Principles of tumorigenesis and emerging molecular drivers of SHH-activated medulloblastomas

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Abstract

SHH-activated medulloblastomas (SHH-MB) account for 25–30% of all medulloblastomas (MB) and occur with a bimodal age distribution, encompassing many infant and adult, but fewer childhood cases. Different age groups are characterized by distinct survival outcomes and age-specific alterations of regulatory pathways. Here, we review SHH-specific genetic aberrations and signaling pathways. Over 95% of SHH-MBs contain at least one driver event – the activating mutations frequently affect sonic hedgehog signaling (PTCH1, SMO, SUFU), genome maintenance (TP53), and chromatin modulation (KMT2D, KMT2C, HAT complexes), while genes responsible for transcriptional regulation (MYCN) are recurrently amplified. SHH-MBs have the highest prevalence of damaging germline mutations among all MBs. TP53-mutant MBs are enriched among older children and have the worst prognosis among all SHH-MBs. Numerous genetic aberrations, including mutations of TERT, DDX3X, and the PI3K/AKT/mTOR pathway are almost exclusive to adult patients. We elaborate on the newest development within the evolution of molecular subclassification, and compare proposed risk categories across emerging classification systems. We discuss discoveries based on preclinical models and elaborate on the applicability of potential new therapies, including BET bromodomain inhibitors, statins, inhibitors of SMO, AURK, PLK, cMET, targeting stem-like cells, and emerging immunotherapeutic strategies. An enormous amount of data on the genetic background of SHH-MB have accumulated, nevertheless, subgroup affiliation does not provide reliable prediction about response to therapy. Emerging subtypes within SHH-MB offer more layered risk stratifications. Rational clinical trial designs with the incorporation of available molecular knowledge are inevitable. Improved collaboration across the scientific community will be imperative for therapeutic breakthroughs.

Introduction

Medulloblastoma (MB) is the most common pediatric brain malignancy, accounting for approximately 20% of childhood brain cancers and 10% of all childhood cancer deaths. Incidence culminates among children younger than 10 years of age, with about half of cases arising before the age of 5.1,2 Up to 40% of patients are diagnosed with metastatic disease,3 with a grim outlook for survival.4 More than one-third of patients die within 5 years after diagnosis, and survivors face treatment-related long-term adverse effects.5

MB treatment strategy involves maximal safe resection followed by craniospinal irradiation and cytotoxic chemotherapy, with specific type and intensity for high- or standard/average-risk disease. Average-risk patients are over 3 years of age with total or near-total resection and no disease dissemination, while patients with suboptimal tumor resection, metastasis, and/or large cell/anaplastic (LCA) histology are treated for high-risk disease.6 Infants...
under 3 years of age require delayed irradiation and are preferably treated by multiagent chemotherapy, with better results after gross total resection with the absence of dissemination compared to patients with residual or metastatic disease.²⁻⁷ Continuing advances in neuroimaging, neurosurgical techniques, radiation therapy, and combined chemotherapy have increased 5-year survival rates to 70–80%,¹⁵ although individual responses to treatment vary considerably and survival rates have reached a plateau.¹⁰ The highly toxic and invasive multimodal therapies frequently induce debilitating adverse effects on the long term.¹¹ Evidently, interventions should be spared in patients likely to be cured and maximized in those with aggressive disease.

The molecular era lead to exciting transformations in patient stratifications with consequences for therapeutic approaches. Based on molecular alterations, four subgroups became widely accepted: sonic hedgehog-activated (SHH-MB), wingless-activated (WNT-MB), Group 3, and Group 4 MBs, each characterized by distinct patterns of somatic mutations, copy number alterations, transcriptional profiles, and clinical outcomes.¹² WNT- and SHH-activated MBs have abnormal activation of the WNT and SHH pathways, respectively, while no dominant signaling pathway alterations were identified in Group 3 and Group 4 MBs and appear as non-WNT/non-SHH in the revised WHO classification.¹³ Subgroup assignment is highly prognostic, with markedly different survival rates.¹⁴ The 5-year overall survival is as high as 95% in WNT-activated MBs. Group 3 patients face the worst 5-year overall survival (45–60%), especially low among infants. Group 4 and SHH-MBs are characterized by an intermediate (75–80%) 5-year overall survival that also depends on disease dissemination, histology, and genetic aberrations, such as mutations and oncogene amplifications.¹⁵⁻¹⁸ Within each primary MBs, additional subtypes are emerging with distinct biology and clinical outcomes,¹⁸⁻²⁰ providing a constructive approach for therapy optimization.¹⁴ Here, we provide a comprehensive overview of SHH-MBs with special focus on emerging prognostication schemes and novel therapeutic approaches.

