UC Irvine

UC Irvine Previously Published Works

Title

Exploring the role and clinical implications of proteasome inhibition in medulloblastoma.

Permalink

<https://escholarship.org/uc/item/0vz3t8vd>

Journal

Pediatric blood & cancer, 68(10)

ISSN

1545-5009

Authors

Hoerig, Clay M
Plant-Fox, Ashley S
Pulley, Michelle D
et al.

Publication Date

2021-10-01

DOI

10.1002/pbc.29168

Peer reviewed

Exploring the role and clinical implications of proteasome inhibition in medulloblastoma

Clay M. Hoerig^{1,3} Ashley S. Plant-Fox^{2,3} Michelle D. Pulley^{1,3} Kaijun Di³
Daniela A. Bota⁴

1 Department of Pediatric Hematology/Oncology, Children's Hospital Orange County, Orange, California, USA

2 Department of Pediatric Oncology, Ann and Robert H. Lurie Children's Hospital Chicago, Illinois, USA

3 University of California, Irvine, California, USA

4 Department of Neurology, University of California, Irvine, California, USA

Correspondence

Michelle Pulley, Pediatric Hematology/Oncology,
Cedar Sinai Medical Center,
8700 Beverly Blvd, Beverly Hills, CA 90048.
USA.

Email: mdpulley@gmail.com

Funding information

National Cancer Institute, Grant/AwardNumber:
P30CA062203

Abstract

Ubiquitin proteasome-mediated protein degradation has been implicated in posttranslational oncogenesis in medulloblastoma. Current research is evaluating the clinical implications of proteasome inhibition as a therapeutic target. In medulloblastoma cell lines, proteasome inhibitors induce apoptosis and inhibit cell proliferation via multiple pathways involving activation of caspase pathways, NF κ B (nuclear factor kappa-lightchain-enhancer of activated B cells) pathway inhibition, reduced AKT/mTOR pathway activity, and pro-apoptotic protein expression. Second-generation proteasome inhibitors demonstrate blood–brain barrier penetration while maintaining antitumor effect. This review summarizes the ubiquitin-proteasome system in the pathogenesis of medulloblastoma and the potential clinical implications.

KEYWORDS

marizomib, medulloblastoma, proteasome, ubiquitin

Abbreviations: BBB, blood–brain barrier; Bmi1, B lymphoma Mo-MLV insertion region 1 homolog; CI, confidence interval; CNS, central nervous system; E3, ubiquitin ligase; GBM,

glioblastoma; GLI, glioblastoma-derived transcription factor; IC50, half maximal inhibitory concentration; MATH1, protein atonal homolog 1 (alias ATOH1); MYC, myelocytomatosis oncogene; OR, odds ratio; PTCH, patched, a family of membrane receptors including PTCH1 and PTCH2; SCF, Skp, Cullin, F-box complex; SCF- β -TrCP, E3 ligase β -transducin repeats-containing protein; SCF-Fbw7, E3 ligase F-box/WD repeat-containing protein 7; SCF-Skp2, E3 ligase S-phase kinase-associated protein 2; SHH, sonic hedgehog; TBR1, T-box, brain, 1; Ub, ubiquitin; UPS, ubiquitin-proteasome system; WNT, Wingless-related integration site gene.

1 INTRODUCTION: MEDULLOBLASTOMA

Medulloblastoma is an embryonal tumor (tumors that start from fetal/embryonic cells; neural cell precursors) occurring in the posterior fossa and is the most common malignant childhood brain tumor.¹ The annual incidence of medulloblastoma has been reported to be six per one million children (450 new cases a year).² Treatment for medulloblastoma is multimodal, including surgery, chemotherapy, with or without intracranial radiation depending on age. While risk stratification is used for treatment decision making, current trends are moving away from this practice and are investigating subgroup-specific treatment plans. Recent molecular studies have demonstrated that medulloblastoma is not a single group, but a collection of related neoplasms originating from the cerebellar stem and progenitor cells with variable clinical features and prognoses.³ Historically, there have been four subgroups of medulloblastoma: Wingless-related integration site gene (WNT; 10% incidence), sonic hedgehog (SHH; 30% incidence), Group 3 (20% incidence), and Group 4 (40% incidence).⁴ As a result of the utilization of similarity network fusion integrating both gene and non-gene datasets into a classification system, there are now 12 described subtypes of medulloblastoma: two WNT (α , β), four SHH (α , β , γ , δ), three Group 3 (α , β , γ), and three Group 4 (α , β , γ)^{3,4} (Table 1).

2 INTRODUCTION: UBIQUITIN-PROTEASOME SYSTEM

The ubiquitin-proteasome system (UPS) is a highly regulated intracellular protein degradation system crucial for cell survival and protein maintenance. The ubiquitin-proteasome pathway utilizes enzymes that link a polypeptide cofactor, ubiquitin (Ub), to proteins marking them for degradation. The Ub is activated by an activating enzyme (E1) and then transferred to a conjugating enzyme (E2), and finally, ubiquitin ligase (E3) adds Ub to a target

protein covalently as a tag. Once tagged with multiple Ub, these tagged proteins are recognized by the 26S proteasome resulting in degradation of the ubiquitinated proteins into smaller peptides⁵ (Figure 1). This system is crucial in preventing cellular dysfunction as it prevents the accumulation of denatured, misfolded, and aged proteins.

In properly functioning tissue, the UPS helps regulate the cell cycle through cellular homeostasis, apoptosis, and immune editing. In particular, the UPS has been shown to play an essential role in cerebellar development.

