



Evaluating the Efficacy of Targeted Inhibitor Therapeutics for Sonic Hedgehog Medulloblastoma: Significant Milestones and Current Limitations

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Abstract

Medulloblastoma (MB) is the most common pediatric brain tumor with the Sonic Hedgehog (SHH) subtype accounting for 30% of all diagnoses. The current standard treatment regimen includes high doses of toxic chemotherapy and radiation, as well as surgical resection, motivating the need for alternative therapies which do not generate deleterious effects on patients. The purpose of this literature review was to evaluate the most recent developments in the efficacy of targeted therapeutics in treatment of SHH MB, specifically focusing on small molecule inhibitors targeting the Sonic Hedgehog pathway. The sources analyzed in this review include case studies, preclinical and clinical studies, and other review papers that investigate the mechanism and value of five SHH inhibitors: vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61. Novel discoveries have highlighted that inhibitor therapeutics effectively target aberrant activity of the SHH pathway at various stages, thereby diminishing tumor progression and metastasis. Through evaluation of the inhibitors, it was determined that they are promising targeted therapeutics for SHH MB, despite their limitations. These limitations include drug resistance, molecular heterogeneity of SHH-driven tumors, and poor drug properties. More research will be needed to overcome these obstacles for clinical use, but the investment is warranted given the promise of these inhibitors. Future research should seek to establish optimal dosage and timing of intervention, further delineate the genetic basis for SHH MB, and investigate potential combination therapies with SHH inhibitors.

Keywords: Medulloblastoma, Sonic Hedgehog Pathway, Inhibitor Therapeutics, Vismodegib, Sonidegib, Glasdegib, Temozolomide, GANT-61, Pediatric, Cancer, SHH, Sonic Hedgehog, SMO, PTCH

1. Introduction

Medulloblastoma (MB) is a malignant brain tumor first described by Harvey Cushing and Percival Bailey in their classification of central nervous system (CNS) tumors in 1925. Although it is the most common pediatric malignant brain tumor, representing about 20% of all pediatric brain tumors,¹ it is very rare in adults, representing 0.4 to 1% of adult brain tumors.² Most cases are diagnosed under age 16 and it is rarely seen after age 40. In the United States, an average of 500 children and 200 adults are diagnosed with MB every year, and it is more common in males than females.³ Medulloblastoma is part of the primitive neuroectodermal tumors (PNET) group, which is classified within the embryonal subtype of CNS tumors. It originates in the cerebellum, in the posterior fossa, and may spread to other regions of the brain and spinal cord. The World Health Organization classified medulloblastoma into four non-histological subgroups according to molecular profiling of the tumors: Wingless (WNT), Sonic Hedgehog (SHH), and groups 3 and 4. This review will focus on the SHH subtype because it accounts for 30% of all MB diagnoses and is the most prominent subtype in both infants (< 3 years of age) and adults (> 17 years of age).⁴

First introduced in the 1930s, surgery was the initial treatment approach. However, the mortality rate following the operation exceeded 30%.⁵ In 1953, craniospinal irradiation was introduced following surgery, and although there was improvement to a 3-year survival rate of 65%, the development of significant motor and cognitive side effects was observed. Non-specific cytotoxic chemotherapy complemented with surgery and/or radiation was then introduced in the 1970s, and it is still the standard treatment today. Unfortunately, the unforeseen long-term use of this high toxicity treatment therapy has caused extensive toxic damage to patients, particularly in younger patients.⁶ Cerebellar mutism, dysarthria, and neurocognitive disorders result in over 25% of patients following treatment, as well as the growth

of secondary tumors due to high intensity of radiation and chemotherapy. Moreover, the current standardized treatment fails to address the root cause of tumor growth, and along with the occurrence of serious adverse effects, validates the need for non-toxic, individualized therapy which tackles the substantial variability of pathological mechanisms among the four subtypes.

Currently, a substantial amount of research is focused on developing targeted therapeutics for SHH MB in order to meet this need. Since the overactivation of the SHH pathway may play a critical role in the formation of this MB subtype, creating therapeutics that can mitigate aberrations to the SHH pathway serves as a putative treatment strategy. Evidence indicates that SHH signaling directs developmental processes, such as cell differentiation and morphogenesis, and has been implicated in several cancers.⁷ Importantly, proper SHH signaling is crucial for neural tube formation and normal cerebellar development, as the pathway mediates the proliferation of cerebellar granule cells (GCs) during embryonic development. The heightened activation of the SHH pathway leads to the overproliferation of cerebellar GCs, which may culminate in tumorigenesis.⁸ This pathway shows the most promise for developing effective inhibitors, as shown by the extensive amount of research that has been dedicated to understanding its mechanism and the role of SHH signaling in generating medulloblastoma.

