasms with an overall dismal survival rate averaging one year [1, 2]. It is characterized by high anaplasticity and heterogeneity of tumor cells together with their high migratory potential, which allows malignant cells to effectively invade the brain [3]. GBM is also known for possessing a variety of genetic alterations including mutations in the main tumor suppressor genes such as *p53* or *Pten* [4] and deletions of some parts of the chromosomes (e.g. 1p36.23, 6q26–27, 17p13.3–12) [5]. Moreover, the latest data show the presence of a subpopulation of self-renewing and pluripotent glioma stem-like cells (GSCs) in the tumor mass, which may be an essential factor in GBM recurrence [2]. These characteristics of GBM together with its aggressiveness and highly infiltrative nature make it complicated to diagnose and treat. Thus, GBM still poses a therapeutic challenge.

Despite the increasing understanding of the pathogenesis of GBM at the molecular and genetic levels, treatment options for this tumor remain limited [1, 2, 6, 7]. So far, the standard of care for GBM has been maximal possible tumor resection, followed by concurrent radiotherapy and chemotherapy with temozolomide (TMZ) [8]. Nevertheless, current clinical regimens also include the use of several other chemotherapeutic agents, such as bevacizumab, cisplatin, lomustine, or carmustine, which may slow down the progression of this tumor, but do not provide full recovery of treated patients [9–13]. In consequence, identification of therapeutically effective agents warrants further investigation. Unfortunately, drug design for brain tumors is challenging and needs to face a lot of handicaps. Ultimately, several major factors have been recognized as reasons for CNS drugs failure, including impermeability of the blood–brain barrier (BBB), emergence of resistance pathways, poor pharmacokinetic properties, and suboptimal clinical trial design [14]. Hence, an urgent need to develop novel, clinically effective pharmacotherapeutics for GBM is still emerging.

To date, many organelles and molecular pathways have been investigated in the context of anticancer drug targets. Given the key role of proteasomes in controlling many cellular processes, not only in normal but also in cancer cells, targeting this organelle has long been believed to provide therapeutic benefits in anticancer treatment. Thus, the ubiquitin–proteasome system (UPS) has now become a well-established drug target in the therapy of malignant diseases [2, 6]. Nonetheless, implementation of the proteasome inhibitor (PI)-dependent therapies against brain tumors encounters many obstacles, one of the major ones being BBB permeability. In this respect, marizomib (MZB) also known as Salinosporamide A or NPI-0052 was the first one to surpass this requirement and was hoped to significantly improve the efficiency of PI-based therapies in GBM management. Therefore, the aim of this work is to summarize the existing knowledge concerning utilization of MZB as potential tool in combating brain malignancies and discuss potential reasons of failure of MZB-based therapies in clinical trials.

Methodology

The literature for this review was searched through the main scientific databases, including PubMed and Scopus, from its inception to January 2025. Articles published exclusively in English were included to preserve the consistency and accessibility of the information. The experimental studies, clinical trials, but also reviews were screened for the keywords such as ‘marizomib’, ‘salinosporamide A’, ‘NPI-0052’, ‘glioma’, ‘glioblastoma’, ‘brain tumors’, or ‘cancer’. Articles directly addressing the role of MZB in different neurooncological entities were selected and comprehensively analyzed. Studies concerning MZB and other cancers, or brain tumors and other proteasome inhibitors were also included to give the context and historical timeline of introducing PIs into therapeutical practice. Studies were selected based on their robust methodologies, clear outcomes, and peer-reviewed status. Additionally, ClinicalTrials.gov website was reviewed for completed/ongoing clinical trials. Preprints and articles focused solely on synthesis of MZB were excluded from the analysis.

The Ubiquitin-Proteasome System (UPS)

The primary mode of action of all proteasome inhibitors (PIs) including MZB is targeting the proteasomes and causing their malfunction. This in consequence results in

defective degradation of cellular proteins and disturbed proteostasis in cancer cells. A key mechanism responsible for maintaining the balance between protein synthesis and degradation is the ubiquitin-proteasome system (UPS). Proteasome-dependent proteolysis is a complex process that controls the removal of damaged or misfolded proteins, and is therefore responsible for the regulation of many cellular functions. In addition to the presence of proteasomes, the process of protein degradation also requires the participation of many enzymes and energy input from the ATP [15]. In mammals, the most abundant proteasomes are 26S proteasomes, which are multiprotein complexes composed of a 20S catalytic core and two 19S regulatory subunits (Fig. 1) [15, 16]. Proteasomes degrade proteins in a highly regulated manner. Strict control of which protein enters their internal compartment is achieved by ubiquitin labeling. Protein polyubiquitination occurs in three stages: (I) activation of ubiquitin and its transfer to the E1 enzyme; (II) transfer of ubiquitin to the E2 conjugating enzyme; (III) transfer of ubiquitin by the E3 enzyme (ubiquitin ligase) to the protein [15]. A protein prepared in such way binds to 19S subunits, which cleave ubiquitin from the protein chain. The protein passes further through the 20S core unit and is degraded into small oligopeptides (<25 amino acids). The 20S proteasome is composed of four rings arranged one on top of the other, creating a barrel-like structure. Each ring is additionally composed of seven different subunits. The inner rings are composed of β subunits, while the outer ones are composed of α subunits. Only the β subunits exhibit proteolytic activity, and are composed of three subunits with different enzymatic activities, i.e. caspase-like (C-L; subunit β1), trypsin-like (T-L; subunit β2), and chymotrypsin-like (CT-L; subunit β5) (Fig. 1) [16].

Proteasome inhibitors in anticancer therapy

Proteasome inhibitors have attracted interest of medical and pharmacological sciences due to their role in regulating protein metabolism. Initially, they were considered to be able to support patients with cancer cachexia - a multifactorial syndrome of metabolic disorders [17]. However, preclinical studies have shown that PIs can be used in anticancer