3 UPS AND NORMAL CEREBELLAR DEVELOPMENT

The cerebellum arises from the dorsal part of the anterior hindbrain during embryogenesis and its cellular organization continues after birth.⁶ The cerebellum contains over 50% of the brain's neurons.⁷ Cerebellar neurons arise from four cerebellar germinal zones: the internal germinal zone, external germinal zone, rostral germinal zones, and upper rhombic lip. Cerebellar granule cells arise from the external germinal zone and upper rhombic lip and then migrate to establish the external germinal zone that serves as a reservoir of noncommitted granule cell precursors.^{8–10} Proliferation in the external germinal zone is tightly regulated by the transcription factor protein atonal homolog 1 (alias ATOH1) (*MATH1*).¹¹ *MATH1* activates *Barhl1*, which plays a role in cerebellar development. In glutamatergic neurons, *MATH1* affects *PAX6* (paired box 6), which is essential in cerebellar development and subsequently induces *TBR1/2*, *Neuro D*, and *reelin* expression.^{12–15} WNT/ β catenin signaling pathway is also involved in proliferation and differentiation of cerebellar granule neurons. The WNT signaling pathway causes nuclear translocation of β -catenin, which activates gene transcription involved in cell fate specification and proliferation of cerebellar granule neuron precursors.¹⁶ The lifespan of β -catenin is determined by Ub-mediated proteolysis, specifically E3 ligase, SCF- β -TrCP (E3 ligase β -transducin repeats-containing protein).^{17,18}

The SHH signaling pathway is involved in proliferation of cerebellar granule cell progenitors. Activation of the Purkinje cell SHH pathway causes cerebellar granule cell precursors to exit the cell cycle and migrate along Bergmann glial fibers, which then promotes proliferation of cerebellar granule cell precursors and maintains cerebellar architecture. SHH signaling occurs when SHH binds Patched-1 (PTCH1) receptor. The binding of

SHH to PTCH1 releases Smoothed (SMO) inhibition, which activates glioblastoma-derived (GLI) transcription factors (Gli1, Gli2, Gli3), leading to the accumulation of GLI in the nucleus and activation of the hedgehog target genes, leading to cell proliferation.^{23,24} UPS is involved in maintenance of Gli3, a repressive transcription factor, and PTCH1. The E3 ligase, SCF- β -TrCP ubiquitinate Gli3 and the E3 ligase, HECT, degrade PTCH1.²⁴ SHH signaling in the external germinal zone protects *MATH1* from degradation by ubiquitin E3 ligase, Huwe1.²⁵

Polycomb group gene *Bmi1* (B lymphoma Mo-MLV insertion region 1 homolog) is associated with cerebellar granule cell precursors' proliferation and induces neural precursor cell self-renewal and proliferation during development.^{5,26} It is often coexpressed with *N-MYC* and *Cyclin D2*.^{5,27} The *Bmi1* gene is described as an oncogene through the regulation of p16 and p19 (transcription inhibitors) that inhibit the expression of ubiquitin E3 ligase, Fbw7.⁵ Mutations in the *Bmi1* gene result in defective hematopoiesis, neurologic function, and cerebellar development (Table S1).

Normal cerebellar development involves multiple gene regulators whose UPS tightly controlled products' concentrations. This system's balance leads to normal cerebellar development, while UPS abnormalities can result in disease, including cancers such as medulloblastoma. UPS disruption and subsequent development of medulloblastoma subgroups are reviewed in the following section.

4 DYSREGULATION OF UPS AND ITS ROLE IN TUMORIGENESIS

Dysregulation of UPS is implicated in tumorigenesis through posttranslational modification of oncogene and tumor suppressor levels.²⁸ In normal cells, the accumulation of damaged proteins results in apoptosis. However, in cancer cells, it is thought that normal apoptotic pathways are bypassed through alteration of the proteasomal pathways. As Nahreini and Vriend et al. described, this can result in inappropriate degradation of signaling and transcription factors, leading to altered fate and function of cells. In the UPS system, the E3 ligases are the primary regulator of cellular processes such as cell proliferation, cellular arrest, and apoptosis. E3 ligases can impact levels of oncogene products and tumor suppressor proteins.

The expression of specific E3 ligases in the UPS is unique in each medulloblastoma subtype.²⁹ Preclinical studies revealed that proteasome-dependent degradation plays a role in progression and maintenance of medulloblastoma.^{30–32} Proteasome inhibition studies demonstrate apoptosis and inhibition of cell proliferation in medulloblastoma cell lines and xenograft models.^{33–36} Targeting proteasome some subunits in medulloblastoma may prove to be effective adjuvant treatment.

When the UPS is interrupted, misfolded, and damaged, proteins accumulate, leading to cell apoptosis. Cancer depends on the abnormal regulation of cell proliferation and anti-apoptotic pathways by the proteasome. Due to an abnormal rate of protein synthesis, cancer cells are theorized to be more sensitive to this process' inhibition. The exact mechanism for efficacy is still unclear, but it is thought that it is due to altered cell cycle proteins' altered degradation, altered pro and anti-apoptotic protein balance, and inhibition of the NFkB pathway. It has also been demonstrated that malignant cells are more sensitive to proteasome inhibitors' cytolytic effect than normal cells, making proteasome inhibitors a potential therapeutic target. Proteasome inhibitors work by specifically targeting the proteasome in the catalytic center, preventing the degradation of tumor suppressors and checkpoint inhibitors.

5 PHARMACOLOGY OF PROTEASOME INHIBITORS

Proteasome is the most central part of the UPS system. It is a large, complex molecule that contains a catalytic protease complex. Proteasome inhibitors are small peptides that fit into a binding site on a catalytic subunit. From there, the activity of the proteasome inhibitor depends on its pharmacophore at the C-terminus. Proteasomes have three types of catalytic sites targeted by the proteasome inhibitors ($\beta 1$, $\beta 2$, $\beta 5$) (Figure 2). Inhibition of all three sites is not required for arresting the proteolytic activity. The $\beta 5$ site is most often targeted as it seems to have the most significant effect on normal protein breakdown. There are currently three proteasome inhibitors that are used in clinical practice and three additional inhibitors that are still in the trial stages of development^{37–45} (Table 2).