The purpose of this literature review is to evaluate the most notable recent developments in targeted inhibitor therapeutics as a possible treatment for SHH MB and suggest further research that would improve the efficacy of the inhibitors. The sources examined describe the mechanism of action and value of five SHH inhibitors: vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61. Significant work has been recently devoted to the development of these inhibitors, and researchers need to understand the extent of this recent progress in order to plan their next steps. Therefore, this review serves as an important resource because it

presents a summary and evaluation of findings regarding the efficacy of SHH inhibitors, as well as potential future steps to eventually use these therapeutics in clinical practice. Overall, novel findings suggest that inhibitors of the SHH pathway exhibit efficacy in suppressing the pathway and diminishing tumor growth in SHH MB; however, further investigation is needed to overcome the limitations that arise with these therapeutics.

2. The Hedgehog Pathway

SHH-MB is named as such because the overactivation of the SHH pathway is the mechanism driving the formation of this tumor. Evidence suggests that mutations in genes that contribute to the SHH pathway may generate tumorigenesis. Some of these genes include the patched homologue 1 gene (PTCH1), smoothened homologue gene (SMO), and the suppressor of fused homologue gene (SUFU). Heightened expression of the GLI zinc finger transcription factors (GLI1, GLI2, GLI3) and MYCN, an oncogene, has also been associated with the formation of SHH MB. When the ligand for the SHH pathway is not present, the Patched 1 (PTCH) protein, a 12 transmembrane receptor protein, represses the smoothened receptor (SMO), thereby inhibiting the pathway. As shown in Figure 1, when a PTCH ligand, such as sonic hedgehog, is present, it binds to PTCH1 and activates the pathway, as SMO is no longer suppressed. Next, SMO is moved to the primary cilium, and it then activates a GLI zinc finger transcription factor, which could be GLI1, GLI2, or GLI3. Once a GLI factor is stimulated, the transcription of target genes for the SHH pathway (e.g. GLI1, PTCH1, cyclin D1, BCL-2, SNAIL) is promoted. Anomalous activity at any stage of this pathway may spur the formation of medulloblastoma. The most prominent drivers of SHH MB include aberrant expression of SHH target genes, PTCH dysfunction, and SMO promotion.⁶ To counter the deleterious effects of

these SHH pathway permutations, targeted small molecule inhibitors that prevent the SHH ligand from binding to PTCH or antagonists of SMO have risen as potential therapeutics against SHH MB.

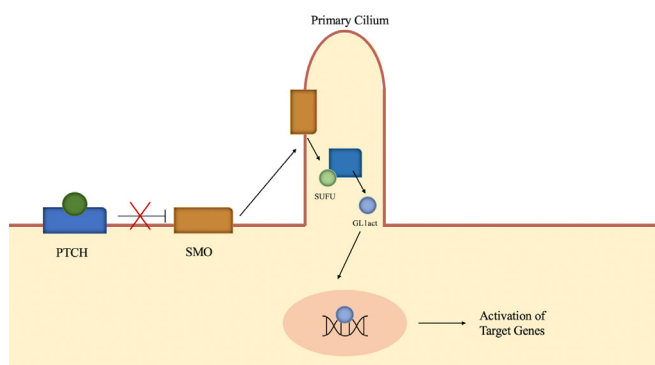


Figure 1. An overview of the hedgehog signaling pathway. In the absence of a PTCH ligand (e.g. sonic hedgehog), PTCH represses SMO. Once sonic hedgehog binds, PTCH no longer inhibits SMO, and SMO is translocated to the primary cilium, where it subsequently activates GLI transcription factors that promote target gene expression of the hedgehog pathway.

3. Mechanism of Action for Small Molecule Inhibitors of The Sonic Hedgehog Pathway

The following SHH pathway inhibitors are potential targeted therapeutics for SHH MB: vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61. As seen in Figure 2, they target and block specific parts of the hedgehog pathway. They are not yet approved for treatment for MB.

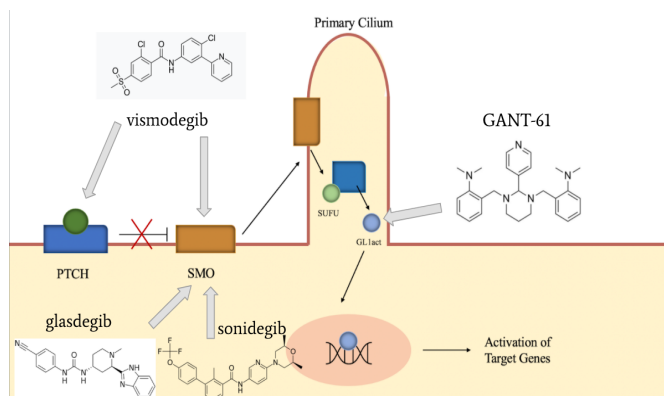


Figure 2. Continued on next page.

