

Inhibition of SHH pathway mechanisms by arsenic trioxide in pediatric medulloblastomas: a comprehensive literature review

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ABSTRACT. Recent innovations in the genomic understanding of medulloblastomas have provided new ways to explore this highly invasive malignant brain cancer arising from the cerebellum. Among the four different medulloblastoma subgroups described to date, the sonic hedgehog (SHH) genetic pathway is the pathway activated in the tumorigenesis of medulloblastoma. SHH-related medulloblastomas are usually of nodular/desmoplastic histology and frequently occur in children under the age of three, an age group highly susceptible to the acute and long-term effects of treatment. Several new drugs aimed at SHH modulation are currently under development. This review focuses on the role of arsenic trioxide, a drug well established in clinical practice and probably an under-explored agent in medulloblastoma management, in the SHH pathway.

Key words: Medulloblastoma; Sonic-hedgehog; Brain cancer; Arsenic trioxide; Treatment

INTRODUCTION

Tumors of the central nervous system (CNS) are amongst the most common pediatric neoplasias, representing about 20 to 23% of all childhood cancers. In some study series, CNS tumors have shown the highest mortality rates, only lower than those for lymphohematopoietic diseases (Rickert and Paulus, 2001; Kool et al., 2012).

Medulloblastomas (MB) are the most common malignant brain tumors in children, corresponding to approximately 20% of all brain tumors in children younger than 15 years and accounting for significant morbidity-mortality rates (Patel et al., 2014). In more than 80% of the cases, they occur as midline tumors primarily originating in the cerebellar vermis. Metastases may be present at diagnosis or occur later (de Bont et al., 2008; Korshunov et al., 2010; Wells et al., 2010). The incidence of MB peaks among 6- to 8-year-old children (Rickert and Paulus, 2001). As a function of their location and embryonic origin, MB tend to disseminate towards the subarachnoid space, giving rise to metastases by dissemination through the cerebrospinal fluid (Rickert and Paulus, 2001).

Histologically, MB can be divided into three subgroups: the classical histological type comprises 70% of all MB; the anaplastic or large cell MB are those of the worst prognosis and lowest frequency (less than 5% of cases); and desmoplastic/nodular type of MB, particularly those affecting infants, have the best chance of cure (Crawford et al., 2007).

Advances in the molecular classification of medulloblastomas

The molecular study of MB has revealed distinct genetic-molecular expression profiles among the different subgroups of the tumor. Current consensus is that there are at least four major molecular subgroups, denoted *Wingless* (WNT), *Sonic Hedgehog* (SHH), Group 3, and Group 4. Therefore, from the anatomopathological viewpoint, MB comprises a set of different subgroups that are part of the same neoplasia (Fellay et al., 2011; Northcott et al., 2011; Kool et al., 2012; Gerber et al., 2014).

The WNT subgroup, corresponding to 10% of all MB, has a major role in the somatic mutation of the *CTNNB1* gene, which codes for the beta-catenin protein. When this protein is not degraded, it accumulates in the cytoplasm, stabilizes, and is then translocated to the nucleus. The accumulation of nuclear beta-catenin leads to the regulation of transcription of a series of target genes mainly involved in cell proliferation. This group may also show germinal mutations in the tumor suppressor *APC*, as well as in chromosome 6 (monosomy). It usually affects older children with the classical histological subtype of MB, with metastases seldom being present at diagnosis. Moreover, MB with mutations in *APC* are associated with Turcot syndrome (de Bont et al., 2008; Fellay et al., 2011; Gerber et al., 2014).

In most cases, the SHH subgroup shows somatic mutations in one or more genes of the SHH pathway (e.g., *PTCH1*, *SUFU*, or *SMO*), which contribute to its constitutive activation. Its histological subtype may be desmoplastic, extensively nodular or classic, and rarely, anaplastic. Its incidence is biphasic, with infants/preschoolers and young adults being affected. The prognosis for infants is excellent when the tumor histology is extensively nodular or desmoplastic. The current tendency in the treatment of this group in infants is to reduce the intensity of therapy in order to increase the likelihood of a cure and to lower the rate of morbidity. Additionally, the MB of the SHH group may be associated with Gorlin syndrome (de Bont et al., 2008; Fellay et al., 2011; Gerber et al., 2014).

Subgroup 3 is almost totally diagnosed in children and very rarely in adults or adolescents. It shows a greater predisposition for males and greater occurrence of metastases at diagnosis. The prognosis for this group is reserved, with the tumor usually belonging to the large cell/anaplastic or classic histological subtypes. *MYC* is amplified in most cases. In the future, the genes involved in this pathway, as is the case for *MYC* and others, might become candidates for pharmacological target therapy (de Bont et al., 2008; Fellay et al., 2011; Gerber et al., 2014).