6 CLINICAL IMPLICATIONS AND CLINICAL TRIALS UTILIZING PROTEASOME INHIBITORS

The clinical utility of proteasome inhibition for cancer is best exemplified in multiple myeloma. Bortezomib and carfilzomib, both irreversible proteasome inhibitors with limited 20S inhibition profile, showed efficacy in multiple myeloma treatment.^{46–49} The use of these proteasome inhibitors for brain tumors has been limited by their inability to cross the blood–brain barrier (BBB).^{50–53} Evidence supports poor blood–brain penetration by bortezomib. In pharmacokinetic (PK) and pharmacodynamic (PD) studies in rat models, brain concentrations of bortezomib were extremely low with a brain-to-blood ratio of 0.02.⁵⁰ There was also no difference in brain proteasome activity observed in the treatment group versus the control group.

Bortezomib (a reversible proteasome inhibitor) has been efficacious in the treatment of multiple myeloma.^{46,47} A meta-analysis of 12 randomized control trials comparing bortezomib versus no bortezomib with the same background therapy for multiple myeloma demonstrated that bortezomib prolonged progression-free survival (PFS) and overall survival (OS) compared to background therapy alone, with an odds ratio (OR) of 0.65, 95% confidence interval (CI) 0.57–0.74, and OR 0.77, 95%CI 0.65–0.92, respectively.⁴⁷ Proteasome inhibitors have shown efficacy in some adult malignancies.

Clinical studies in adult gliomas demonstrate improved outcomes with combined proteasome inhibitor therapy. Preclinical data on glioma cell lines suggested the therapeutic efficacy of bortezomib and carfilzomib in combination.^{51–53} A phase I clinical trial of 66 patients with recurrent malignant glioma showed only two partial responses to bortezomib. A phase II trial showed no clinical activity of bortezomib in malignant glioma treatment with or without temozolomide.⁵⁸ Intratumoral delivery of bortezomib in orthotopic glioblastoma (GBM) murine models showed an increase in efficacy of bortezomib.^{54,55} One explanation for this discrepancy is insufficient BBB penetration of these agents.⁵⁵

Marizomib (NPI-0052) is a second-generation, lipophilic, irreversible proteasome inhibitor. Its structure varies significantly from bortezomib and carfilzomib with its bicyclic β -lactam structure. Recent work has shown an in vivo antitumor effect of marizomib in orthotopic xenograft models of human GBM.⁵⁶ The same study showed marizomib effectively penetrates the BBB in cynomolgus monkeys and rats. Marizomib levels were seen at 30% of blood levels in rats, and inhibited chymotrypsin-like proteasome activity in monkey brain tissue.⁵⁶ There are

multiple clinical trials involving marizomib for the treatment of adult GBM. Phase 1/2 clinical trial evaluating the use of marizomib with or without bevacizumab in recurrent GBM treatment showed promising results (NCT02330562).⁵⁷ A phase 1b multicenter study of marizomib in combination with radiotherapy and temozolomide showed safety and tolerability of the combination (NCT02903069).⁵⁸ A phase III study is now open for newly diagnosed GBM to study marizomib's impact on overall survival (NCT03345095).

Upon reviewing clinicaltrials.gov, there are currently 66 clinical trials for pediatric aged patients using bortezomib, five trials using ixazomib, five trials for carfilzomib, and one trial for marizomib. There are no trials for pediatric aged patients using oprozomib or delanzomib. Of these trials, three include central nervous system (CNS) malignancies (two using bortezomib and one using marizomib). There are various adult trials using proteasome inhibitors, but there are currently only three trials utilizing bortezomib or marizomib in CNS malignancies (Table S2).

Although the results for the pediatric trials looking at the use of proteasome inhibitors as a monotherapy versus combination therapy for CNS tumors have not yet come (NCT01132911 and NCT00994500), the adult phase trials using bortezomib in combination with vorinostat for recurrent GBM were not successful.⁶² The poor results were thought to be due to the findings of Hemeryck et al., which demonstrated low CNS penetration of bortezomib in models. Other proteasome inhibitors have shown early efficacy in some adult clinical trials and are discussed in the later sections.

7 PROTEASOME INHIBITOR STUDIES USING MEDULLOBLASTOMA CELL LINES AND XENOGRAFT MODELS

Given the role of UPS in oncogenesis, proteasome inhibitors have been the topic of recent therapeutic research. As previously mentioned, multiple clinical studies have shown efficacy in utilizing proteasome inhibitors in the treatment of adult cancers such as multiple myeloma.^{46–48,59} Here, recent studies using proteasome inhibition to elucidate mechanisms of tumorigenesis in mouse models and studies evaluating the utility of proteasome inhibition for brain tumor treatment will be discussed.

Taniguchi et al. described a medulloblastoma mouse model with haploinsufficiency for PTCH1.⁶⁰ Development of tumors in these mice confirmed presence of PTCH1 messenger RNA (mRNA) transcripts, but a relative paucity of PTCH1 protein. The study hypothesized this was due to posttranscriptional regulation of PTCH1, including degradation by UPS. The mice were treated with bortezomib and found restored levels of the PTCH1 protein, resulting in extended survival of these mice.