Figure 2. Overview of where the inhibitors target the pathway. As shown, vismodegib targets PTCH1 and SMO. Glasdegib and sonidegib are SMO antagonists. GANT-61 is a GLI inhibitor.

However, both vismodegib and sonidegib were approved for metastatic non-resectable Basal Cell Carcinoma in 2012 and 2015, respectively. Glasdegib was approved in 2018 for acute leukemia treatment.⁶

Vismodegib is a small molecule drug taken orally. In regard to the SHH pathway described above, it interacts with SMO and PTCH by specifically blocking activities of hedgehog-ligand cell surface receptors PTCH and/or SMO. By blocking the process here, hedgehog signaling is suppressed. When vismodegib (also known as GDC-0449) blocks PTCH, SMO can continue to be repressed, and not further the cycle.⁹ Vismodegib acts as an SMO antagonist, blocking the SMO receptor. This prevents SMO from activating the GLI zinc finger transcription factors, further inhibiting the pathway. Vismodegib is also a kinase inhibitor, meaning that it inhibits activity of the enzyme kinase (kinases can add phosphate groups to proteins and change their functions). In an *in vivo* study, vismodegib has been shown to affect complete tumor regression in mice with doses of 12.5 mg/kg administered twice per day.^{10,11} Additionally, vismodegib has been shown to yield vast but impermanent tumor regression and relief of symptoms when given orally with a dose of 540 mg/day for 3 months. This data is important in evaluating the efficacy of targeted inhibitors to the SHH pathway as therapeutics for SHH MB.

Sonidegib is also a SMO antagonist. Sonidegib works by penetrating the blood-brain barrier and blocking the SMO receptor and

therefore the SHH pathway, making it a potentially effective treatment for SHH MB. It has inhibited tumor growth in mice when administered at 5 mg/kg/day and is shown to allow for more regression at higher doses. However, these are also transient effects, and after being exposed to sonidegib for a long time, it has been shown that resistance/relapse occurs due to mutations in SMO that are formed. Moreover, while preclinical research has demonstrated momentary efficacy of sonidegib in SHH MB, the beneficial effects dwindle over time due to SMO mutations and serve as a significant limitation.¹² Additionally, glasdegib is a SMO antagonist. It functions due to its benzimidazole scaffold which has been shown to be used as an anticancer agent.¹³ It has a very high potency, indicating that it may effectively inhibit tumor growth in SHH MB. As mentioned before, it has been FDA approved along with low doses of cytarabine for treating acute leukemia.^{14,15}

In addition to preclinical research, clinical trials have evaluated the efficacy of sonidegib and vismodegib at mitigating MB tumor growth, as well as their safety profile.¹⁶⁻²¹ In a phase I trial conducted from 2007 to 2008, vismodegib was administered to 68 patients who had refractory, locally advanced, or metastatic solid tumors caused by aberrant hedgehog pathway signaling.

One of these patients had SHH MB. The SHH MB patient demonstrated a partial but unconfirmed response to increasing doses of vismodegib, as well as an acceptable safety profile. Resistance to vismodegib occurred in this patient as a result of SMO mutations, revealing vismodegib's potential as a therapy for SHH MB while highlighting mutations as a drawback. This clinical trial is not recent, however. Much more research has been conducted on vismodegib's efficacy in SHH MB patients since 2008, producing conclusions that are more revealing.¹⁷ A more recent clinical trial regarding vismodegib was conducted in 2013. Vismodegib was provided to 6 patients at a dose of 85 mg/m², and 7 patients received a 170mg/m² dose. There was no bone

toxicity documented from the drug, although dose-limiting toxicities did manifest. This study served to evaluate the safety, the toxicity, and efficacy of vismodegib in pediatric patients with recurrent or refractory MB. Ultimately, the study showed that this SMO inhibitor is safe and feasible in children, which is significant considering the lack of pediatric clinical trials with SMO inhibitors. A subsequent study administered a phase II dosage to three out of seven SHH MB patients from the first phase. Notably, antitumor activity was observed in 1 of these patients.¹⁸ These clinical findings suggest vismodegib's robust safety and efficacy in SHH MB patients.