Subgroup 4 is the most common subgroup, occurs at all ages, can be metastatic at diagnosis, and its prognosis is intermediate. As is the case for group 3, there is no exact description of genetic predisposition. Although in most cases isochromosome 17 is a cytogenetic marker of this subgroup, this is still a poorly characterized group from a molecular viewpoint compared to all the other MB subgroups (de Bont et al., 2008; Fellay et al., 2011; Gerber et al., 2014).

Analysis of the overall survival of patients with these MB subgroups showed that survival of up to 5 years has been observed in up to 90% of children with the WNT subgroup, as opposed to that observed in 39-58% of children with the 3 and 4 subgroups. This rate of survival depends on the age at tumor occurrence, presence of metastases, and unfavorable coexisting cytogenetic changes. Patients with the SHH subgroup of MB have an intermediate survival rate, while it is particularly longer among infants with the SHH subgroup. This finding is probably associated with the high frequency of desmoplastic histology among SHH tumors in infants since desmoplastic/nodular histology is an isolated marker of favorable prognosis in this age group (Northcott et al., 2011; Fellay et al., 2011; Kool et al., 2012). Conversely, patients with the SHH subgroup who do not present desmoplastic/nodular histology have a poorer survival rate. This subgroup of MB patients, along with group 3 patients, require new therapeutic approaches that could improve their chance at a cure and reduce the side effects caused by current treatment methods (Northcott et al., 2011; Fellay et al., 2011; Kool et al., 2012).

Hedgehog signaling pathway

Activation of the SHH signaling pathway is essential during embryonic development for the formation of different tissues and organs. Following this period, the pathway contributes to homeostasis and repair of adult tissues by maintaining stem and progenitor cells and control of cell proliferation and fate. However, when this pathway is activated aberrantly, it could lead to tumor development in different organs (De Smaele et al., 2008).

MB represents one of the different types of tumors that may originate from SHH pathway deregulation (Fellay et al., 2011). This pathway is responsible for the proliferation of cerebellar cells during development, and the maintenance of its activation after the embryonic period may give rise to MB (Fellay et al., 2011; Gerber et al., 2014).

The SHH pathway may be activated through signaling by specific ligands and by mutation of the pathway components. The three main ligands that are classified as morphogenic are *Sonic hedgehog* (SHH), *Desert hedgehog* (DHH), and *Indian hedgehog* (IHH); the first being the one most extensively studied (Buczkwicz et al., 2011).

SHH protein has an initial molecular weight of 45 kDa and autocatalytic activity and may be cleaved into two portions, N-terminal and C-terminal (20 kDa and 25 kDa, respectively) (Shahi et al., 2012). The carboxyl group at the N-terminal of SHH undergoes a change, forming cholesterol that facilitates its binding, secretion, and transit activities. Involvement of the Dispatched protein is necessary for the paracrine action of SHH. When SHH binds to the patched 1 (PTCH1) transmembrane receptor, smoothened (SMO) (a receptor usually inhibited

by PTCH1) is released, undergoes a conformational change, and translocates to the cytoplasm. SMO binds to suppressor of fused homolog (SUFU) in the cytoplasm, consequently releasing proteins belonging to the GLI family. These proteins, GLI1 (activator), GLI 2 (activator), and GLI 3 (inhibitor), translocate to the nucleus, where they act as transcription factors that regulate the expression of different targets such as *GLI1*, *PTCH1*, *CYCLINE D*, and *MYC* involved in cell survival, proliferation, and differentiation (Figure 1) (Buczkwicz et al., 2011).

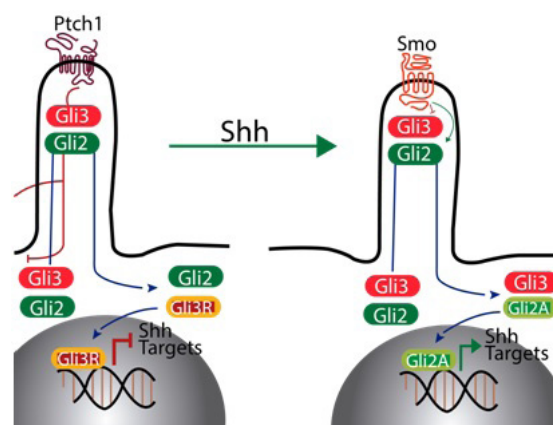


Figure 1. Schematic representation of the SHH transmembrane signaling pathway (Based on Han and Alvarez-Buylla, 2010).