The study included in vitro cell growth assays of bortezomib on three human medulloblastoma cell lines (DaOY, D283, and D341)⁶⁰ (Table 3). This study confirmed human medulloblastoma cell lines were sensitive to bortezomib with an half maximal inhibitory concentration (IC₅₀) ≤10 nM. The level of Gli1, a downstream target of PTCH1, was decreased in these cells treated with bortezomib, suggesting PTCH1 function restoration with bortezomib. Overall, these studies suggest that proteasome-dependent degradation of PTCH1 plays a role in progression and maintenance of medulloblastoma.^{60,61}

Preclinical data on glioma cell lines have suggested bortezomib and carfilzomib's therapeutic efficacy, although this has not been replicated in xenograft models.^{51–54} One study confirmed proteasome inhibition in two human malignant glioma xenografts (TCG3 and U87) treated with three dosing levels of bortezomib, but there was no increase in apoptotic rate, growth delay, or modification of cell cycle distribution.⁵³ This was likely due to the lack of BBB penetration as opposed to testing cell cultures.

A study performed by Ohshima-Hosoyama et al. evaluated bortezomib's mechanism in a model of SHH-activated medulloblastoma.³⁰ Gene expression microarray analysis was conducted, and 44,000 mouse transcripts were used to identify E3 ubiquitin ligases with high expression. No direct upstream mediator of bortezomib's efficacy was identified. Next, the study evaluated post-translationally modified proteins known to be downstream targets of bortezomib in other cancers. MEK5 stabilization and subsequent ERK5 activation were identified as a possible mechanism. They also noted protein, NOXA, accumulated in the presence of bortezomib and was dose-dependent. NOXA mediates apoptosis in response to reactive oxidative stress (ROS) and is degraded by ubiquitination. The stabilization of NOXA was confirmed in PTCH1, p53 double-mutant medulloblastoma cells, suggesting the induction of apoptosis is p53 independent.³¹

In primary culture of human medulloblastomas and in human medulloblastoma xenografts, Yang et al. demonstrated that bortezomib induced apoptosis and inhibited cell proliferation in DaOY and D283 medulloblastoma cell lines.³¹ Yang theorized that apoptosis was induced by activation of the caspase system and the endoplasmic reticulum response system pathway. These studies revealed cell proliferation was inhibited by accumulation of growth inhibitory molecules p21Cip1 and p27Kip1, cyclin-dependent kinase inhibitors degraded by UPS, and inhibition of phosphorylation of protein kinase B (AKT) and mTOR. Yang's team found that bortezomib treatment resulted in the accumulation of phosphorylated I κ B, and blocked NF κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) nuclear translocation and its effects on transcription.

Based on these laboratory studies, bortezomib activity was demonstrated in established cell lines, primary culture, and mouse xenograft model of human medulloblastoma, suggesting potential therapeutic benefit of proteasome inhibition in medulloblastoma.³²

Preliminary studies in the Bota laboratory examined the effect of marizomib on the 20S proteasome activity of stable malignant pediatric medulloblastoma cell lines, CHLA-259, and MED-2112FH. CHLA-259 is a Group 3, anaplastic medulloblastoma, MYCN amplified medulloblastoma stable cell line. MED-2112FH is a Group 3, anaplastic large cell, 17p, 17q, 9q, myelocytomatosis oncogene (MYC) amplified medulloblastoma stable cell line. The viability of MED-2112FH and CHLA-259 medulloblastoma cells was measured following exposure to increasing concentrations of marizomib. The IC₅₀ was calculated to be 28 nM for MED-2112FH and 10 nM for CHLA-259. In vitro studies demonstrated that marizomib reduces cell viability in a dose-dependent manner. In vivo studies are ongoing.

8 DISCUSSION

The UPS is intricately involved in the normal neurogenesis of cerebellum. It also plays a crucial role in regulating intracellular protein degradation, including regulatory proteins in signaling pathways of cell growth, cell cycle progression, and apoptosis that if interrupted, can lead to oncogenesis. As previously mentioned, cancer cells have rapid proliferation rates compared to normal cells, which demonstrates increased proteasome activity. The

increase in proteasomal activity would prevent the accumulation of proteins within the cell, which allows the cancer cell to avoid apoptosis, and continue to “survive” and proliferate. When specific mutations are present, such as those seen in the E3 ligases, cells (such as cancer cells) may have an increased ability to drive proteasomal activity, such as in the case of medulloblastoma.

In medulloblastoma, given that specific subgroups are associated with mutations of the E3 ligases of the UPS or their associated proteins, one could infer that medulloblastoma cells with these mutations propagate due to increasing proteasome activity. The associated mutations of the E3 ligases within each medulloblastoma subgroup lead to increased transcription proteins or decreased tumor suppressors. Therefore, proteasome inhibitors would theoretically cause these proteins to accumulate, leading to apoptosis.

Multiple institutions have demonstrated the proteasome’s role and the subsequent effect and therapeutic benefit of proteasome inhibitors in cancers such as leukemia, lymphoma, multiple myeloma, and some types of solid tumors. Little is known about the effect of proteasome inhibitors in pediatric CNS tumors, especially medulloblastomas. While there have been some promising results in xenograft models, one of the most considerable obstacles from those studies remains the low BBB penetration, which is problematic when treating CNS malignancies. However, marizomib, a second-generation proteasome inhibitor, is a new agent that has shown promise in preclinical trials for crossing the BBB.

9 CONCLUSION

Given the significant involvement of UPS in normal cerebellar development and pathogenesis of medulloblastoma, next-generation proteasome inhibitors may possess therapeutic benefit for these childhood tumors. Efficacy may be subgroup-specific depending on the molecular subgroup characteristics and its relation to the UPS. To our knowledge, no studies have investigated proteasome activity in each subgroup of medulloblastoma. However, given the role of Fbw7 and Huwe1 in MYC levels and the presence of Fbw7 mutations in the SHH subgroup, it is theorized that UPS has a more significant effect in the SHH and Group 3 subgroups of medulloblastoma. This potentially suggests that the SHH and Group 3 medulloblastomas may have a higher susceptibility to proteasome inhibitors. Although there are currently no open pediatric trials looking at

marizomib for pediatric medulloblastoma, preclinical studies have shown promising results regarding marizomib's utilization in the treatment of pediatric medulloblastoma. It will be beneficial to evaluate the results of adult trials using marizomib for CNS tumors. These trials could provide the essential data needed to further fund research utilizing proteasome inhibitors in pediatric CNS tumors like medulloblastoma.