Clinical Attributes

SHH-MBs account for ~30% of all MBs and occur in a bimodal age distribution encompassing the majority of infant and adult, but relatively fewer childhood cases.¹⁵,²¹,²² (Fig. 1A). Pediatric and adult tumors are molecularly and clinically distinct.¹²,²³ Approximately 21% of SHH-MBs are enriched with TP53-mutations, delineating a distinct subcategory – “SHH-activated TP53-wild-type” is more frequent among adults and young children and confers a good prognosis with an 81% 5-year overall survival (OS). In contrast, the “SHH-activated TP53-mutant” subtype occurs typically among older children between ages 5 and 18 and is associated with a dismal prognosis, including a 41% 5-year OS. In children older than 5 years, tumors with TP53-mutations account for two-third of deaths.²⁴

The majority of SHH-MBs are nodular/desmoplastic but can also have a classic or LCA histology, the latter especially frequent among children.²⁵,²⁶ The nodular-desmoplastic subtype predicts increased survival in infants, and in this young group of patients, radiation therapy may be successfully abolished.²⁷⁻²⁹ Radiation-sparing treatments involve systemic chemotherapy with intraventricular therapy (HIT-SKK’92 protocol),⁷ or high-dose chemotherapy with stem cell rescue (CCG-99703 protocol).³⁰ Sequential high-dose chemotherapy following the CCG-99703 protocol resulted in excellent outcome for SHH-MB patients with classical histology.³¹ Nevertheless, conventional chemotherapy, excluding intraventricular methotrexate, is not feasible as The Children’s Oncology Group clinical trial (ACNS1221) for nonmetastatic infants under 4 years of age with nodular-desmoplastic MB was closed prematurely due to an excessive number of relapses.³²

Molecular Biology

SHH-MBs frequently arise in the cerebellar hemisphere from cerebellar granule neuron precursors (CGNPs) or the cochlear nuclei of the brainstem.³³ Constitutive SHH-signaling leads to overproliferation,³⁴⁻³⁶ therefore SHH expressed during early postnatal development from Purkinje neurons promotes the rapid expansion of CGNPs in the external granule layer differentiating as granule neurons migrate into the internal granule layer.

Distinct regulatory processes are altered in different age groups. Developmental processes and DNA/histone methylation include the most frequently disturbed pathways in infants, while chromatin organization and transcription regulation are most heavily involved in adults.³⁶ Despite the age group-specific molecular features, SHH tumors share common traits, such as high expression of SHH-signaling target genes (e.g., Gli family of transcription factors) or CGNP specification genes (e.g., ATOH1) and relatively low expression levels of neuronal differentiation genes.²⁶,³⁷⁻³⁹

Genetic predispositions

SHH-MBs have the highest, 14–20% prevalence of destructive germline mutations among all MBs, although less than half are projected based on family history and medical records. Genetic predispositions influence both progression-free and overall survival.⁴⁰ Gorlin syndrome is associated with mutations affecting PTCH1 and SUFU genes of the SHH pathway, and Li–Fraumeni syndrome is...
linked to TP53-mutations predisposing carriers to multiple familial cancers.\textsuperscript{41} Germline PTCH1 and SUFU mutations are more common among infants with a median age of 2.0 years and are present in 21% of all infant SHH-MBs. Hereditary TP53-mutations are most frequent among children (median age of 9.8 years), present in \textasciitilde 21% of all SHH-MBs patients aged 5–16, and are coupled with a low, 27% 5-year survival.\textsuperscript{40} Li–Fraumeni syndrome-related SHH-MBs are associated with an increased incidence of chromothripsis, a massive genomic rearrangement during a single catastrophic event, resulting in gene fusions and/or highly amplified copy number states of recognized oncogenes.\textsuperscript{40} These findings suggest that TP53-mutations predispose cells to catastrophic DNA rearrangements or facilitate cell survival after such events.\textsuperscript{42} Accordingly, an outstandingly high mutational rate characterizes Li–Fraumeni syndrome-associated SHH-MBs.\textsuperscript{37} Rare heterozygous germline mutations in BRCA2 and PALB2 genes were also identified among SHH-MB patients, coupled with homologous recombination repair deficiency-like mutation spectrum.\textsuperscript{40}