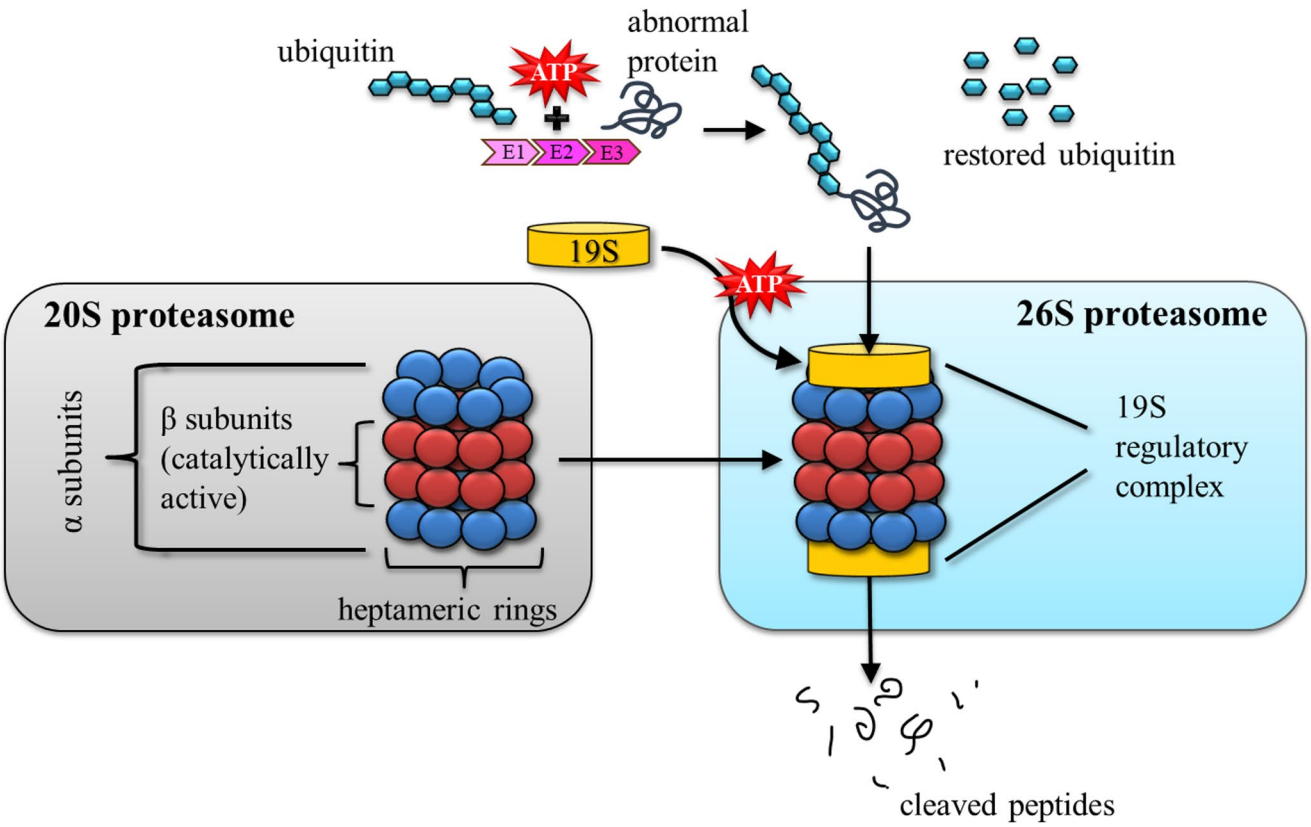


Fig. 1 Proteasomal structure and protein degradation. The 20S proteasome is composed of four rings. Each ring is additionally composed of seven subunits. The inner rings are composed of β subunits; the outer ones are composed of α subunits. The β subunits exhibit proteolytic activity (caspase-like, trypsin-like, and chymotrypsin-like). The two 19S regulatory complexes bind to the 20S catalytic core to form the 26S proteasome structure in an ATP-dependent manner. Proteins destined for the degradation are labeled with ubiquitin, in a process catalyzed by E1, E2, and E3 enzymes. Ubiquitin-labeled proteins bind to 19S subunits and are degraded by proteolytic β subunits into small oligopeptides (<25 amino acids). E1 - ubiquitin-activating enzyme; E2 - ubiquitin-conjugating enzyme; E3 - ubiquitin ligase

therapy per se, due to their ability to induce apoptosis. To date, numerous excellent papers analyzing the development of PIs in anticancer research have been published [18–21]. Thus, only a brief summary of key well-recognized PIs is presented in this work. As such, bortezomib - a first-generation PI, was FDA-approved for the treatment of multiple myeloma in 2003. Over time, after successfully completing clinical trials, the second-generation inhibitors such as carfilzomib (2012) and ixazomib (2015) were approved as drugs for multiple myeloma and mantle cell lymphoma (MCL) [22]. Furthermore, several other PIs such as delanzomib, oprozomib and marizomib are currently intensively investigated in both preclinical and clinical settings. However, despite promising results of the preliminary studies, none of these PIs was yet approved as an of care treatment in oncological patients.

PIs can be categorized based on their chemical structure and mechanism of action (Table 1). Structure-wise, they can be divided into boronic acid derivatives, epoxyketones, and beta-lactones, whereas in terms of their mode of action, PIs can be separated into reversible/irreversible inhibitors of various subunits (C-L, T-L or CT-L) of proteasome [19]. As such, bortezomib (PS-341) is a boronic acid derivative. It presents high specificity, while rapidly and reversibly inhibiting the chymotrypsin-like (CT-L) activity of the 20S proteasome complex. In preclinical studies, bortezomib has been shown to be active against many types of cancer, including multiple myeloma [23], mantle cell lymphoma [24], breast cancer [25], lung cancer [26] and more. Additionally, it has been shown to have greater cytotoxicity against proliferating cancer cells rather than normal cells, and was reported to evoke therapeutic effect while administered both intravenously and subcutaneously [20]. Ixazomib (MLN9780/ MLN2238) and delanzomib (CEP-18770) are also boronic acid derivatives. They are reversible proteasome inhibitors active after intravenous, subcutaneous or oral administration [21, 27, 28]. Of note, ixazomib was the first oral PI to undergo clinical trials in patients with multiple myeloma. Moreover, both delanzomib and ixazomib inhibit the CT-L subunit of the proteasome, while ixazomib can also block C-L and T-L subunits at higher concentrations [29]. These inhibitors differ significantly in terms of the duration of the elimination phase. Hence, delanzomib was reported to have an exceptionally long half-life of nearly 60 h, while the half-life for ixazomib was demonstrated to be as short as 18 min [27, 30]. To date delanzomib was tested in preclinical models of various cancers such as triple-negative breast cancer [31], hepatocellular carcinoma [32] or multiple myeloma [33], showing mainly antiproliferative and proapoptotic activity. Accordingly, ixazomib was found to suppress the proliferation of colorectal cancer cells [34] and was effective in the treatment of multiple myeloma [35]. Other group of compounds is the epoxyketone-based PIs. Accordingly, carfilzomib (PR-171) and oprozomib (ONX0912/ PR-047) are composed of an epoxide ketone that reacts with the catalytic threonine residue present in the proteasome molecule, blocking it irreversibly [36]. This results in a longer duration of proteasome inhibition, and restoration of the activity of this structure is only possible if the new proteasome is synthetized. Both compounds belong to the second generation PIs, strongly inhibiting the CT-L subunit of the 20S proteasome (although certain reports indicate weak inhibition of the C-L activity by carfilzomib) [37]. They also exhibit reduced off-target effects and corresponding adverse toxicities [36, 38]. Furthermore, carfilzomib can be administered intravenously, while oprozomib represents an orally bioavailable derivative of carfilzomib. On top of that, both inhibitors present similar half-life times ranging between 1 h and 1.5 h [29, 39]. The antitumor activity of carfilzomib has been demonstrated in preclinical models of various cancers such as colorectal cancer [40] or breast cancer [41, 42] and presented promising results in clinical trials against multiple myeloma [43, 44]. Likewise, oprozomib was found to suppress the proliferation of hepatocellular carcinoma [45] and lung cancer [46], and proved to be effective in clinical trials against multiple myeloma [47–49]. Finally, a separate group of PIs of β-lactone origin is represented by marizomib (MZB; salinosporamide A) [50]. MZB is an irreversible, II class PI active after both intravenous and oral administration. It suppresses the activity of all three subunits of the proteasome (CT-L, T-L and C-L), which makes it a unique PI amongst other inhibitors [51]. However, the half-life of MZB is quite short and is limited to merely half an hour [50]. A detailed description of MZB is included further in this work. A summary of the main properties of key PIs has been listed in Table 1.