10 HIGHLIGHTS

1. The UPS system is integral to cerebellar development.
2. Data suggest subgroup-specific E3 ligases are involved in the pathogenesis of medulloblastoma.
3. Dysfunction of the UPS can result in abnormal cell proliferation and imbalance of pro- and anti-apoptotic signals.
4. Cancer cells have shown susceptibility to proteasome inhibitors, and newer agents like marizomib are BBB penetrant.
5. Proteasome inhibitors warrant further research as a potential therapeutic strategy for medulloblastoma.

11 METHODOLOGY OF LITERATURE REVIEW

Electronic searches were performed in PubMed with final searches performed by October 9, 2020. Articles were limited to publications between the years 1998 and 2020. The primary search strategy used a combination of keywords for proteasome inhibition in medulloblastoma (proteasome inhibition or UPS) or E3 ligases or proteasome inhibitors or UPS cerebral development or UPS medulloblastoma or proteasome medulloblastoma or Ub pathway and medulloblastoma or medulloblastoma subgroups or medulloblastoma development or medulloblastoma xenograft models or medulloblastoma xenografts proteasome inhibition or marizomib or marizomib medulloblastoma). We also cross-referenced our search with articles cited in several reviews and other papers found with our search criteria. The authors conducted searches and article selection. Electronic searches in clinicaltrials.gov were also conducted to identify relevant clinical trials using proteasome inhibitors as a treatment modality in treating both pediatric and adult malignancies. Criteria used to find clinically relevant trials included "proteasome inhibitors," "marizomib," "bortezomib," "carfilzomib," "Ivazomib," "oprozomib," and "delanzomib." No age ranges were excluded during the search. All statuses for enrollment were also selected.

ORCID

Michelle D. Pulley <https://orcid.org/0000-0002-4593-8700>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Hoerig CM, Plant-Fox AS, Pulley MD, Di K, Bota DA. Exploring the role and clinical implications of proteasome inhibition in medulloblastoma. *Pediatr Blood Cancer*. 2021;e29168. <https://doi.org/10.1002/pbc.29168>

Table 1. Medulloblastoma subgroups overview.

Subgroups (% prevalence)		WNT (9%)		SHH (29%)			
Subtypes		WNT α	WNT β	SHH α	SHH β	SHH γ	SHH δ
Clinical Characteristics	Subtype Prevalence	70%	30%	29%	16%	21%	34%
	Population (age)	3-17yo	10yo-adult	2-17yo	0-3yo	0-3yo	adults
	Metastases	8.6%	21.4%	20%	33%	8.9%	9.4%
	5-year survival	97%	100%	69.8%	67.3%	88%	88.5%
Biological Characteristics	Proposed cell origin	Lower rhombic lip and embryonic dorsal brainstem		Granule precursors of the external granule layer			
	Histology	Classic, rarely large cell anaplastic		Desmoplastic/nodular (MBEN), large cell anaplastic			
	Cytogenetics	6* (monosomy 6)	unknown	9q*, 10q*, 17p*	unknown	unknown	10q*
	Subgroup Genetics and Mutations	CTNNB1 mutation		PTCH1/PTCH2/SMO/SUFU mutation 3q*, 14q*			
	Subtype specific Mutations	unknown	unknown	MYCN amp GLI2 amp YAP1 amp	PTEN loss	MBEN	10q22- 11q23.3
	Other characteristics			TP53 mut.			TERT pro.
Subgroups (% prevalence)		Group 3 (19%)			Group 4 (43%)		
Subtypes		Group 3 α	Group 3 β	Group 3 γ	Group 4 α	Group 4 β	Group 4 γ
Clinical Characteristics	Subtype Prevalence	47%	25%	28%	30%	33%	37%
	Population (age)	0-10yo	3-17yo	0-10yo	3-17yo	3-17yo	3-17yo
	Metastases	43.4%	20%	39.4%	40%	40.7%	38.7%
	5-year survival	66.2%	55.8%	41.9%	66.8%	75.4%	82.5%
Biological Characteristics	Proposed cell origin	Neural stem cells			Unipolar brush cells		
	Histology	Large Cell/Anaplastic, classic (rarely)			Large Cell/Anaplastic, classic (rarely)		
	Cytogenetics	7*, 8*, 10q*, 11*, i17q	unknown	8*, i17q	7q*, 8p*, i17q	i17q	7q*, 8p*, i17q
	Subgroup Genetics	PVT1 SMARCA4 1q*, 18q*, 16p*			KD6MA MLL3 11p*		
	Subtype Specific Mutations	unknown	OTX2 gain DDX31 loss	MYC amp	MYCN amp CDK6 amp	SNCAIP dup	CDK6 amp
	Other characteristics		GFI1/1B exp				

Note: For cytogenetics; – indicates “loss” whereas + indicates “gain.” Specific subtypes have been found to have a higher prevalence of certain genetic markers/expression. There are some mutations found within the subgroup that have not yet been found to have a higher prevalence in any of the specific subtypes. As a result, they were grouped together. Regrading TP53 mutations, although the recent WHO classification includes SHH-activated p53 mutant tumors as a separate category, there is a high prevalence of the mutation in SHH α group. Adapted from a previous study by Cavalli et al.³

Abbreviations: amp, amplification; dup, duplication; exp, expression; i17, isochromosome 17; MBEN, medulloblastoma with extensive nodularity; Mets, metastasis; mut, mutation; pro, proliferation; SMO, Smoothed; SUFU, suppressor of fused homolog.