Another mechanism leading to the activation of the SHH pathway is the mutation of inhibitory regulatory genes. An example of this mechanism is somatic mutation and loss of heterozygosity in the gene that codes for the *PTCH1* homologue (Robinson et al., 2015). As is the case for the other components of the SHH signaling pathway, the *PTCH1* mutation seems to be sufficient for the development of MB. *PTCH* mutations have been reported in a subset of pediatric MB (Raffel et al., 1997), with the observed mutations affecting both the germinal and somatic cell lines, particularly in *SUFU* (Taylor et al., 2002). The downstream activity of the GLI protein family is intimately linked to tumorigenesis. The inactivation of both *GLI* alleles has been reported to lead to MB formation in mice heterozygous for *PTCH*, suggesting that SHH signaling may direct tumorigenesis in an independent manner (Raffel et al., 1997).

Even though similar mutation levels are observed in the four groups under study, the genetic sequencing of MB suggests that the different disease types affect individuals belonging to different age brackets (Fellay et al., 2011; Gerber et al., 2014). *SUFU* and *SMO* mutations have most frequently been detected in infants and adults, respectively. *TP53* mutations have been detected in almost 50% of patients aged 4-17 years. These results suggest that the treatment of MB can be better directed by considering the patient age range and genetic alteration present (Taylor et al., 2002; Kool et al., 2012).

Arsenic trioxide (ATO)

The therapeutic application of arsenic has a prodigious history. Knowledge of its medicinal benefits dates back to 400 B.C., with reports on its use by Hippocrates, Aristoteles, Dioscorides, and Pliny (Au, 2011).

ATO is a known drug that has been studied and used to treat different diseases. Its carcinogenic action is believed to have been observed first in Paris (1822), where cattle grazing in the proximity of foundries developed neoplasias in their flanks. The onset of these tumors was attributed to gases impregnated with large amounts of these substances emitted by the foundries. In 1888, Hutchinson reported six cases of skin cancer in patients who had ingested arsenic for the treatment of dermatoses, to the London Pathology Society (Au, 2011). Interestingly, ATO was also used on battlefields in World War II in the form of a lethal gas called Lewisite in honor of the American chemist W. Lee Lewis (Au, 2011).

After decades of minimal use, it is interesting to note that ATO emerged again in the 21st century as one of the drugs used for the treatment of acute promyelocytic leukemia M3, refractory to first line treatment, with promising results (Nasr et al., 2008).

ATO is a metal element known to exert its biological effect by direct interaction of its trivalent anion AsO_3^- and the thiol groups of cysteines in the proximity of target proteins (Nasr et al., 2008) (Figure 2).

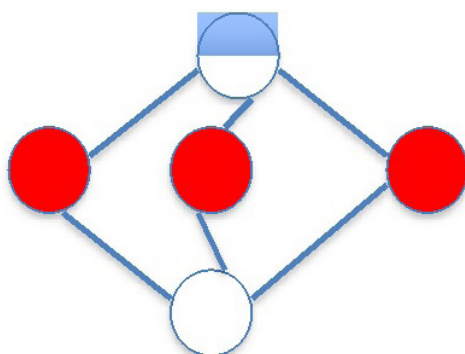


Figure 2. Chemical structure of arsenic trioxide (As_2O_3) (Based on Au, 2011).

In the Zn-finger protein involved in acute promyelocytic anemia (PML), the N-terminal domain is rich in cysteine and includes a principal ring (R) and a helix domain (Lu et al., 2007).

In a study of patients with PML with CNS relapse or under intrathecal prophylaxis, Au (2011) demonstrated that enterally administered arsenic reached a satisfactory concentration in the CNS. The author also demonstrated a correlation between serum and cerebrospinal fluid levels of ATO, with the CNS concentration being 17.7% of the plasma level.

ATO has been approved by the food and drug administration for the treatment of PML. This drug shows good permeability in the CNS and is implicated in the inhibition of a central pathway of genes activated in MB. Overall, all of these characteristics cause ATO to be a considerably attractive drug for the study of its therapeutic potential against MB of the SHH subgroup *in vitro* and in animal models (Nakamura et al., 2013).