Proteasome inhibitors in brain malignancies

Taking into account the promising therapeutic potential of proteasome inhibition in many types of cancer, several PIs have been investigated in preclinical models of glioma, while some of them even entering clinical trials [58–60]. As such, early reports demonstrate that a reversible PI–MG132, caused apoptotic death in several glioblastoma cell lines [61, 62]. Later, after being successfully implemented for treatment of hematological malignancies, bortezomib was also tested in various models of solid tumors including GBM. Accordingly, it has been extensively investigated against many GBM cell lines showing prominent antiproliferative and proapoptotic effects [63–66]. Additionally, bortezomib was found to sensitize GBM cells to TMZ and bevacizumab enhancing the antiproliferative effect in vitro and prolonging survival of tumor-bearing mice [65, 67, 68].

Table 1 A summary of the main properties of key proteasome inhibitors based on their chemical structure and mechanism of action

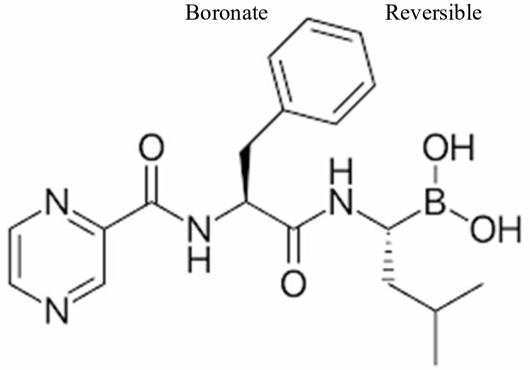
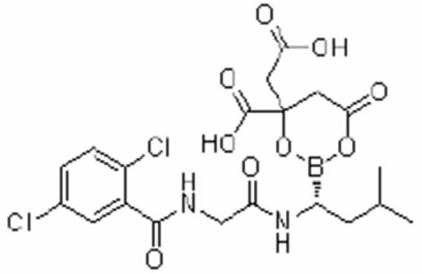
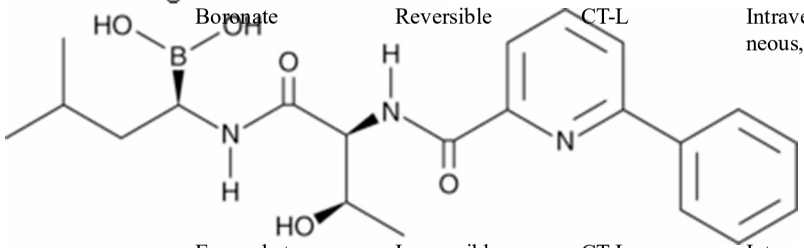
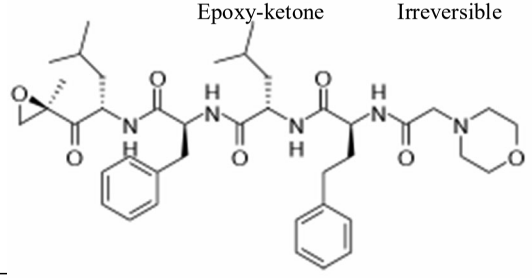
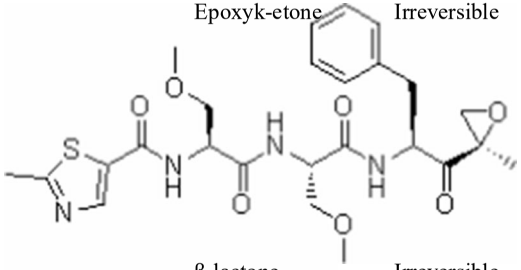
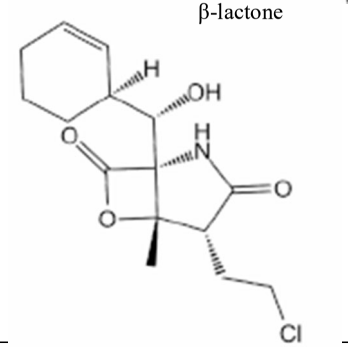
Proteasome inhibitor	Structure	Chemical origin	Inhibition	Targeted proteasome subunit	Administration	Class	Half-life/ applied dosage	References
Bortezomib (PS-341)		Boronate	Reversible	CT-L	Intravenous, Subcutaneous	I	110 min (1 µmol/L for 30 min) up to 6.8 h (1 mg/m^2, first day)-32.5 h (1 mg/m^2, eleventh day)*	[18, 27, 52]
Ixazomib (MLN9780/ MLN2238)		Boronate	Reversible	CT-L	Intravenous, Oral	I	18 min (1 µmol/L for 30 min) up to 3.6–11.3 days (once weekly 0.8–3.95 mg/m^2)*	[27, 53, 54]
Delanzomib (CEP-18770)		Boronate	Reversible	CT-L	Intravenous, Subcutaneous, Oral	I	62 h (0.1–1.8 mg/m^2)	[28, 30, 55]
Carfilzomib (PR-171)		Epoxy-ketone	Irreversible	CT-L	Intravenous	II	< 60 min (2–8 mg/kg)	[29, 39, 56]

Table 1 (continued)

Proteasome inhibitor	Structure	Chemical origin	Inhibition	Targeted proteasome subunit	Administration	Class	Half-life/ applied dosage	References
Oprozomib (ONX0912/ PR-047)		Epoxyk-etone	Irreversible	CT-L	Intravenous, Oral	II	< 90 min (30 mg/kg)	[29, 39, 57]
Marizomib (salinosporamide A/ NPI-0052)		β-lactone	Irreversible	CT-L, T-L, C-L	Intravenous, Oral	II	< 30 min (0.3–0.8 mg/m^2)	[50]

*The reported values of the half-life vary dependently on the administrated dose, therapy regimen, duration of treatment and the day of sample collection