In 2 phase II studies conducted in 2015, 31 adult patients and 12 pediatric patients were treated with 150-300 mg/d of vismodegib. Responses were evaluated with neuroimaging and molecular tests. Protocol defined response outlined that a complete or partial response must be sustained for 8 weeks. A complete response was achieved when all lesions targeted disappeared, and a partial response was achieved when 30% reduction in the sum of the diameter of long lesions targeted. 3 adult patients and 1 pediatric patient with SHH-MB achieved the protocol-defined response. Progression-free survival was longer for patients with SHH-MB than non-SHH-MB. Prolonged disease stabilization was achieved in 41% of patients with SHH-MB. It was concluded that vismodegib acts against adult recurrent SHH-MB and not non-SHH-MB. For pediatric patients, there was no conclusion. It was also concluded that SMO inhibitors depend on the deviations in the genome of the tumor.¹⁹ Taken together, vismodegib seems to work by specifically targeting the SHH pathway, which explains its inefficacy against non-SHH-MB tumors. While this clinical data is promising, vismodegib needs to be studied much more extensively in pediatric patients and its efficacy in mitigating mutated SHH MB tumors needs to be explored.

In a similar manner to vismodegib, sonidegib has also been clinically assessed as an inhibitor of the SHH pathway in MB patients.^{20, 21} In 2014, a

phase I clinical trial was conducted to assess the safety and efficacy of sonidegib taken orally in patients with MB and basal cell carcinoma (BCC). The prominent dose-limiting toxicity manifested as a 3/4 increase in creatine kinase in blood serum, which developed in 18% of patients. Nonetheless, sonidegib displayed an adequate safety profile and decreased expression of GLI1 mRNA in a dose-dependent manner.²⁰ Therefore, this clinical evidence suggests sonidegib's safety and efficacy in curtailing MB tumor growth by inhibiting progression of the SHH pathway. In a 2017 phase II trial, 60 pediatric patients and 16 adult patients with recurrent tumors received oral sonidegib treatment. Pediatric patients received a 680mg/m² daily dose, while adults received 800mg. Out of all the pediatric patients, 39 had MB. A 5-gene Hh signature assay was conducted to determine the genetic driver of tumors in complete responders, partial responders, and non responders to sonidegib treatment. Notably, it was found that among the complete responders, 2 children and 2 adults, had SHH-driven tumors. This same outcome was found in the one partial responder.

Out of the 50 non-responders, none demonstrated an SHH-driven tumor.²¹ This is significant because it indicates that sonidegib shows efficacy in mitigating MB tumor growth, specifically by inhibiting the SHH pathway. Overall, these clinical trials indicate that while both vismodegib and sonidegib show substantial promise as SHH inhibitors in MB, several limitations to their efficacy still need to be overcome. Thus, the clinical investigation of other potential SHH inhibitors is also warranted.

Temozolomide - another SHH small molecule inhibitor - is not yet approved, but there are ongoing studies about its use as a monotherapy or in combination with vismodegib. It functions by preventing DNA duplication in cells during proliferation, causing cell death.²² Therefore, temozolomide works to disrupt division of tumor cells and consequently, hinders tumor growth. Though SHH MB seems amenable to temozolomide's mechanism of action, the most

effective dosage has not yet been established. For instance, vismodegib and temozolomide monotherapies were studied in a patient with recurrent SHH MB, and were taken at a dose of 150 mg. The patient responded to both vismodegib and temozolomide over a significant period of time, though the efficacy of both treatments eventually waned due to drug resistance. Several mutations occurred to SMO: SMO-L412P, SMO-G477L, and PIK3CA-H1065L mutations, which indicates the importance of a treatment regimen that targets multiple aspects of the SHH pathway. Determining the optimal dosage of these SHH inhibitors in preclinical studies is critical before moving to clinical trials, where patient safety is at risk. Additionally, since the benefits of the pharmaceuticals seem to dwindle over time, delineating time of intervention over the course of the disease progression is also essential to better treating patients.²³

Lastly, GANT-61 is a GLI inhibitor. Inhibiting GLI in the SHH pathway may be an effective anti-cancer therapeutic. It has been studied in Daoy cells, a medulloblastoma cell line. The study found that GANT-61 succeeded at inhibiting GLI, a key transcription factor in the SHH pathway, downregulated the Bcl-2 target genes, and even made the tumor cells more sensitive to cisplatin (a chemotherapy drug).²⁴ This yielded a significant inhibition of cell proliferation, which would theoretically inhibit tumor growth in vivo.