MATERIAL AND METHODS

This is an integrative literature review and conducted in order to answer the following guiding question: Which inhibitory mechanism of subgroup 2 of medulloblastoma (SHH) involves the Gli gene? To this end, a bibliographic study was performed using four databases:

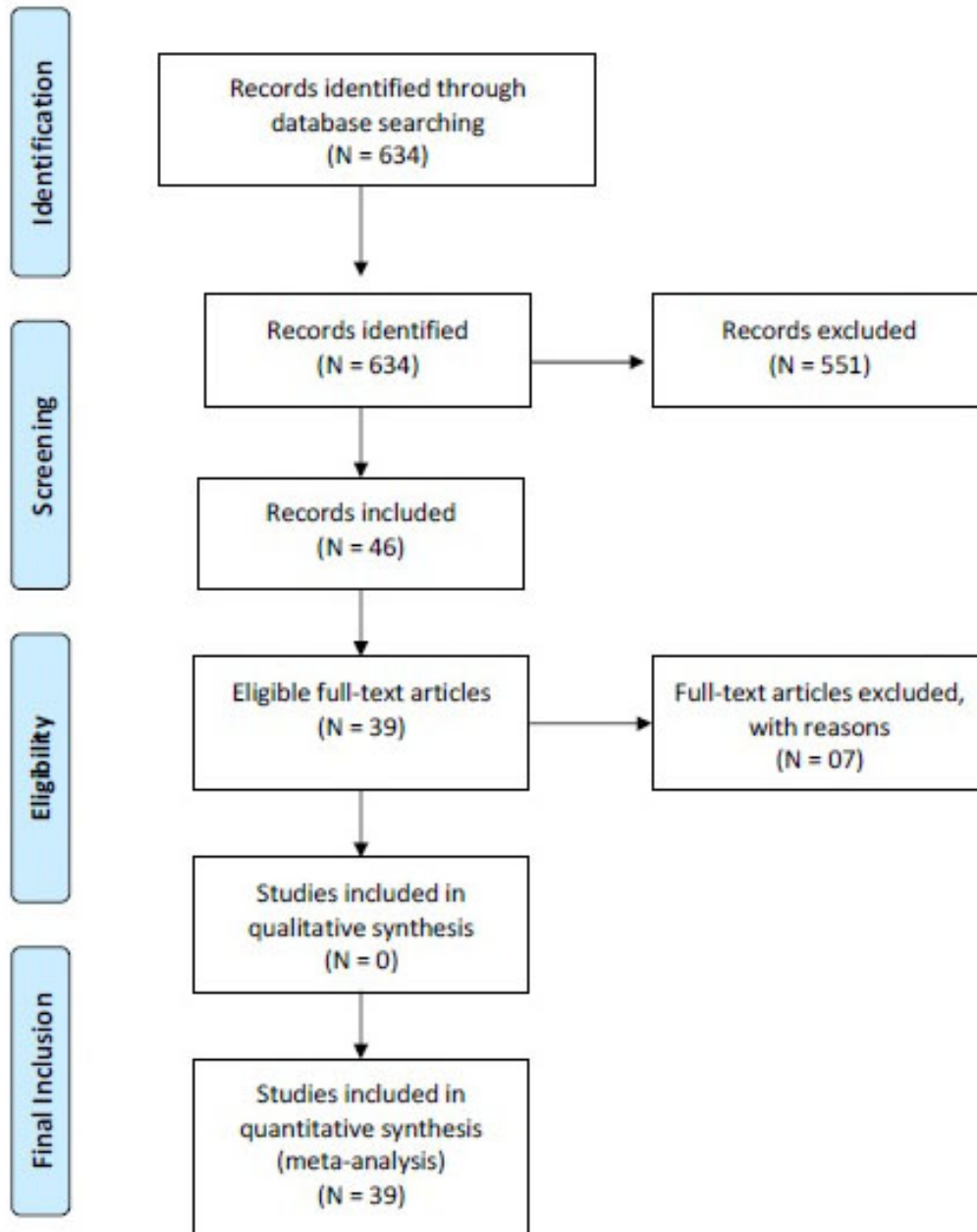
PRISMA 2009 Flow Diagram

Figure 3. PRISMA flow diagram detailing the sample selection process of articles for the review.

Table 2. List of the primary studies included in this integrative review with their author/s, title, year of publication, study method, and study classification.