In contrast, Labussiere et al. demonstrated that despite the effective inhibition of proteasome function, bortezomib did not show tumor growth-suppressing effect in either TCG3 and U87 malignant glioma xenograft models [69]. Further reports demonstrated that carfilzomib reduced viability, migration, and invasion of GBM cells in vitro and diminished tumor growth in BALB/c nude mice [70, 71]. As opposed to this, Zang et al. found that carfilzomib and oprozomib induced prosurvival autophagy via activation of the ATF4-dependent branch of the UPR in head and neck squamous cell carcinoma, which could counteract the proapoptotic effect [72]. Despite these ambiguous results certain PIs have entered clinical trials [73–76]. Thus, bortezomib was tested in several trials with mixed results [73–75, 77]. The phase I study established the dose required for maximal inhibition of the whole blood 20S proteasome and the maximal tolerated dose of bortezomib in patients with recurrent malignant glioma [75]. Unfortunately, the clinical therapeutic effect of that regimen was questionable [75]. Another phase I study tested the co-administration of bortezomib with TMZ. This trial showed that sequential treatment with bortezomib + TMZ was safe and promoted Th1-driven immunological responses in selected patients, which improved clinical outcomes [74]. Finally, a phase II trial aimed at assessing the safety profile and efficacy of an upfront treatment using bortezomib combined with TMZ and standard radiation therapy in twenty four patients with newly diagnosed GBM. No unexpected adverse effects of bortezomib were identified in this study, while some promising data concerning the progression-free survival and overall survival of patients was reported [73]. Nevertheless, further clinical investigations in a larger cohort of patients are required to confirm these findings. Additionally, a single phase 0 clinical trial of ixazomib was performed in three GBM patients [76]. Studies revealed that at the time of tumor resection ixazomib was detectable in tumor tissues at measurable concentrations, confirming target tissue delivery, which warrants further phase I study of this PI in recurrent GBM [76].

Despite the preliminarily promising results of clinical investigations, none of the tested PIs has yet been approved in therapeutic practice in GBM-suffering patients. The most limiting factor for developing efficient CNS drugs is usually their BBB-penetration activity. The majority of the PIs in this context are BBB impermeable, so their delivery to the brain tissue is hindered and their therapeutic efficiency is significantly reduced [78]. However, current view on the BBB impermeability supports the concept that many pathologic conditions, including brain tumors, can disrupt the integrity of this barrier [79] In this respect, consideration of drug distribution across the BBB would lose its relevancy in designing therapies for GBM, making majority of the PIs good candidates for CNS drugs. Nevertheless, in the light of current knowledge, the BBB is not uniformly disrupted across the whole tumor area and still possesses fragments with an intact structure [79]. This, in consequence, does not provide therapeutically effective drug exposure to all fractions of tumor cells, which is required for efficient treatment of cancer. As such, drug distribution across an intact BBB should be a priority in developing effective therapies for brain malignancies and should be a key requirement in designing any clinical trial for newly diagnosed or recurrent GBM. Taking this into account, efforts should be directed towards establishing PIs to use as anti-GBM therapy. One such PI able to cross the BBB is marizomib. MZB has been already tested in some preclinical studies and several clinical trials in brain tumors, but the knowledge of its molecular mode of action is still sparse and requires further examination. In this respect, a comprehensive analysis of MZB's effects in brain tumors might contribute to a better understanding of its functioning and might be helpful in designing future investigations to maximize its therapeutic potential.

Marizomib in preclinical models of brain tumors

Marizomib (also called salinosporamide A or NPI-0052) is a secondary metabolite of the marine actinomycete bacteria *Salinispora tropica* [80, 81]. The compound was first discovered over 20 years ago [82, 83] and was isolated as a colorless crystalline solid showing antitumor and antimicrobial properties during initial screening tests [81]. Further chemical studies revealed structural similarity of MZB to omuralide (one of the earliest identified PIs), shifting the attention of researchers towards questioning its interaction with the proteasome. Indeed, MZB was shown to irreversibly target all three (CT-L, T-L, and C-L) activities of the 26S proteasome with the IC_{50} values within the nanomolar range [82, 84]. As such, MZB was tested in oncopharmacology displaying potent cytotoxic effects in several models of hematological cancers including multiple myeloma [85, 86], acute lymphocytic leukemia [87], and acute myeloid leukemia [88], as well as solid tumors such as cervical cancer [89], pancreatic cancer [90], breast cancer [91], or renal cell carcinoma [92]. Moreover, due to its BBB-penetrant capacity, MZB has opened new possibilities for the treatment of brain malignancies [80]. As such, MZB has already been shown to be effective in several preclinical models of GBM [51, 80, 93–97]. However, the exact molecular mode of action of this PI in brain tumors is still incompletely understood. It has been demonstrated that the BALB/c nu/nu mice intracranially implanted with glioma xenografts showed significantly prolonged survival when treated with MZB in comparison with the untreated counterparts [80]. At the cellular level, MZB inhibited proliferation and invasion