Figure 1. Ubiquitin-proteasome system (UPS) and protein selection. There are three types of ubiquitination of substrates: mono-, multi-, or poly-ubiquitination. Mono- or multi-ubiquitination is typically involved in the process of receptor endocytosis, DNA repair, nuclear export, gene expression, or histone regulation. Poly-ubiquitination is involved with proteasomal degradation, intracellular trafficking, DNA damage response, cell cycle control, TNF α signaling, protein kinase control, ribosomal biogenesis, NG-kB activation, mitochondrial activation, and endoplasmic reticulum-associated protein degradation

Figure 2. Proteasome structure. The proteasome is heavily involved in many cellular processes as cell cycle proteins rely on the degradation of these proteins for cell cycle progression. Tumor cells rely on increased proteasomal activity for the degradation of the tumor-suppressive proteins. This increased proteasomal activity was the basis for marking the proteasome as a target for therapy, specifically the catalytic components (20S). $\beta 1$, caspase-like activity; $\beta 2$, trypsin-like activity; $\beta 3$, chymotrypsin activity
Note: the 19S subunit has a regulatory function at this time.

Table 2. Proteasome inhibitor pharmacology^{37–45}

Drug (trade name)	Gen.	Pharmacophore binding site	Binding mode	Indication	Main adverse effects	Crosses BBB?
Bortezomib (Velcade)	First	Boronic acid $\beta 5$, partial $\beta 1$	Reversible	Multiple myeloma (MM), mantle cell lymphoma	Neutropenia, peripheral neuropathy	^a No (~[5%–7%])
Carfilzomib (Kyprolis)	Second	Epoxyketone $\beta 5$	Irreversible	Relapse/refractory MM	Cytopenias, respiratory symptoms, fatigue, headache	No
Ixazomib (Ninlaro)	Second	Boronic acid $\beta 1$, $\beta 2$, $\beta 5$	Reversible	Relapsed/refractory MM	Diarrhea, constipation, nausea, vomiting, swelling of hands/feet, peripheral neuropathy	No
Oprozomib	Second	Epoxyketone $\beta 5$	Irreversible	Trials for MM and Waldenstroms macroglobulinemia (WM)	Diarrhea, constipation nausea, vomiting, fatigue	No
Marizomib (Salinosporamide A)	Second	B-lactone $\beta 1$, $\beta 2$, $\beta 5$	Irreversible	Trials for MM, WM, lymphomas, leukemias, colon cancer, pancreatic cancer, prostate cancer, melanoma, glioma, non-small cell lung carcinoma, and renal cell carcinoma	Diarrhea, constipation, nausea, vomiting, cytopenias, headache, dizziness, infusion site pain, hallucinations, ataxia	Yes
Delanzomib	Second	Boronic acid $\beta 1$, $\beta 5$	Reversible	Trials for non-Hodgkin lymphoma and solid tumors	Skin toxicity	No

Abbreviations: BBB, blood–brain barrier; Gen, generation; MM, multiple myeloma; WM, Waldenstroms macroglobulinemia; $\beta 1$, caspase-like activity; $\beta 2$, trypsin-like activity; $\beta 5$, chymotrypsin-like activity.

^aIn some studies, bortezomib had low BBB penetrance, while the other proteasome inhibitors, excluding marizomib, had no penetrance.

Table 3. Medulloblastoma cell line and xenograft information

Identifier	Cell line or xenograft	Diagnosis	Subgroup subtype	Date of original isolation	Patient demographics	Other relevant information
DaOY (HTB-186)	Cell line	Desmoplastic cerebellar Medulloblastoma	SHH	1985	4-year-old Caucasian Male	
D283 MED (HTB-185)	Cell line	Medulloblastoma	Group 4 β or 4 γ	1985	6-year-old Caucasian Male	Line derived from metastatic site No MYC amplification High-level gain GABRA5 expression 8q+, 17p+, 20q+ *Gained an extra copy of chromosome 11 after initial implant
D341 MED	Cell line	Medulloblastoma	Group 3 γ	1988	3.5-year old Caucasian Male	MYC amplification
TCG3	Mouse xenograft	Anaplastic oligodendroglioma	NA	2001	58-year-old Caucasian Female	GEFR amplification P53 status = mutant PIK3CA mutation
U87	Mouse xenograft	Stage 3 glioblastoma	NA	1966	44-year-old Caucasian Male	
MED-2112FH	Cell line	Anaplastic medulloblastoma	Group 3	Unknown	7-year-old Caucasian Male	
CHLA-259	Cell line	Anaplastic medulloblastoma	Group 3	2001	14-year-old Hispanic Male	MYCN amplified