4. Evaluation: Therapeutic Potency of SHH Inhibitors in SHH-MB

Targeted small molecule inhibitors of the SHH pathway have proven to be a promising therapeutic for SHH MB. Many studies and reviews indicate that these inhibitors are effective at halting proliferation.^{24, 25} Overall, there are three main benefits of these inhibitors that contribute to their efficacy: their capacity to directly target the

SHH pathway, their infrequent toxicities, and their intimate connection with the SHH pathway genes. However, significant limitations exist. Resistant mutations and the heterogeneity of the disease are two major limitations, and poor drug properties is a minor (but important) limitation. This section will address these benefits and limitations.

4.1 Precision Yet Resistance

Novel investigations evaluating the efficacy of targeted inhibitor therapeutics in SHH MB have demonstrated the capacity of these inhibitors to bind specifically to various factors in the SHH pathway, thereby preventing its progression. This makes targeted inhibitors an effective therapeutic because it attacks the cancer at the heart of its mechanism for proliferation. Poisoning the cells that carry the mechanism through chemotherapy, an alternative treatment, is not as precise. Sonidegib illustrates this. MB growth in mice decreased by 33% more than the control group following sonidegib treatment.²⁵ By targeting the SMO protein, sonidegib prevented SMO's translocation to the primary cilium, thereby halting the pathway. As a result, tumor progression significantly declined at a dose dependent rate, indicating its potential for treating SHH MB.²⁵ Another example is a 2016 study examining the efficacy of GANT-61, a GLI transcription factor inhibitor in SHH MB. The overexpression of this gene is associated with several cancers. In the study, varied concentrations of GANT-61 were administered to Daoy cells, which serves as the in vitro model of MB. By inhibiting GLI, GANT-61 promoted apoptosis of the Daoy cells and downregulated the Bcl-2 target gene, substantially inhibiting cell proliferation.²⁴ Both GLI and Bcl-2 are components of the SHH pathway, illuminating how these inhibitors attack the MB cancer at the site of tumor initiation. In summary, the examples of sonidegib and GANT-61 illustrate that SHH targeted inhibitors are effective because they target the MB cancer at its source: the SHH pathway.

Although the targeting nature of small molecule inhibitors is a significant benefit, mutations in the SHH pathway genes often lead to resistance. These mutations create proteins in the SHH pathway that interfere with the inhibitors' effects, rendering them ineffective at halting tumor growth. For example, multiple sources reveal that SMO point mutations result in proteins that do not allow for inhibitor binding.^{1,6} Overall, patterns in current literature indicate that SMO, SUFU, GLI2, and MYCN are the genes that primarily experience inappropriate amplifications or mutations resulting in resistant effects. Patterns in these mutations and how each mutation results in resistance is still unclear, but multiple studies report that SUFU, GLI2, and MYCN are all downstream from SMO.^{1, 19, 26} For example, one clinical trial found that patients with the downstream genes did not respond to SMO antagonists at all or initially responded to the antagonists but later experienced recurrence.²⁶ Conversely, this clinical trial and a separate clinical trial found that SHH MB patients with mutations in the upstream PTCH1 gene did respond to the inhibitors.^{19, 26} These variable clinical responses are due to the heterogeneity of the disease and mutations that lead to resistance.^{23, 26} Furthermore, a pattern in clinical trials has emerged where SHH MB patients' tumors will initially shrink, only to be followed with recurrent growth.^{6, 26, 27} These patterns are supported by in vitro experimentation. Inhibitors such as sonidegib and novel Artemisinin derivatives significantly halted MB proliferation during early stages of experimentation but were unable to overcome resistant mutations that led to recurrent proliferation.^{12, 28-30} It should be noted that not all of the sources agree on which gene mutations result in resistance. Most sources comment on SUFU, GLI2, and MYCN, but one review also discusses truncations of GLI1, amplifications of GLI2, cyclin D1, and upregulation of the ATP binding cassette transporter p-glycoprotein substrate. Given that the other sources did not mention these mutations, it is unclear whether they pose a significant

limitation. Overall, resistant mutations pose a serious limitation to the efficacy of the inhibitors because they undo the exact mechanism used to halt proliferation of the MB cells. More research is needed to clarify which mutations most contribute to resistance and how they do so. However, the following additional benefits of SHH inhibitors still make these therapeutics a promising option.