and caused apoptosis of U-251MG and D-54MG cells. This effect was dependent on the overproduction of reactive oxygen species (ROS) as the addition of N-acetyl cysteine (NAC) reduced the overexpression of cleaved forms of caspase 3 (cl-casp3) and PARP (cl-PARP) and reversed the pro-apoptotic effect [80]. Likewise, in LN18 cells treated with MZB, the pre-treatment with NAC attenuated the release of mitochondrial cytochrome c (Cyt C), diminished the activity of caspase 3/7 and cleavage of caspase 9, and reduced fragmentation of the DNA, suggesting an important role of ROS in MZB-dependent cytotoxicity [98]. Moreover, MZB was found to activate caspase cascade in LN18 cells. On top of that, experiments with the inhibitors of caspase 8 (z-IETD-fmk) and 9 (z-LEHD-fmk) revealed that caspase 9 acted upstream of caspase 8 to induce GBM cell death upon proteasome inhibition. Notably, elevated levels of p27 and p21 were observed in lysates from the tumor-bearing portion of the brain of MZB-treated mice [98]. Nevertheless, further consequences of this overexpression have not been studied. Furthermore, Kusaczuk et al. demonstrated that in LN229 and U118MG cells treatment with MZB resulted in diminished expression of antiapoptotic Bcl-2 and elevated levels of cl-casp 3, cl-PARP, Noxa, Cyt C, and death receptor 5 (DR5) indicative of apoptotic cell death [51]. This effect was accompanied by the induction of the ER stress as shown by increased expressions of GRP78, p-EIF2α, IRE1α, c-Jun, p-SAPK/JNK, *Atf6α*, *Atf4* and CHOP. However, no significant overproduction of ROS nor increased expressions of LC3 II, Beclin 1, and *Atg5* were detected, suggesting that neither oxidative stress nor autophagy was activated upon MZB treatment [51]. These results partially agree with the observations of Lin et al., who studied the effect of MZB with panobinostat (PAN, a well-known histone deacetylase inhibitor) in diffuse midline gliomas [96]. They noticed that stimulation of SU-DIPGXIII cells with 20 nM MZB alone increased the expression of p21, cl-casp 3 and cl-PARP, and this effect was accompanied by an overexpression of GRP78, IRE1α, PERK and CHOP. Interestingly, when a combination of MZB and PAN was applied, the key divergent metabolites included decreased levels of reduced glutathione and increased levels of oxidized glutathione, suggesting oxidative stress as a mechanistic driver of the MZB + PAN cytotoxicity [96]. However, the addition of NAC did not restore the cell viability, which may indicate that the induction of ROS was not a primary cause of the cytotoxicity evoked by the combination of these drugs [96]. Furthermore, MZB altered the metabolic profile of glioma cells by decreasing the relative NAD + levels, lowering basal respiration, and reducing spare respiratory capacity. The combination with PAN exacerbated these effects. Altogether, these results indicate that diffuse midline glioma cells are dependent on NAD + availability, and that the effect of MZB might be at least partially driven by its impact on cellular metabolism [96]. In line with this, Jane et al. studied the effect of co-treatment of MZB with PAN in pediatric (SJG2, KNS42, SF8628-stem cells, DIPG-007, DIPG-013) and adult (U87, LNZ308, T98G) glioma cell lines [97]. They noticed that stimulation with 25 nM MZB alone resulted in a weak proapoptotic effect in all tested cells, although pediatric cell lines seemed to respond to MZB better than the adult GBM cells. In line with previous research, PAN potentiated the effect of MZB, significantly depleting ATP and NAD + content, increasing mitochondrial permeability and ROS generation, and finally promoting apoptosis in all tested cell lines. Nevertheless, the effect of MZB as a single agent was not profoundly explored [97]. Further investigations have shown that MZB, together with IZI1551 (a second-generation TRAIL-receptor agonist), were effective in causing apoptotic cell death in a panel of patient-derived GBM cell lines [93]. Again, the responsiveness to the single agent treatment was generally poor, but the combination of both drugs substantially lowered cell viability in the majority of cell lines [93]. The exposure of GBM cells to 80 nM MZB alone did not result in the initiation of apoptosis after just 4 h of incubation, but the proapoptotic effect was more pronounced when the stimulation time was prolonged to 24 h. Nevertheless, pre-incubation with MZB significantly sensitized cells to IZI1551 treatment, indicating its good synergy with other drugs [93]. An additional set of results concerning MZB in brain malignancies comes from medulloblastoma, which is the most common solid pediatric brain tumor [99]. Frisira et al. demonstrated that MZB caused the accumulation of ubiquitinated proteins after 3 h of treatment in a range of patient-derived medulloblastoma cells, confirming the proteasome-inhibitory effect of MZB [99]. Prolonged exposure to MZB (up to 24 h) resulted in S phase cell cycle arrest and induction of apoptosis in the most aggressive medulloblastoma subgroups. Mechanistically, MZB was found to alter the mitochondrial membrane potential (ΔΨm) causing mitochondrial hyperpolarization, which was accompanied by overproduction of ROS and substantial reduction in total glutathione levels with a decreased ratio of reduced glutathione/oxidized glutathione, suggesting the occurrence of oxidative stress. To further clarify the mechanism of MZB-dependent apoptosis, the p53 and p73 on transcriptomic and proteomic levels were evaluated in CHLA-01-MED, ICb-1299, and DAOY medulloblastoma cell lines. Higher expressions of both p53 and p73 were observed in all tested malignant cells in comparison with the normal cerebellar cells. These findings were confirmed in tumor organoids derived from primary ICb-1299 cells, showing stabilization of p53 and increased expression of cl-casp 3. Additionally, high doses of MZB induced significant overexpression of γ-H2AX – an early

marker of DNA damage, suggesting that high doses of MZB may induce significant ROS-mediated DNA damage in medulloblastoma organoids. These results were enhanced by the pre-treatment with γ-radiation, which sensitized medulloblastoma cells to MZB and increased cell death [99]. A tentative model of the molecular mode of action of MZB in brain tumor cells is depicted in Fig. 2. Noteworthy, glioma stem-like cells (GSCs), recognized as a self-renewal, pluripotent population of tumor cells, are known to be more sensitive to proteasomal inhibition than the fully differentiated glioma cells [80]. Since GSCs are currently believed to be an essential factor responsible for GBM recurrence, their efficient elimination might bring a prominent benefit in stabilizing the progression of brain malignancies [2]. In this respect, MZB efficiently blocked the activity of proteasomes and impacted the viability of GSCs [80]. Thus, although low-grade GSCs were insensitive to MZB, high-grade GSCs showed roughly 40% reduction in cell survival at 20 nM concentration of MZB, which was similar to that observed in stable GBM cultures [80]. These results suggest that MZB may hold some potential in reducing GBM recurrence, however, further analyses are necessary to confirm these findings. Additional set of studies confirmed cytotoxic activity of MZB in twelve patient-derived IDH1-mutant glioma cell cultures [94] and reported survival benefit in MZB-treated zebrafish implanted with human GBM xenografts (however, without significant reduction in tumor growth) [95].

Despite over twenty years since the discovery of MZB, still little is known about its molecular mode of action in