Study	Authors	Study title	Year of publication	Methods, series, and main findings	Type of study
1	Al-Halabi et al.	Preponderance of sonic hedgehog pathway activation characterizes adult medulloblastoma	2011 Acta Neuropathol.	A study of 31 samples of medulloblastomas occurring in adults in order to establish the biological characteristics and differences from pediatric medulloblastomas. These particularities can be considered for the choice of therapy for this group	Functional assays and study of the biological characteristics of medulloblastomas occurring in adults
2	Au et al.	Determinants of cerebrospinal fluid arsenic concentration in patients with acute promyelocytic leukemia on oral arsenic trioxide therapy	2008 Blood	Patients with AML M3 and CNS relapse or undergoing chemoprophylaxis with ATO were monitored and the plasma and CSF concentrations of ATO were determined	Clinical study
3	Au	A biography of arsenic and medicine in Hong Kong and China	2011 Hong Kong Med. J.	Historical review of the use of ATO	Review article
4	Au et al.	Oral arsenic trioxide for relapsed acute promyelocytic leukemia in pediatric patients	2012 Pediatr. Blood Cancer	Four patients with AML M3 relapse were treated with oral ATRA + ATO and all of them had molecular remission	Case series report
5	Beauchamp et al.	GLI1 is a direct transcriptional target of EWS-FLI1 oncoprotein	2009 J. Biol. Chem.	Functional assays with Ewing sarcoma cell lines	Preclinical study, functional assays with cell cultures
6	Beauchamp et al.	Arsenic trioxide inhibits human cancer cell growth and tumor development in mice by blocking Hedgehog/GLI pathway	2011 J. Clin. Invest.	Functional assays with Ewing sarcoma cell lines and a xenographic model in animal models	Preclinical study, functional assays with cell cultures and animal models
7	Boehme et al.	Targeting hedgehog signalling by arsenic trioxide reduces cell growth and induces apoptosis in rhabdomyosarcoma	2016 Int. J. Oncol.	Functional assays with rhabdomyosarcoma cell lines	Preclinical study, functional assays with cell cultures
8	Bont et al.	Biological background of pediatric medulloblastoma and ependymoma: a review from a translational research perspective	2008 Neuro Oncol	Review article with emphasis on the biology of the pediatric tumor medulloblastoma and ependymoma and future perspectives	Review article
9	Buczkoewicz et al.	GLI2 is a potential therapeutic target in pediatric medulloblastoma	2011 J. Neuropathol. Exp. Neurol.	Functional assays for determination of the action of ATO in the GLI family, correlating GLI concentrations with the survival of 124 patients	Functional assays and survival analysis
10	Chang et al.	Arsenic trioxide inhibits cancer stem-like cells via down-regulation of Gli1 in lung cancer	2016 Am J Transl Res	Functional assays with lung cancer cell lines of the small cell type and observation of the action of ATO on these cell lines and on tumoral stem cells	Functional assays with cell lines
11	Crawford et al.	Medulloblastoma in childhood: new biological advances	2007 Lancet Neurol	Literature review regarding new biological advances in the treatment of medulloblastomas Greater understanding of the molecular biology of medulloblastoma is needed As newer biological agents are translated from the lab to the bedside, clinicians need to understand the fundamental signaling pathways that are target during therapy	Review article
12	Elamin et al.	Curcumin inhibits the Sonic Hedgehog signaling pathway and triggers apoptosis in medulloblastoma cells	2010 Mol. Carcinog.	Curcumin reduced beta-catenin levels and activated the phosphorylated form of Akt and NF- kappa B, leading to negative regulation of the three main common effectors, i.e., C-myc, N-myc, and cyclin D1. Consequently, apoptosis was triggered by curcumin through the mitochondrial pathway by the negative regulation of the Bcl-2 protein, an antiapoptotic downstream effector of Shh	Functional assays using the drug curcumin

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Table 2. Continued.

Study	Authors	Study title	Year of publication	Methods, series, and main findings	Type of study
				signaling. Importantly, the resistant cells that did not exhibit any reduction of Shh or Bcl-2 levels were sensitized to curcumin by the addition of the Shh antagonist cyclopamine	
13	Enguita-Germán et al.	CD133+ cells from medulloblastoma and PNET cell lines are more resistant to cyclopamine inhibition of the sonic hedgehog signaling pathway than CD133- cells	2010 Tumour Biol.	Functional assays with cell lines using positive and negative CD133 lines, and a search of inhibitors of the SHH pathway and its sensitivity to tumoral stem cells	Functional assays with cell lines
14	Falchi et al.	The evolution of arsenic in the treatment of acute promyelocytic leukemia and other myeloid neoplasms: Moving toward an effective oral, outpatient therapy	2015 Cancer	The authors discuss the oral use of ATO in AL subtype M3 and other types of myeloid leukemia	Review article
15	Fellay et al.	Medulloblastomas in adults: prognostic factors and lessons from paediatrics	2011 Curr. Opin. Neurol.	Review article with emphasis on the biological markers of each medulloblastoma subgroup, the prognosis for each group, as well as treatment	Review article
16	Hong-yan et al.	VEGFA expression is inhibited by arsenic trioxide in HUVECs through the upregulation of Ets-2 and miRNA-126	2015 PLoS One	Investigation of the mechanism by which ATO inhibits VEGFA in HUVEC through the upregulation of miRNA-126	Functional assays with cell lines
17	Gerber et al.	Recent developments and current concepts in medulloblastoma	2014 Cancer Treatment Reviews	Literature review on new biological advances in treatment	Review article
18	Götschel et al.	Synergism between Hedgehog-Gli and EGFR signaling in Hedgehog-responsive human medulloblastoma cells induces downregulation of canonical Hedgehog-target genes and stabilized expression of Gli1	2013 PLoS One	Investigation of the canonical SHH/Gli pathway by intervention between HH and EGF	Functional assays with cell lines
19	Han et al.	Arsenic trioxide inhibits viability of pancreatic cancer stem cells in culture and in a xenograft model via binding to SHH-Gli	2013 Onco. Targets Ther.	Demonstration of the <i>in vitro</i> and <i>in vivo</i> action of ATO using pancreatic cancer cell lines	Preclinical study, functional assays with cell lines and animal models
20	Han and Alvarez-Buylla	Role of primary cilia in brain development and cancer	2009 Curr. Opin. Neurobiol.	Review article about primary cilia and defects associated with genetic syndromes and tumorigenesis	Review article
21	Kim et al.	Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector	2010 Proc. Natl. Acad. Sci.	ATO as an antagonist of the SHH pathway prevents cilia accumulation and reduces Gli2 stability in transcription	Preclinical study, functional assays with cell cultures and animal models
22	Kool et al.	Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas	2012 Acta Neuropathol.	International meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 with new biological advances in the treatment of medulloblastomas	Review article