4.2 Less Toxic Yet Less Stable

Notable advancements have revealed that targeted inhibitor therapeutics of SHH MB are much less invasive and toxic than the current standardized treatment. A 2010 investigation demonstrated that SHH inhibitors are an effective mode of treatment for other SHH-dependent cancers, such as Basal Cellular Carcinoma, Lung cancer, and Liver cancer.³¹ Since 2015, SHH inhibitors have been a validated treatment option, specifically for treating metastatic or locally advanced non-resectable Basal Cellular Carcinoma.²⁵ Therefore, SHH inhibitors demonstrate rehabilitative potential in SHH MB, which is driven by aberrations in the SHH pathway. Administration of SHH inhibitors have been shown as safe with limited adverse effects, as indicated by its clinical approval for Basal Cell Carcinoma. Moreover, these inhibitors' low toxicity and limited invasiveness suggest its greater therapeutic capacity in comparison with current standardized treatment. For example, vismodegib and temozolomide have exhibited a promising safety index in recent investigations. One vismodegib clinical trial found that the patients revealed a low toxicity profile and that none of the patients withdrew from the clinical trial because of toxicity.¹⁹ Another clinical trial found that a 16-year old patient suffering from SHH MB exhibited a steady response to vismodegib and temozolomide with limited adverse effects. Although the benefits eventually declined due to mutations, this study demonstrates that adverse events were not due to toxicity.²³ Nonetheless, surgical resection and subsequent radiation and

chemotherapy are still the most prevalent treatment regimen for all MB subtypes. Given that the invasive nature of radiation and chemotherapy often generate chronic cytotoxic effects and tumor recurrence, inhibitors are a promising alternative.

Although this data indicates that inhibitors improve upon the standard treatment regimen for SHH MB in terms of toxicity and invasiveness, evidence suggests that developing inhibitors without these cytotoxic effects and optimal pharmacokinetic properties has posed a challenge. For example, two SHH pathway inhibitors that resulted in negative consequences are cyclopamine and HhAntag: cyclopamine generated cytotoxic effects in healthy cells and a preclinical study of HhAntag resulted in permanent developmental defects in the bones of the mouse models.^{32, 33} Given that SHH is a pathway critical for development, the latter study has raised concerns about the use of SHH inhibitors in infants and young children. Cyclopamine also had poor pharmacokinetic properties, another limitation of SHH MB inhibitors. Adequate concentration and stability in circulation are two pharmacokinetic properties that have been commented on in the literature. For example, rat livers cleared away N-Phenylbenzamide too quickly, preventing adequate concentration. This ended its testing although it was initially promising.³⁴ Other examples are GANT-61 and vismodegib. GANT-61 has proven effective in vitro but is less stable than its GANT-58 alternative under physiological conditions.³⁵ Vismodegib has demonstrated encouraging pharmacokinetic characteristics in animal models, but its pharmacokinetic properties can also be improved upon. An in vivo study demonstrated that these properties can be further bolstered when hydrogen ions in the active sites are replaced with deuterium. This allowed for sustained benefits at lower doses.³⁶ Overall, although poor drug properties are important limitations, literature suggests that they are not as significant as genetic based limitations such as resistant mutations and heterogeneity. Most of the sources analyzed did not comment extensively on

the cytotoxicity and pharmacokinetic properties, if at all. These challenges should be addressed, however, as the development of novel SHH inhibitors progresses.

4.3 Genetic Connections Yet Persistent Heterogeneity

Recent developments in the use of SHH inhibitors have also elucidated connections between the genetics of the disease and the efficacy of inhibitors. These connections have allowed researchers to craft treatment regimens that target specific genetic mutations, bolstering their efficacy. In 2015, a clinical study reported results from two Phase II trials that evaluated the safety and efficacy of vismodegib in patients with recurrent or refractory SHH MB.¹⁹ It was found that the position of the genomic deviation in relation to SMO and PTCH1 were predictors of the response to SMO inhibitor activity. Both the transgressions of SMO and PTCH1 culminated in favorable outcomes with respect to attenuating tumor progression. This discovery is critical because it demonstrates that knowing the genetic basis of the patients' SHH MB tumor can predict whether or not they respond favorably to the inhibitor. Secondly, it was discovered that robust P53 diffuse staining in SHH MB was associated with a substantially less significant response to the inhibition of SMO. The mechanism underlying this finding is not yet understood because the relationship between P53 and SMO is unclear. However, results indicated that mutations of this protein generate chromothripsis, where thousands of chromosomal rearrangements occur, possibly upregulating the expression of SHH signaling oncogenes.³⁷ With this information, paired with the relationship between P53 and SMO that still requires further investigation, it is concluded that alterations to the P53 protein expression may be correlated with anomalous SMO activity.¹⁹

Moreover, inhibitors that can mitigate both P53 and SMO aberrancy, which are driven by genetic mutations, serve as a potential therapeutic strategy for SHH MB. This underscores the

relationship between SHH MB's genetics and efficacy of inhibitors. A third key finding from this study was the delineation of the target population for vismodegib therapy, which was conducted via complete molecular profiling of SHH MBs. The evidence obtained demonstrated the importance of distinguishing SHH MBs that are driven by mutations downstream in the SHH pathway, which are not amenable to the inhibition of SMO. This serves as a significant milestone because it suggests the need for novel inhibitor therapeutics that target different proteins of the pathway while underscoring that inhibitor efficacy has genetic connections. This emphasizes the importance of further delineating the genetic basis of SHH MB in order to provide more effective individualized therapies.