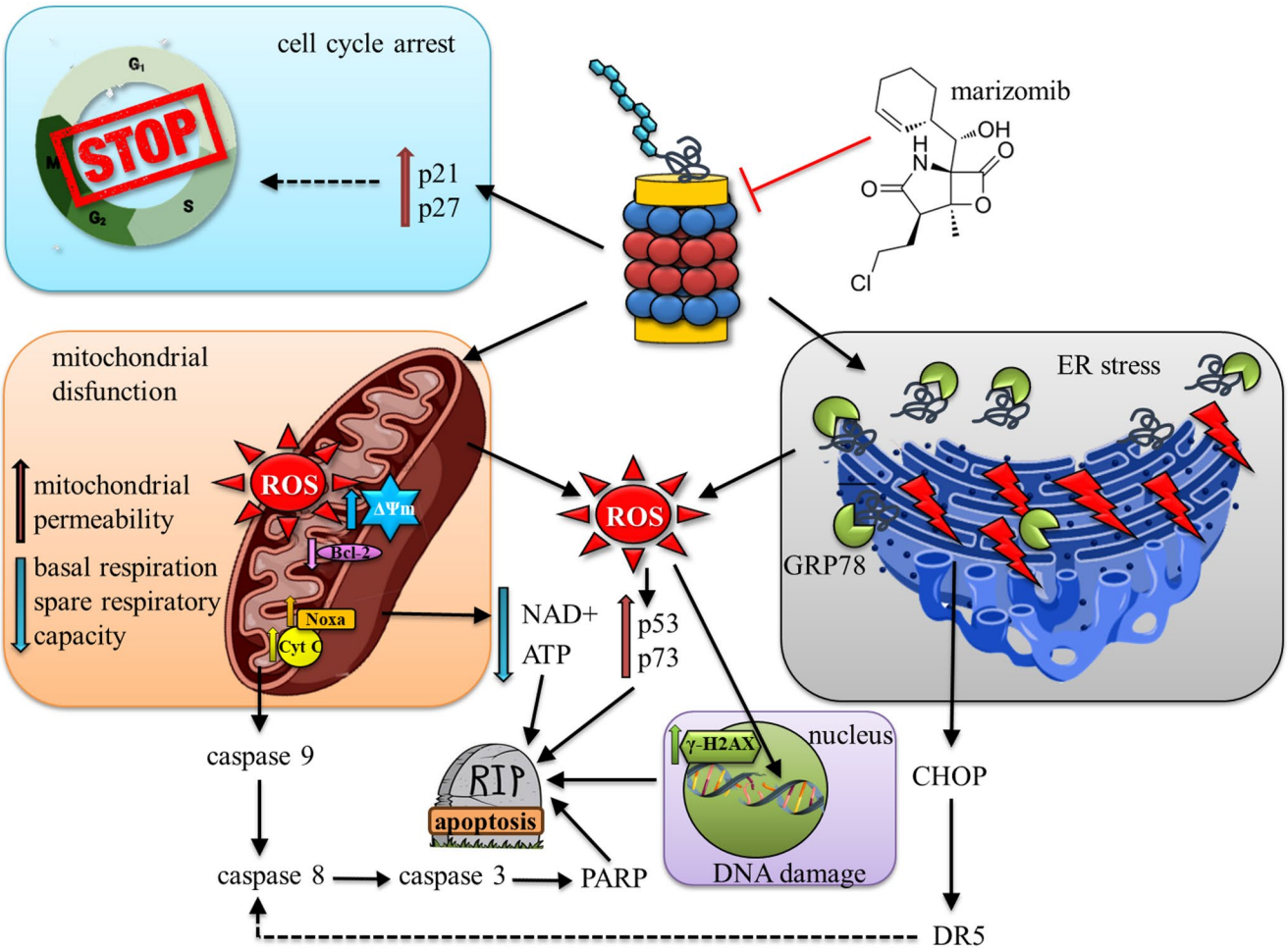


Fig. 2 Tentative mechanistic model of MZB functioning in brain tumors. The MZB-dependent inhibition of proteasome may cause cell cycle arrest via accumulation of p21/p27. The MZB treatment may result in proapoptotic effect occurring via several pathways: MZB may cause ER stress initiating apoptosis via CHOP-dependent mechanism and ROS generation; MZB may cause mitochondrial disfunction resulting in low basal respiration, reduced spare respiratory capacity, and decreased NAD+/ATP levels; MZB may cause elevation of ΔΨm, augmentation of mitochondrial permeability and stimulation of ROS overproduction; MZB may cause overexpression of p53 and p73; MZB may cause overexpression of γ-H2AX resulting in DNA damage. Continuous arrows symbolize well-established molecular pathways. Dashed arrows show possible interactions. Bcl-2 – antiapoptotic B-cell leukemia/lymphoma 2 protein; Cyt C – cytochrome C; DR5 – death receptor 5; ER – endoplasmic reticulum; NAD+ – nicotinamide adenine dinucleotide; ΔΨm–mitochondrial membrane potential; PARP – poly(ADP-ribose) polymerase; ROS – reactive oxygen species; γ-H2AX – phosphorylated histone H2AX

brain malignancies. To date, the proapoptotic activity of MZB in vitro has been relatively well established, but the reports exploring the precise mechanism underlying this effect are sparsely represented. Unfortunately, even less is known about the influence of MZB on other cellular events such as autophagy, cell cycle arrest, or premature senescence, which may be a key factor limiting successful translational attempts and hindering the selection of efficient co-therapeutic strategies. As such, studies ought to be continued to fully recognize cellular effects and molecular pathways activated upon treatment with MZB.

Marizomib in clinical trials for glioma patients

The promising results of the preliminary preclinical studies encouraged further exploration of MZB in clinical trials for patients with several types of cancer. Therefore, MZB was evaluated in patients with lymphoma, leukemia, relapsed/refractory multiple myeloma, and solid tumors (e.g., melanoma, colorectal cancer, stomach cancer, prostate cancer, and cervical carcinoma), showing an overall good tolerability and relative stabilization of the progress of certain malignancies [50, 100–104]. Notably, clinical activity of MZB was observed in multiple myeloma patients with tumor manifestations in the CNS [105, 106]. Based on the BBB-penetrant activity of MZB, it has entered clinical trials in patients with brain tumors [60, 107–109]. Hence, a phase I/II clinical study in patients with recurrent GBM was performed (NCT02330562) [60]. MZB was then tested either as a single agent or in combination with bevacizumab. In part A of the trial, the preliminary efficacy and safety profile of MZB in monotherapy was assessed, whereas part B consisted of the escalating doses of MZB in combination with bevacizumab. Finally, part C of the study included an intra-patient dose escalation of MZB for the combination of both drugs. A 10-minute intravenous infusions administered once a week for three weeks in 28-day cycles at a dose of 0.8 mg/m^2 were applied for MZB alone. Later, a 10-min intravenous infusions in dose cohorts ranging from 0.55 to 0.8 mg/m^2 on days 1, 8, and 15 of each 28-day cycle of MZB were administered together with a fixed dose (10 mg/kg) of bevacizumab (on days 1 and 15 of each 28-day cycle) in all patients. Eventually, MZB was given once weekly with escalating doses from 0.8 mg/m^2 up to 1.2 mg/m^2 for three weeks, and bevacizumab (10 mg/kg) was given every 2 weeks in 28-day cycles. Overall, the most often experienced adverse effects after treatment with MZB were headache, fatigue, hallucination, and insomnia, and the maximum tolerated dose was set at 0.8 mg/m^2, which is in line with previous studies [50, 110]. The co-therapy with bevacizumab showed a nonoverlapping safety profile when used concomitantly. The analysis of patients' response revealed that in part A of the trial, the median progression-free survival was longer among patients with a methylated version of the *MGMT* (O^6-methylguanine-DNA-methyltransferase) gene in comparison to those with the unmethylated status of the *MGMT* promoter (4.9 vs. 1.8 months). However, the median overall survival seemed independent of the *MGMT* methylation status. In the pooled group of patients from parts B and C of the study, the overall response rate was similar regardless of the *MGMT* methylation profile, while the median overall survival was longer in patients with the methylated gene. Altogether, despite the proof that MZB crosses the BBB, the preliminary evaluation of its efficacy did not demonstrate a relevant benefit of the addition of this PI to bevacizumab for the treatment of recurrent GBM (Table 2) [60, 109]. Other clinical trials evaluated MZB in combination with standard therapeutic protocol in patients with newly diagnosed glioblastoma (NCT02903069; NCT03345095) [107, 111]. As such, MZB was combined with standard treatment with radiotherapy (RT) and TMZ-based chemotherapy (TMZ/RT→TMZ) in newly diagnosed GBM (NCT02903069), to determine the recommended dose [107]. Patients were enrolled in several cohorts: (I) TMZ/RT + MRZ→TMZ + MRZ (N = 15); (II) TMZ/RT→TMZ + MRZ (N = 18) + TMZ/RT + MRZ at recommended dose → TMZ + MRZ at recommended dose (N = 20). A separate group of patients (III) received TMZ/RT→TMZ + MRZ at recommended dose with tumor treating fields (TTF, N = 13). The MZB was infused intravenously on days 1, 8, 15, 29, 36 (of 42-day TMZ/RT + MRZ cycle) at 0.55, 0.7, 0.8, and 1.0 mg/m^2 and days 1, 8, 15 (of 28-day TMZ + MRZ cycle). The recommended dose for MZB was established to be 0.8 mg/m^2. Moreover, out of 66 treated patients, the most frequently reported side effects were fatigue, nausea, hallucination, vomiting and headache. The median overall survival for patients receiving MZB with TMZ/RT→TMZ was 14.8 months (Table 2) [107]. Furthermore, a multicenter, randomized, controlled, open-label phase III superiority trial was conducted (NCT03345095). The primary aim of the study was to compare the overall survival in patients receiving MZB in addition to standard treatment (TMZ/RT→TMZ) with those subjected to the standard therapeutic regimen only. The study was performed either in the whole population, as well as in the subgroup of patients with tumors characterized by an unmethylated *MGMT* promoter. A total of 866 patients were recruited to this 2-year long project, of which 117 were scored out of the trial at the beginning for not meeting the eligibility criteria. A 749 of the remaining patients were further divided to receive standard treatment plus MZB (375 patients, 50.1%), or just the standard radiochemotherapy (374 patients, 49.9%). The result of a long-term follow-up analysis showed that 538 patients out of 749 enrolled to