Because of TP53’s crucial role in DNA repair, genome maintenance, and cell death, radiation therapy may accelerate tumor growth, which is particularly important for patients with Li–Fraumeni syndrome.\textsuperscript{43} Radiation also increases the likelihood of secondary malignancies, such as basal cell carcinomas and other tumors of the skin in patients with Gorlin syndrome (germline PTCH1 mutation).\textsuperscript{44} Genetic counseling is recommended for families in case of TP53-mutations. Additionally, genetic testing is recommended for germline PTCH1 and SUFU mutations for children with MB, in children <3 years old, or those whose tumors show nodular or desmoplastic histologic features and/or somatic changes in the SHH pathway.\textsuperscript{45}

**Copy number variations**

Losses of 9q, 10q, and 17p, frequently co-occurring with TP53-mutations, are the most frequent large-scale chromosomal aberrations in SHH-MB. Focal somatic copy number aberrations (SCNA) include MDM4 and PPP1ID amplifications and focal deletions of TP53, all involved in

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**Figure 1.** Age-specific distribution of childhood medulloblastomas. (A) Infants, children, and adults are represented differently within each medulloblastoma subgroup. (B) Dominant mutations across three different age groups in SHH-activated medulloblastomas (SHH-MB). (C) Schematic representation of major mechanisms most frequently affected by somatic alterations contributing to SHH-MB development.
TP53-signaling; amplification of GLI2 and deletion of PTCH1, exclusive for SHH tumors; and amplifications of MYCN and CCND2. Some SCNAS (including IGFIR, PIK3C2G, PIK3CB, KIT, MD4, PDGFRα, and deletion of PTEN) are potentially targetable with already available small molecule inhibitors.26

**Mutations**

Over 95% of SHH-MBs contain at least one driver event. However, the types of mutations are highly variable22,26. Activating mutations almost permanently involve the SHH-signaling pathway,26 but alterations beyond canonical SHH-signaling, such as mutations of the IDH1 gene with epigenetic regulatory function, have also been recently described.22

The most frequently mutated genes include PTCH1 (~43%), SNUF (~10%), and SMO (~9%), and the presence of these activating mutations is mutually exclusive and age group specific22,26,37,39 (Fig. 1B). In a significant proportion of patients, where PTCH1, SMO, and SNUF mutations are absent, alternative mechanisms are responsible for SHH-pathway activation.46 PTCH1 mutations are roughly equally numerous in adults, children, and infants, while SMO and SNUF mutations are more abundant in adults and SNUF mutations in infants (0–4 years), with both mutations barely present in children.26 Infants harboring germline PTCH1 mutations are diagnosed with Gorlin syndrome (nevoid basal cell carcinoma).41

SHH-MBs enriched with TP53-mutations (~20%) are coupled with the highest overall mutational rate of all MBs. Chromosome 17p loss is common in TP53-mutant cases.26 Interestingly, WNT-MBs are also enriched with TP53-mutations, with the second highest mutation rate, but without the survival disadvantage observed in SHH-MBs.37 In children, TP53-mutations are mutually exclusive with PTCH1 mutations, but frequently co-occur with GLI2 and MYCN amplifications, which are essential regulators of transcription22 (Fig. 1B); for example, GLI2 is the main transcription effector of SHH-signaling in granule cell precursors.47

Chromatin modulation is frequently affected in SHH-MBs (Fig. 1C). KMT2D and KMT2C methyltransferase mutations occur with relatively high frequency in both pediatric and adult samples.22 Mutations deregulating histone-acetyltransferase (HAT) complexes involving genes such as CREBBP, KAT6B, EP300, BRPF1, and KANSL1 are present in approximately 19% of all SHH-MB patients.22 Frequently mutated genes also include the nuclear receptor corepressor complex encoding BCOR, GPS2, LDB1, GABRG1, and NCOA2.39,48

Mutations of various genes are almost exclusively specific to the adult subgroup, including alterations in BRPF1/3 associated with SMO mutations or CREBBP and KDM3B in PTCH1-mutated tumors.26 Fifty-four percent of adults and 7% of pediatric samples carry DDX3X mutations affecting RNA metabolism.26 Telomerase reverse transcriptase (TERT) promoter mutations that drive telomerase activity occur with high frequency in adult SHH-MBs (83% of older patients),39 including the most-frequent C228T and the less-frequent C250T variants.26 TERT-mutant SHH tumors carry very few previously described SCNAS and are mostly mutually exclusive with 10q loss, possibly underlying the comparatively favorable prognosis.39 Recurrent mutations of the PI3K/AKT/mTOR pathway involving PIK3CA, PTEN, and PIK3C2G mutations also occur mainly in adult patients, with a surprisingly high frequency; pathway activation based on p-AKT and p-S6 positivity assessed by IHC was identified in about 30% of adult SHH patients, with a strong association with poor outcome.26 We summarize the most common SHH-MB-specific genetic alterations in Table S1.