Although there are useful connections between inhibitor efficacy and disease genetics, not all of the SHH MB genes have been discovered and important patterns in expression have yet to be identified. Much of the literature describes SHH MB as a heterogeneous disease, meaning that different genes are associated with tumor growth across patients. There is evidence for this in the variability of responses to inhibitors. For example, two adult males suffering from SHH MB had extremely different responses to vismodegib. One had a very favorable response while the other developed resistance multiple times. Furthermore, the second adult male even had a genetic profiling more similar to the childhood version of the disease, making it difficult to predict how he would respond to the treatment.²⁷ This highlights the complexity of the disease's genetic basis and suggests that there are subgroups of SHH MB based on combinations of affected genes. However, these nuances are still unclear because it is a rare cancer; more patients are required to detect patterns with certainty. This makes it difficult for researchers to develop effective inhibitors and assign patients to optimal clinical trials.⁶ Furthermore, some genes might even be better suited for use as a diagnostic tool while other genes (such as GLI) have shown promising results as a

therapeutic target.²⁴ Overall, the heterogeneity of SHH MB limits the efficacy of inhibitors in that they might not target the pathway optimally in each patient. Fortunately, studies have successfully identified preliminary patterns of tumor-inducing gene expression in SHH MB patients using transcriptome sequencing and whole genome analysis. These studies have found that there are patterns across age groups.^{1,26} One study found that infants, children, and adults had instances of PTCH1 mutations, only infants had the SUFU mutations, and only adults had SMO mutations. Mutations in SUFU, GLI2, and MYCN genes have shown primary resistance to SMO inhibition.²⁶ This suggests that patients in clinical trials should be assigned inhibitors according to age, and that genetic sequencing of SHH MBs should be a part of the treatment planning process. Ultimately, a better understanding of the responsible genes is still needed.

Overall, the potential of SHH inhibitors makes them a promising therapeutic for SHH MB in the clinical setting, despite their limitations. Future research must address these limitations if the inhibitors are to be approved for clinical use. However, the promise of these inhibitors warrants the investment of such research. Given how they have improved upon the standard treatment regimen for SHH MB, small molecule inhibitors of the SHH pathway are likely the future of SHH MB treatment.

Table 1: Milestone Studies Illuminating the Therapeutic Potential of SHH inhibitors in SHH MB

Type of study (preclinical/clinical)	Year	Type of inhibitor	Significant findings
Clinical	2014	SMO inhibitors	Genomic analysis of infant, children, and adult SHH MB demonstrate significant molecular heterogeneity. Among adult patients, 82% of those in the study exhibited PTCH1 or SMO mutations, indicating that these tumors would be amenable to SMO antagonist treatment. ²⁵
Clinical	2015	Vismodegib	Pediatric and adult recurrent SHH MB patients received 150-300mg of vismodegib daily. Compared to patients with non-SHH MB tumors, SHH MB patients demonstrated tumor amelioration to a greater extent. Vismodegib treatment induced genetic transgressions in the SMO gene, indicating anti-tumor capacity. ²⁶
Preclinical	2016	GANT61	In Daoy MB cell culture, GANT-61 administration attenuated tumor growth by hindering cell proliferation, migration, and infiltration. GANT-61 also stimulated apoptosis induced by mitochondria and the caspase-3 pathway. qPCR confirmed that GANT-61 effectively impeded GLI factor expression. ⁸
Clinical	2018	Temozolomide, vismodegib, sonidegib	A SHH MB pediatric patient with the PTCH1 mutation received three different treatment regimens of Hh inhibitor. He first received vismodegib and demonstrated a partial metabolic response. However, vismodegib resistance eventually developed, as indicated by tumor recurrence. Temozolomide had a similar effect. Sonidegib resulted in exacerbated tumor growth and the need for emergency surgery. ¹⁹
Clinical	2019	Vismodegib	Whole-genome analysis of two patients with SHH MB was conducted. While the tumors from both patients exhibited a mutation in PTCH1, only one of the patients was responsive to Vismodegib treatment. The expression of MYCN and GLI1, absence of PTEN, and depletion of P53 may explain the vismodegib resistance that manifested in the patient. ²³
Preclinical	2020	eHNP (nanoparticle)	Nanoparticles with SHH inhibitors loaded on can cross the blood-brain barrier (BBB). They can be used as a delivery carrier to cross the BBB and to the SHH MB. ³⁹