Table 2 A summary and baseline characteristics of clinical trials of Marizomib in glioblastoma

Trial number	Cancer type	Phase	Clinical setting		Sample size	Study endpoints	Response rate	Progression-free/median overall survival	References
			Treatment	Dose and timeframe					
NCT02330562	Recurrent glioblastoma	I/II	Part A: MZB in monotherapy	Part A: MZB administrated as 10-min IV infusion once weekly for 3 weeks in 28-day cycles at a dose of 0.8 mg/m^2	Part A: 30	Part A: primary endpoint: best response of MZB in monotherapy. Secondary endpoint: safety and efficacy (ORR, PFS, OS)	Part A: 3.3%	Part A: OS: 11.4 months	[60, 108, 109]
			Part B: escalating doses of MZB (0.5–0.8 mg/m^2)+BEV	Part B: MZB administered as 10-min IV infusion in dose cohorts ranging from 0.55 to 0.8 mg/m^2 on days 1, 8, and 15 of each 28-day cycle+BEV administered at a fixed dose of 10 mg/ kg IV on days 1 and 15 of each 28-day cycle	Part B: 36	Part B: primary endpoint: MTD/MAD and RPIID of MZB in combination with BEV. Secondary endpoint: safety and efficacy (ORR, PFS, OS)	Part B: 44.4%	Part B: OS: 9.4 months	
			Part C: escalating doses of MZB (0.8–1.0 mg/m^2)+BEV	Part C: MZB given once weekly for 3 weeks (at starting dose of 0.8 mg/m^2 to 1.0 mg/m^2, and increased up to 1.2 mg/m^2 if tolerated)+BEV (10 mg/kg) given every 2 weeks in 28-day cycles	Part C: 41	Part C: primary endpoint: OS. Secondary Endpoint: safety (adverse effects and dose-limiting toxicities) + ORR and PFS	Part C: 22%	Part C: OS: 8.3 months	

Table 2 (continued)

Trial number	Cancer type	Phase	Clinical setting		Sample size	Study endpoints	Response rate	Progression-free/median overall survival	References
			Treatment	Dose and timeframe					
NCT02903069	Newly diagnosed glioblastoma	I	Cohort I: TMZ/RT+MRZ→TMZ+MRZ, Cohort II: TMZ/RT→TMZ+MRZ, followed by dose-expansion with TMZ/RT+MRZ at RD → TMZ+MRZ at RD Cohort III: TMZ/RT→TMZ+MRZ at RD with TTF	MRZ infused IV as 10-min infusion at 0.55, 0.7, 0.8, and 1.0 mg/m^2 on days 1, 8, 15, 29, 36 (of 42-day of CT) and days 1, 8, 15 (of 28-day of AT); TMZ administered once daily, 7 days/week, for 6 weeks, starting on day 1, at a dose of 75 mg/m^2 during CT and once daily on days 1–5 every cycle (150–200 mg/m^2 during AT; RT administered once daily, 5 days/week, for 30 doses over 6 (60 Gy, starting on day 1 during CT)	66 (Cohort I: *N* = 15 Cohort II: *N* = 38, Cohort III: *N* = 13)	Primary endpoint: toxicity. Secondary endpoint: OS	NR	OS: 14.8 months	[107]
NCT03345095	Newly diagnosed glioblastoma	III	Group I: RT+TMZ →TMZ Group II RT+TMZ →TMZ+MZB	Group I: RT (60 Gy in 30 fractions over 6 weeks)+TMZ (75 mg/m^2 once daily for 6 weeks) followed by maintenance treatment with TMZ (150–200 mg/m^2 once daily on days 1–5 of a 28-day cycle for up to 6 cycles) Group II: the same treatment as Group I+MZB given as a 10-min infusion IV at a starting dose of 0.8 mg/m^2 on days 1, 8, 15, 29, and 36 during RT and on days 1, 8, and 15 of each 28-day cycle during maintenance therapy with TMZ. After completion of the maintenance therapy, MZB given on days 1, 8, and 15 of each 28-day cycle for additional 12 cycles.	Group I: 374 Group II: 375	Primary endpoint: OS. Secondary endpoint: PFS, best overall response, objective response rate, complete response rate, duration of response, frequencies, and percentages of worst adverse events, or laboratory event grades, quality of life, and cognition.	NR	Group I: OS: 17 months; PFS: 6 months Group II: OS:16.5 months; PFS: 6.3 months	[111]

Abbreviations: AT – adjuvant treatment; BEV – bevacizumab; CT – concomitant treatment; IV – intravenous; MAD – maximum administered dose; MTD – maximum tolerated dose; MZB – marizomib; NR – not reported; ORR – overall response rate; OS – overall survival; PFS – progression-free survival, RD – recommended dose; RPIID – recommended phase II dose; RT – radiotherapy; TMZ – temozolomide; TTF – tumor treating fields

the trial had died and the median overall survival time was 29.1 months in the TMZ/RT→TMZ + MZB group, and 27.5 months in the standard TMZ/RT→TMZ group. However, the overall survival and the progression-free survival did not differ significantly between either MZB-supplemented and non-supplemented groups of the intend-to-treat population of patients. Moreover, despite the preliminary data suggesting therapeutic benefit of MZB in patients with the unmethylated MGMT promoter [60], no therapeutic progress was found within the group of TMZ/RT→TMZ + MZB-treated patients with *MGMT* promoter-unmethylated tumors in comparison with those possessing the methylated version of this gene (Table 2) [111]. In general, addition of MZB to the standard therapeutic regimen in patients with GBM did not significantly prolong patients survival, and more so, resulted in additional toxicity and adverse effects. In this respect, contemporary translational programs ought to focus on the identification of the mechanisms underlying failure of the MZB-based therapy to confer a significant clinical benefit in neuro-oncology. The discovery of biomarkers specific for tumors amenable to proteasome inhibition alone or in combination with other treatments would allow for the identification and selection of patients prone to respond to such treatment. Hence, further studies are indispensable to continue the therapeutic progress in brain malignancies.