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Table 2. Continued.

Study	Authors	Study title	Year of publication	Methods, series, and main findings	Type of study
23	Korshunov et al.	Adult and pediatric medulloblastomas are genetically distinct and require different algorithms for molecular risk stratification	2010 J. Clin. Oncol.	Analysis of medulloblastomas occurring in the adult age range and detection of markers differing from those of medulloblastomas occurring in children. This study states the need for different treatments for adults	Functional assays, evaluation of biological markers, survival curve
24	Leonard et al.	Sonic Hedgehog signaling impairs ionizing radiation-induced checkpoint activation and induces genomic instability	2008 J. Cell. Biol.	The results suggest that inappropriate SHH activation promotes tumorigenesis by deactivating a key signaling pathway that helps maintain genomic stability and inhibits tumorigenesis	Preclinical study, functional assays with cell cultures and animal models using irradiation for DNA damage
25	Lu et al.	Targeting thioredoxin reductase is a basis for cancer therapy by arsenic trioxide.	2007 Proc. Natl. Acad. Sci.	An attempt to detect the mechanism by which ATO acts on medulloblastoma	Functional assays, evaluation of the mechanism of action of ATO
26	Nakamura et al.	Arsenic trioxide prevents osteosarcoma growth by inhibition of GLI transcription via DNA damage accumulation	2013 PLoS ONE	Demonstration in human osteosarcoma lines using real time PCR that ATO reduces the expression of genes linked to the SHH pathway including <i>PTCH1</i> , <i>GLI1</i> , and <i>GLI2</i>	Preclinical study, functional assays with cell cultures
27	Nasr et al.	Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation	2008 Nat. Med.	Analysis of the action of ATO and ATRA in animal and <i>in vitro</i> models relating their respective mechanisms of action to the degradation of the PML-RARA protein	Preclinical study, functional assays with cell cultures
28	Northcott et al.	The miR-17/92 polycistron is up-regulated in sonic hedgehog-driven medulloblastomas and induced by N-myc in sonic hedgehog-treated cerebellar neural precursors	2009 Cancer Res.	Analysis of 90 primary medulloblastoma samples. miR-17/92 expression increased in the medulloblastoma subgroup associated with SHH activation compared to subgroups in which miR-17/92 was also elevated due to increased MYC/MYCN expression	Functional assays, evaluation of biological markers and of survival curve
29	Northcott et al.	Medulloblastoma comprises four distinct molecular variants	2011 J. Clin. Oncol.	Classification of 104 primary medulloblastoma samples based on similarities in their biological characteristics in 4 subgroups	Analysis of the biological characteristics of medulloblastoma samples
30	Patel et al.	Are pediatric brain tumors on the rise in the USA? Significant incidence and survival findings from the SEER database analysis	2014 Child's Nerv. Syst.	Analysis of epidemiological data of childhood CNS tumors in the United States up to 2008	Historical cohort
31	Pizem et al.	Expression of Gli1 and PARP1 in medulloblastoma: an immunohistochemical study of 65 cases	2011 J. Neurooncol.	Survival rates were better in patients with a strong nuclear reaction for Gli1 than in patients with GLI1-negative medulloblastomas. Immunohistochemical detection of Gli1 may be useful for the identification of medulloblastomas with activation of the SHH pathway. As revealed by nuclear reaction for Gli1, the SHH pathway is activated in about 60% of all medulloblastomas. In some medulloblastomas, both SHH and WNT seem to be activated. PARP1 is highly expressed in medulloblastomas and may be useful as a target for increasing the efficacy of treatment modalities	Analysis of the biological characteristics of medulloblastoma samples
32	Raffel et al.	Sporadic medulloblastomas contain PTCH mutations	1997 Cancer Res.	A study of 24 medulloblastomas with heterozygosity for PTCH suggesting that inactivation of PTCH function is involved in medulloblastomas	Analysis of the biological characteristics of medulloblastoma samples

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Table 2. Continued.