5. Further Research Needed

The current limitations of SHH inhibitors in SHH MB illuminate crucial areas of future research. First, optimal dosage and timing of intervention need to be established. In a case study, vismodegib and temozolomide were taken at a dose of 150mg. The therapeutic benefits of both inhibitors dwindled as mutations arose. The new tumors that formed varied genetically from the initial tumor. Following failure of those treatments, sonidegib was taken at a dose of 400mg, which resulted in the need for emergency surgery.²³ Therefore, optimizing dosage and timing of intervention are crucial for translating preclinical findings to clinical practice. Importantly, optimal timing and dosage of inhibitor treatment should be examined for both pediatric and adult patients, especially since clinical trials with pediatric SHH MB subjects are lacking.

Compared to the standard treatment regimen for SHH MB (radiation, chemotherapy, and surgery), SHH inhibitors demonstrate greater therapeutic promise. Nevertheless, future research should focus on improving the efficacy of these inhibitor therapeutics to combat resistant mutations. Resistance to both SMO antagonists and inhibitors of PTCH frequently develop in patients, which can exacerbate tumor growth and spur other adverse events. Moreover, more downstream inhibitors of the SHH pathway, such as GLI inhibitors, should be explored in studies. Investigation into various SHH inhibitors is also critical for treatment of SHH MB in pediatric patients, as SHH MB in the pediatric setting most often occurs due to genetic mutations downstream of SMO in the pathway.²⁶ It is also important to consider the use of combination inhibitor therapies for SHH MB. Since the efficacy of SHH inhibitors, such as vismodegib, may diminish over time due to drug resistance, synergistic inhibitor treatment with other therapies may elicit a more robust rehabilitative response. It may be beneficial to target both the SHH pathway and other pathways that interact with SHH signaling, such as P53, cAMP, Atoh1, Boc, CxCl12, CxCR4, and PI3K.^{1,19} For example, though the interaction between P53 and the SHH pathway remains unclear, an in vivo study found that development of MB increased from 14% to more than 95% when PTCH loss was coupled with P53 loss.³⁸ Moreover, administration of SHH inhibitors with P53 mediators should be explored as a putative combination therapy for SHH MB. Additionally, cholesterol homeostasis may contribute to the overactivation of the SHH pathway and so, modulated inhibitor therapies that can also obstruct cholesterol regulation may serve as an important area of future research. Indeed, a recent milestone investigation not only demonstrated that lipid-based nanoparticles effectively crossed the blood brain barrier and delivered the therapeutic cargo to the tumor site, but also effluxed cholesterol from the cytosol of tumor cells.³⁹ Lower levels of cytoplasmic cholesterol

generated cytotoxic effects in the SHH MB cells. Moreover, the use of lipid-based nanoparticles to carry targeted inhibitor therapeutics may bolster the efficacy of these pharmaceuticals and reduce the drug dosage needed to elicit a response.

Lastly, the pathological mechanisms of SHH MB are still largely unknown due to substantial molecular heterogeneity in these tumors. There are still a multitude of genes that drive this tumor that have not yet been identified, making it difficult to determine which inhibitor therapeutic would be most effective.⁶ Among infants, children, and adult patients, the genetic basis of the tumor differs significantly. Future investigations should focus on further elucidating the genetic basis of SHH MB tumors to determine whether there is an age-dependent factor underlying the molecular disparities. Therefore, individualized targeted treatment aiming to alleviate the specific aberration in the SHH pathway is critical for formulating the most effective treatment of SHH MB in both pediatric and adult patients. All in all, extensive genetic profiling of SHH MB tumors serves as a pivotal area of future research.

6. Summary

In summary, in understanding the SHH pathway and inhibitors vismodegib, sonidegib, glasdegib, temozolomide, and GANT-61, benefits as well as limitations were exposed. Drug resistance due to SMO mutations, molecular heterogeneity, and poor drug properties were discussed. Gaps in knowledge for future research include establishing a more thorough understanding of the genetic basis of the disease, determining optimal dosage of the inhibitors, as well as time of intervention, pathological mechanisms, and synergistic treatments to improve efficacy. In conclusion, targeted inhibitors of the SHH pathway are a promising treatment method, though there are limitations that must be further explored to improve the efficacy and safety as a therapeutic treatment for Sonic Hedgehog Medulloblastoma.

Acknowledgements

We acknowledge the NHS and Dr. Tilan.

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