REFERENCES

1. Ostrom QT, de Blank PM, Kruchko C, et al. Alex's Lemonade Stand Foundation infant and childhood primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. *Neuro Oncol.* 2015;16(10):x1-x36.
2. Mahapatra S, Amsbaugh MJ. *Medulloblastoma. In: StatPearls [Internet].* Treasure Island, FL: StatPearls Publishing; 2020.
3. Cavalli FMG, Remke M, Rampasek L, et al. Intertumoral heterogeneity within medulloblastoma subgroups. *Cancer Cell.* 2017;31(6):737-754.e6.
4. Kijima N, Kanemura Y. Molecular classification of medulloblastoma. *Neurol Med Chir (Tokyo).* 2016;56(11):687-697.
5. Vriend J, Ghavami S, Marzban H. The role of the ubiquitin proteasome system in cerebellar development and medulloblastoma. *Mol Brain.* 2015;8(1):64.
6. Hibi M, Shimizu T. Development of the cerebellum and cerebellar neural circuits. *Dev Neurobiol.* 2012;72(3):282-301.
7. Yeung J, Ha T, Swanson D, Choi K, Tong Y, Goldowitz D. Wls provides a new compartmental view of the rhombic lip in mouse cerebellar development. *J Neurosci.* 2014;34(37):12527-12537.
8. Carletti B, Rossi F. Neurogenesis in the cerebellum. *Neuroscientist.* 2007;14(1):91-100.
9. Leto K, Arancillo M, Becker E, et al. Consensus paper: cerebellar development. *Cerebellum.* 2015;15(6):789-828.
10. Wang V, Zoghbi H. Genetic regulation of cerebellar development. *NatRev Neurosci.* 2001;2(7):484-491.
11. Machold R, Fishell G. Math1 is expressed in temporally discrete pools of cerebellar rhombic-lip neural progenitors. *Neuron.* 2005;48(1): 17-24.
12. Englund C. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J Neurosci.* 2005;25(1):247-251.
13. Fink A. Development of the deep cerebellar nuclei: transcription factors and cell migration from the rhombic lip. *J Neurosci.* 2006;26(11):3066-3076.
14. Bulfone A, Martinez S, Marigo V, et al. Expression pattern of the Tbr2 (Eomesodermin) gene during mouse and chick brain development. *Mech Dev.* 1999;84(1-2):133-138.
15. Lee J, Cho J, Hwang W, Lee Y, Reu D, Suh-Kim H. Expression of neuroD/BETA2 in mitotic and postmitotic neuronal cells during the development of nervous system. *Dev Dyn.* 2000;217(4):361-367.
16. Wodarz A, Nusse R. Mechanisms of WNT signaling in development. *Annu Rev Cell Dev Biol.* 1998;14(1):59-88.
17. Gibson P, Tong Y, Robinson G, et al. Subtypes of medulloblastoma have distinct developmental origins. *Nature.* 2010;468(7327):1095-1099.
18. Lehman N. The ubiquitin proteasome system in neuropathology. *Acta Neuropathol.* 2009;118(3):329-347.
19. Cheng F, Fleming J, Chiang C. Bergmann glial sonic hedgehog signaling activity is required for proper cerebellar cortical expansion and architecture. *Dev Biol.* 2018;440(2):152-166.
20. Haldipur P, Bharti U, Govindan S, et al. Expression of sonic hedgehog during cell proliferation in the human cerebellum. *Stem Cells Dev.* 2012;21(7):1059-1068.
21. Wechsler-Reya R, Scott M. Control of neuronal precursor proliferation in the cerebellum by sonic hedgehog. *Neuron.* 1999;22(1):103-114.
22. De Luca A, Cerrato V, Fucà E, Parmigiani E, Buffo A, Leto K. Sonic hedgehog patterning during cerebellar development. *Cell Mol Life Sci.* 2015;73(2):291-303.
23. Taipale J, Cooper M, Maiti T, Beachy P. Patched acts catalytically to suppress the activity of Smoothened. *Nature.* 2002;418(6900):892-896.
24. Lu X. The C-terminal tail of the Hedgehog receptor Patched regulates both localization and turnover. *Genes Dev.* 2006;20(18):2539-2551.
25. Forget A, Bihannic L, Cigna S, et al. Shh signaling protects Atoh1 from degradation mediated by the E3 ubiquitin ligase Huwe1 in neural precursors. *Dev Cell.* 2014;29(6):649-661.
26. Behesti H, Bhagat H, Dubuc A, Taylor M, Marino S. Bmi1 overexpression in the cerebellar granule cell lineage of mice affects cell proliferation and survival without initiating medulloblastoma formation. *Dis Model Mech.* 2012;6(1):49-63.
27. Leung C, Lingbeek M, Shakhova O, et al. Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. *Nature.* 2004;428(6980):337-341.

28. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev*. 2002;82(2):373-428.
29. Cogen P, Daneshvar L, Metzger A, Edwards M. Deletion mapping of the medulloblastoma locus on chromosome 17p. *Genomics*. 1990;8(2):279-285.
30. Ohshima-Hosoyama S, Davare M, Hosoyama T, Nelon L, Keller C. Bortezomib stabilizes NOXA and triggers ROS-associated apoptosis in medulloblastoma. *J Neurooncol*. 2011;105(3):475-483.
31. Yang F, Jove V, Xin H, Hedvat M, Van Meter T, Yu H. Sunitinib induces apoptosis and growth arrest of medulloblastoma tumor cells by inhibiting STAT3 and AKT signaling pathways. *Mol Cancer Res*. 2010;8(1):35-45.
32. Zaky W, Manton C, Miller C, Khatua S, Gopalakrishnan V, Chandra J. The ubiquitin-proteasome pathway in adult and pediatric brain tumors: biological insights and therapeutic opportunities. *Cancer Metastasis Rev*. 2017;36(4):617-633.
33. Burnett M, White E, Sih S, von Haken M, Cogen P. Chromosome arm 17p deletion analysis reveals molecular genetic heterogeneity in supratentorial and infratentorial primitive neuroectodermal tumors of the central nervous system. *Cancer Genet Cytogenet*. 1997;97(1): 25-31.
34. Huse J, Holland E. Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. *Nat Rev Cancer*. 2010;10(5):319-331.
35. Taylor M, Northcott P, Korshunov A, et al. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol*. 2011;123(4):465-472.
36. Thompson M, Fuller C, Hogg T, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol*. 2006;24(12):1924-1931.
37. Park JE, Miller Z, Jun Y, Lee W, Kim KB. Next-generation proteasome inhibitors for cancer therapy. *Transl Res*. 2018;198:1-16.
38. Franken B, van de Donk NW, Cloos JC, Zweegman S, Lokhorst HM. A clinical update on the role of carfilzomib in the treatment of relapsed or refractory multiple myeloma. *Ther Adv Hematol*. 2016;7(6):330-344.
39. Huehnchen P, Springer A, Kern J, et al. Bortezomib at therapeutic doses poorly passes the blood-brain barrier and does not impair cognition. *Brain Commun*. 2020;2(1):fcaa021.
40. Varga G, Mikala G, Gopcsa L, et al. Multiple myeloma of the central nervous system: 13 cases and review of the literature. *J Oncol*. 2018;2018:3970169.
41. Dou QP, Zonder JA. Overview of proteasome inhibitor-based anticancer therapies: perspective on bortezomib and second generation proteasome inhibitors versus future generation inhibitors of ubiquitin proteasome system. *Curr Cancer Drug Targets*. 2014;14(6):517-536.
42. Franken B, van de Donk NW, Cloos JC, Zweegman S, Lokhorst HM. A clinical update on the role of carfilzomib in the treatment of relapsed or refractory multiple myeloma. *Ther Adv Hematol*. 2016;7(6):330-344.
43. Ghobrial IM, et al. A Phase Ib/II study of oprozomib in patients with advanced multiple myeloma and Waldenström macroglobulinemia. *Clin Cancer Res*. 2019;25(16):4907-4916.
44. Narayanan S, Cai C-Y, Assaraf YG, et al. Targeting the ubiquitin proteasome pathway to overcome anti-cancer drug resistance. *Drug Resist Updat*. 2020;48:100663.
45. Thibaut TA, Smith DM. A practical review of proteasome pharmacology. *Pharmacol Rev*. 2019;71(2):170-197.
46. Dimopoulos M, Moreau P, Palumbo A, et al. Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-label multicentre study. *Lancet Oncol*. 2016;17(1):27-38.
47. Dimopoulos M, Wang M, Maisnar V, et al. Response and progression free survival according to planned treatment duration in patients with relapsed multiple myeloma treated with carfilzomib, lenalidomide, and dexamethasone (KRd) versus lenalidomide and dexamethasone (Rd) in the phase III ASPIRE study. *J Hematol Oncol*. 2018;11(1):49.
48. Scott K, Hayden PJ, Will A, Wheatley K, Coyne I. Bortezomib for the treatment of multiple myeloma. *Cochrane Database Syst Rev*. 2016;4:CD101816.
49. Accardi F, Toscani D, Bolzoni M, et al. Mechanism of action of bortezomib and the new proteasome inhibitors on myeloma cells and the bone microenvironment: impact on myeloma-induced alterations of bone remodeling. *Biomed Res Int*. 2015;2015:172458.
50. Phuphanich S, Supko J, Carson K, et al. Phase 1 clinical trial of bortezomib in adults with recurrent malignant glioma. *J Neurooncol*. 2010;100(1):95-103.
51. Pinel S, Labussiere M, Delfortrie S, Chastagner P. Proteasome inhibition by bortezomib does not translate into efficacy on two malignant glioma xenografts. *Oncol Rep*. 1994;34:171-174.

52. Bota D, Alexandru D, Keir S, Bigner D, Vredenburgh J, Friedman H. Proteasome inhibition with bortezomib induces cell death in GBM stemlike cells and temozolomide-resistant glioma cell lines, but stimulates GBM stem-like cells' VEGF production and angiogenesis. *J Neurosurg.* 2013;119(6):1415-1423.
53. Areeb Z, Stylli S, Ware T, et al. Inhibition of glioblastoma cell proliferation, migration and invasion by the proteasome antagonist carfilzomib. *Med Oncol.* 2016;33(5):53.
54. Raizer J, Chandler J, Ferrarese R, et al. A phase II trial evaluating the effects and intra-tumoral penetration of bortezomib in patients with recurrent malignant gliomas. *J Neurooncol.* 2016;129(1):139-146.
55. Wang W, Cho H, Rosenstein-Sisson R, et al. Intratumoral delivery of bortezomib: impact on survival in an intracranial glioma tumor model. *J Neurosurg.* 2018;128(3):695-700.
56. Di K, Lloyd G, Abraham V, et al. Marizomib activity as a single agent in malignant gliomas: ability to cross the blood–brain barrier. *NeuroOncol.* 2015;18(6):840-848.
57. Bota D, Desjardins A, Mason W, et al. ACTR-71. Full enrollment results from the phase 1/2, multicenter, open-label study of marizomib (MRZ) ± bevacizumab (BEV) in recurrent WHO grade IV malignant glioma (glioblastoma, RGBM). *Neuro Oncol.* 2017;19(6):vi16.
58. Bota D, Kesari S, Piccioni D, et al. A phase 1, multicenter, open-label study of marizomib (MRZ) with temozolomide (TMZ) and radiotherapy (RT) in newly diagnosed WHO grade IV malignant glioma (glioblastoma, ndGBM): dose-escalation results. *J Clin Oncol.* 2018;3615_suppl:e14083.
59. Wang Z, Fukushima H, Gao D, et al. The two faces of FBW7 in cancer drug resistance. *Bioessays.* 2011;33:851-859.
60. Taniguchi E, Cho M, Arenkiel B, et al. Bortezomib reverses a posttranslational mechanism of tumorigenesis for patched1 haploinsufficiency in medulloblastoma. *Pediatr Blood Cancer.* 2009;53(2):136-144.
61. Vriend J, Ghavami S, Marzban H. The role of the ubiquitin proteasome system in cerebellar development and medulloblastoma. *Mol Brain.* 2015;8(1):64.
62. Friday BB, Anderson SK, Buckner J, et al. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. *Neuro Oncol.* 2012;14(2):215-221.