Challenges, future perspectives, and outlook

Despite potentially promising results of preclinical studies, the reasons behind the lack of activity of MZB in clinical trials are yet to be determined. One of the presumable causes of failure of the in vivo activity of MZB might be related to the pharmacokinetics of this PI. Indeed, although MZB was demonstrated to penetrate to the brain and inhibit proteasome activity, it has also been found to display a very short half-life and rapid clearance possibly occurring due to the extensive extrahepatic metabolism, instability at physiological pH, or partitioning to blood cells [50, 60]. Indeed, MZB displayed a very high volume of distribution, which may be indicative of an extensive partition into peripheral tissues and/or binding to blood components. Simultaneously, a clearance rate highly exceeded the average human liver blood flow (21 mL/min/kg or 1470 mL/min) indicating extensive extrahepatic metabolism [60]. Moreover, despite preclinical studies showing that MZB can penetrate to the CNS and cerebrospinal fluid, experiments on cynomolgus monkeys revealed that the achievable bioavailability of MZB was roughly 30–40% after oral administration, which may be insufficient to evoke clinically relevant effects [112, 113]. These findings indicate that there are still many obstacles to overcome before using MZB as therapeutically successful drug in brain malignancies. However, constant progress of pharmacological and medical sciences brings hope for the future. The new era of genomic, computational, and nano-based research makes it possible to map glioma genomes of individual patient, predict potential drug targets, and design novel drug delivery systems [114–116]. These therapeutic advances hold the potential to develop agents characterized by better efficacy and lower toxicity in patients with brain tumors. Current approaches focus on the development of non-invasive methods to prevail over therapeutic limitations and improve the poor prognosis of existing therapies. One such approach is to use nanoparticle-based delivery systems that would facilitate MZB's performance. Therefore, Sui et al. aimed at engineering the MZB-loaded polymeric nanoparticles as potential therapeutic tools against hepatocellular carcinoma [116]. They managed to encapsulate MZB in poly lactic-co-glycolic acid (PLGA) nanoparticles, which preserved the proapoptotic activity of this PI, while simultaneously markedly improving its safety profile in animal studies [116]. Furthermore, Xu et al. fabricated MZB-loaded chitosan-coated hydroxyapatite nanocarriers as potential system for treatment of ovarian cancer [117]. They focused on developing nanoparticle-based technology able to increase low bioavailability of MZB. In this respect, a pH-sensitive biopolymer was used to encapsulate hydroxyapatite nanoparticles with chitosan to increase MZB's bioavailability and efficiency. It has been demonstrated that the nano-drug release of MZB was gradual and slow, with a considerably high level of stability, which may prolong drug exposition and increase its effectiveness. Finally, Jing et al. examined nanotechnological approach as drug delivery system in glioma cells [118]. In this study, MZB-loaded zeolitic imidazolate framework-8@manganese dioxide (MZB-ZIF-8@MnO_2) nanoparticles were designed and tested for cytotoxic properties in C6 and U87 GBM cell lines. Indeed, ZIF-8@MnO_2-bound MZB caused overproduction of ROS, loss of mitochondrial membrane potential and finally evoked proapoptotic effect in tested cells. Notably, MZB-ZIF-8@MnO_2 did not result in any cytotoxicity in non-cancerous NIH3T3 fibroblasts, which indicates that these nanoparticles might be highly suitable for improving the biocompatibility of MZB alone [118]. Collectively, these findings suggest that MZB-carrying nano-formulations might improve bioavailability and biocompatibility, while simultaneously reducing the toxic effects of this drug alone. Unfortunately, despite preliminarily optimistic results, nano-based medicine needs to face its own challenges, such as establishing routes of administration, tempering biodistribution, or deciphering degradation and elimination of nanoparticles. Hence, further widespread analyses are required to fully unravel the potential of nanostructures in neuro-oncology and particularly the possibility of exploiting them as MZB-specific carriers.

Altogether, brain malignancies still pose a therapeutic challenge, and yet MZB has not occurred as an efficient remedy for suffering patients. Unfavorable pharmacokinetic and pharmacodynamic parameters, relatively low bioavailability, and very limited knowledge of the molecular pathways underlying its effects may stand behind MZB's failure in clinical trials. Given this, extensive efforts should be undertaken to identify effective therapy for brain malignancies, and MZB definitely warrants further investigations.

Study limitations

One of the major obstacles hindering the development of potential cures for GBM is the difficulty in emulating the complex nature of the brain and its surrounding microenvironment. The translational aspect of investigational drug research is often compromised by the genetic and cellular heterogeneity of GBM, which can impede the desired therapeutic outcomes. Additionally, further restrictions in drug delivery to the tumor-affected sites of the brain are caused by the presence of the BBB, which exacerbates the limitations already imposed on therapeutic strategies [119]. As such, although the in vitro models engaged to study brain tumors pose many beneficial attributes, these studies are also burdened with certain inherent limitations. The cell-based research often present the reductionist approach by oversimplifying the complexity of tumor microenvironment in vivo. Thus, application of the optimal culture conditions, neglection of the metabolic reactions, or disregarding the immune system interactions, present the undisputable limitations hindering efficient translation of the in vitro results into clinical practice. Moreover, animal models of GBM, despite being more faithful representation of tumor biology, still hold several drawbacks, such as interspecies discrepancies, which prevents a direct prediction of drug responses in humans. Given this, faithful reflection of the full spectrum of heterogeneity of human brain tumors in the in vivo settings still poses a major challenge. In this respect, optimization of the translational potential of the preclinical laboratory analyses warrants further attention and critical overview of such data should always be done when interpreting these results. However, preclinical studies have always been a good starting point in drug-development research, and despite certain inconveniences and flaws of the available GBM models, investigations concerning MZB and other drugs in brain malignancies sought to be continued.

Author contributions M.K. provided the concept of the manuscript, wrote the main manuscript text and prepared all figures. W.M.P. and J.D. constructed the table. W.M.P. scoured through the literature and provided further additions. All authors reviewed and edited the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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