Study	Authors	Study title	Year of publication	Methods, series, and main findings	Type of study
33	Robinson et al.	Vismodegib exerts targeted efficacy against recurrent sonic hedgehog-subgroup medulloblastoma: results from phase ii pediatric brain tumor consortium studies PBTC-025B and PBTC-032	2015 J. Clin. Oncol.	Phase II study of the drug Vismodegib showing activity in adult patients with medulloblastomas of the SHH group refractory to first line treatment	Phase II clinical study
34	Roboz et al.	Arsenic trioxide induces dose- and time-dependent apoptosis of endothelium and may exert an antileukemic effect via inhibition of angiogenesis	2000 Blood	Treatment with arsenic inhibits VEGF in HELA cell lines and incubation of HUVEC cells with arsenic prevents the formation and <i>in vitro</i> differentiation of endothelial cells. Therefore, arsenic interrupts a reciprocal stimulatory circuit between leukemic and endothelial cells, causing apoptosis of both cell types and inhibiting the VEGF production of leukemic cells	Preclinical study, functional assays with cell cultures
35	Shahi et al.	Regulation of sonic hedgehog-GLI1 downstream target genes PTCH1, Cyclin D2, Plakoglobin, PAX6 and NKX2.2 and their epigenetic status in medulloblastoma and astrocytoma	2012 BMC Cancer	The expression and silencing of GLI1 resulted in increased regulation of all target genes in the medulloblastoma cell line, while only PTCH1 was regulated in astrocytomas. Methylation of the D2 cyclin promoter was observed in a significant number of astrocytoma cell lines (63%) and in primary astrocytoma samples (32%), but not in all the samples of any type of medulloblastomas. PTCH1 methylation of the promoter was less frequently observed than methylation of the cyclin D2 promoter in astrocytomas, and in some medulloblastomas	Preclinical study
36	Shahi et al.	The sonic hedgehog-GLI1 signaling pathway in brain tumor development	2012 Expert Opin. Ther. Targets	Proteins such as Ptch, SMO, and Gli are central for the Shh pathway. Other proteins such as HHIP, Sufu, Bmi -1, cyclin D2, placoglobin, PAX6, Nkx2.2, and SFRP1 are not well understood in Shh regulation as downstream target Gli-1 genes. The study attempted to elucidate this complex relationship	Preclinical study
37	Smaele et al.	An integrated approach identifies Nhh1 and Insm1 as Sonic Hedgehog-regulated genes in developing cerebellum and medulloblastoma	2008 Neoplasia	Profile of the expression of the cerebellar gene of rats aged 1 to 13 days revealing a group of genes whose expression is correlated with hedgehog (hh) activity levels. In this set, Insm1 and Nhh1/NSCL1 were identified as new HH targets induced by SHH treatment in progenitors of cultured cerebellar granular cells. The Nhh1 promoter was observed to bind to and get activated by the Gli1 transcription factor	Functional assays
38	Yang et al.	Arsenic trioxide inhibits the Hedgehog pathway which is aberrantly activated in acute promyelocytic leukemia	2013 Acta Haematol.	The Hh pathway is abnormally activated in APL and is associated with poor prognosis. ATO can effectively inhibit the expression of the Hh pathway. The data obtained provided the first clinical evidence for the application of ATO in tumors that exhibit an aberrantly activated Hh pathway	Functional assays
39	Yoon et al.	Defining a role for Sonic hedgehog pathway activation in desmoplastic medulloblastoma by identifying GLI1 target genes	2009 Int. J. Cancer	The authors attempted the identification of genes linked to Gli1 in the activation of the SHH pathway in desmoplastic medulloblastomas	Functional assays

AML M3 = acute promyelocytic leukemia M3; CNS = central nervous system; ATO = arsenic trioxide; CSF = cerebrospinal fluid; ATRA = all-trans retinoic acid; PCR = polymerase chain reaction.

DISCUSSION

ATO as a Gli inhibitor

Strategies for the inhibition of SHH pathways are being constantly developed. Initially, the idea was to develop a drug that would function as a central regulator of the SHH pathway by directly acting on the SMO protein. Within this context, cyclopamide was considered. Cyclopamide was first described as a steroidal alkaloid and later found to be an SMO receptor antagonist (Kim et al., 2010). Several other SMO inhibitors were then developed, four of them, i.e., Vismodegib (GDC-0449), NVP-LDE225 (Novartis), IPI-926 (Infinito), and XL-139 (BMS/Exelis) progressing to phase II clinical assays. All of these compounds act as competitive inhibitors of the cyclopamide ligand (Kim et al., 2010). The response to treatment with these drugs was initially positive; however, cases of drug resistance were observed after a certain period of use. Possible causes of this resistance were reported to be Gli 2 changes, cyclin D1 amplification, and PI3K-AkT-mTOR signaling pathway activation (Kim et al., 2010).

New strategies for the inhibition of the SHH pathway were investigated based on the observation that GLI1 and GLI2 expressions confer a poor prognosis to pediatric MB, with GLI2 playing a central role in SHH activation. GLI2 expression leads to a change in morphology and a reduction in viability, cell numbers, and apoptosis in MB. The GLI1 protein is transcribed together with GLI2 but has no principal action, only representing an activation marker of the pathway, a fact indicating GLI2 as a possible target of therapy (Buczkwicz et al., 2011).

It had been reported that ATO acts on Ewing sarcoma cell lines and MB because of its interaction with GLI; however, the exact mechanism of action was unknown. Beauchamp et al. (2009) demonstrated that the relevant mechanism included direct intranuclear binding of ATO to DNA, with no change in the intracytoplasmic or nuclear GLI protein concentrations (Beauchamp et al., 2011).

The effect of ATO on PML has been well established, with the drug being used widely to achieve remission in induction failure (Nasr et al., 2008). ATO integration with Zn-fingers of the PML protein alters the conformation of the protein and promotes its degradation. Similarly, ATO inhibits tumor growth by inhibiting the ciliary accumulation of Gli 2, promoting its degradation. The combination of itraconazole and ATO for the treatment of MB has been tested in PTCH-positive and TP 53-negative animals, demonstrating synergism with a reduction in tumor volume. ATO in monotherapy or in combination with itraconazole inhibits the SHH pathway by distinct mechanisms (Nasr et al., 2008; Kim et al., 2010).

It has been demonstrated that ATO inhibits tumor growth by blocking the transcription of GLI pathway components. ATO also reduced the expression of genes of the SHH pathway, such as *PTCH1*, *GLI1*, and *GLI2* in human osteosarcoma cell lines (Nakamura et al., 2013). The same study also reported that treatment with ATO promoted apoptosis of neoplastic cells caused by the accumulation of direct DNA damage. Studies of bone tumor cell lines (Ewing sarcoma) also revealed sensitivity to the use of ATO, with direct binding of GLI1 to the EWS-FLI1 oncoprotein (Beauchamp et al., 2011).

Roboz and his collaborators demonstrated that ATO has the ability to induce apoptosis of leukemia cells and endothelial blood vessel cells in a dose- and time-dependent manner (Roboz et al., 2000). In this study model, ATO inhibits endothelial cells by vascular endothelial growth factor (VEGFA) inhibition via a vascular receptor, with the consequent suppression of angiogenesis and tumor growth (Ge et al., 2015). Using the microRNA profile of endothelial

cells derived from stem cells, miR-126 has been reported to directly and negatively regulate VEGFA signaling through PI3KR2 and SPRED1, both regulators of this pathway (Ge et al., 2015). One of the possible study limitations is the retrospective nature of study reviews on the subject. Although we opted to use several databases with ample literature coverage, some inclusive articles may have escaped our search criteria.

CONCLUSIONS

New strategies for the treatment of MB are being explored. The SHH pathway and its components, PTCH1, SMO, and SUFU, were first investigated in an attempt to develop a specific drug for the blockade of these pathways. Initially, clinical results of this intervention were positive; however, drug resistance was observed after a period of therapeutic use. In continued attempts at blockade, the Gli gene has been a little explored target. Drugs that directly block Gli could potentially be included in the standard therapeutic scheme (polychemotherapy and/or radiotherapy). With a singular history, ample clinical use, and good CNS penetration, arsenic trioxide is still being investigated today in a field beyond that of leukemias. Preclinical studies of ATO in brain tumors, in which the participation of the SHH pathway is involved, have demonstrated an acceptable profile for the assessment of this drug in animal models of MB both in combination with chemotherapy and as a radiosensitizing agent.

Conflicts of interest

The authors declare no conflict of interest.

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