High expression of genes often cannot be traced back to specific mutations or chromosome aberrations, instead, might be regulated by epigenetic mechanisms, as methylation events are more common compared to sequence mutations. For example, high expression of cMET in SHH-MBs is not linked to recurrent mutations or amplifications, but is among the most frequently hypo- or hypermethylated genes in MBs,50 also associated with prognostic significance.51

**Molecular classification**

According to the consensus of the International Medulloblastoma Working Group, two of four requirements are suggested to be met for verified SHH activation: (1) GAB1 immunoreactivity, (2) SHH-signaling-specific mutation, (3) methylation profiling, or (4) gene expression profiling consistent with SHH activation either based on genome-wide arrays or focused gene expression panels.38,52 Combined immunoreactivity for GAB1, YAP1, and filamin A distinguishes WNT- and SHH-MBs,53 based on genome-wide arrays or focused gene expression panels.38,52 Combined immunoreactivity for GAB1, YAP1, and filamin A distinguishes WNT- and SHH-MBs from Group 3 and 4 MBs, but GAB1 immunoreactivity characterizes only SHH-activated tumors.25 The Nano-String 22 gene signature array determines SHH-pathway activation based on the differential expression of five genes (PDLIM3, EYA1, HHIP, ATOH1, and SFRP1) optimized for FFPE samples.38 A second diagnostic multi-gene signature utilizes the expression of eight genes (BCHE, GLI1, ITIH2, MICAL1, PDLIM3, PTCH2, RAB33A, and SFRP1) for SHH-subgroup identification.53 Another unique five-gene signature (GLI1, SPHK1, SHROOM2, PDLIM3, and OTX2) identifies SHH-MBs even in the absence of SHH-pathway mutations.54 The
signature developed by Shou et al. detects all SHH-MBs, but unable to predict the response to SMO inhibitors26 (Table 1).

**Subtypes of SHH-Activated MBs**

Based on the presence of metastatic dissemination, TP53-mutation, and MYCN amplification, the proposed consensus stratifies SHH-MBs into three risk categories. Standard risk tumors are nonmetastatic and harbor wild-type TP53 and no MYCN amplification. Metastatic and/or MYCN-amplified tumors belong to the high-risk category, while very-high-risk tumors contain either somatic or germline TP53-mutations55 (Fig. 2). Amplification of MYCN is generally restricted to SHH and Group 4 patients but only associates with poor prognosis in SHH-MBs and is frequently accompanied by TP53-mutations.14,15,26

Additional prognostic biomarkers, such as GLI2 amplification and LCA histology, strongly affect outcomes in pediatric samples, but are extremely unusual in adult tumors.15,26 A large-scale cytogenetic study analyzing biomarkers in a subgroup-specific manner identified GLI2 amplification, 14q loss, and leptomeningeal dissemination, but not anaplastic histology, as key biomarkers differentiating clinically high- and low-risk SHH-MBs.14

Integration of methylation- and gene expression-based data suggests four subtypes: two in infants and one each in children and adults. SHH \( \alpha \) appears primarily in children aged 3–16 years, has the worst prognosis of all SHH-MBs, harbors TP53-mutations, MYCN and GLI2 amplifications, 9q loss, 10q loss, 17p loss, and YAP1 amplifications. TP53-mutations are highly prognostic only within this subtype. SHH \( \gamma \) is present among infants and is enriched for MBEN histology, representing a low-risk group potentially suitable for therapy de-escalation. SHH \( \beta \) is also present in infants with high rates of metastasis and associates with focal PTEN deletions with worse survival compared to SHH \( \gamma \). SHH \( \delta \) is mainly present in adults and is associated with TERT promoter mutations and a favorable prognosis19 (Fig. 2). A methylomic profile-based analysis divided SHH-activated childhood MBs into infant and children subtypes with a cutoff at 4.3 years of age. In children, the presence of MYCN amplification, LCA pathology, metastasis, and incomplete resection separated very-high-risk disease from tumors with a favorable outcome20 (Fig. 2). Two methylation subtypes were identified in infants with markedly different molecular alterations and prognosis in another study: 5-year progression-free survival was 27.8% for the iSHH-I-subtype that harbored SUFU alterations and chromosome (chr) 2 gains versus 75.4%.

**Table 1.** Identifying subgroup affiliation in childhood medulloblastomas.

| IHC       | WNT | SHH | Group 3 | Group 4 | Source | Comments            | Ref. |
|-----------|-----|-----|---------|---------|--------|---------------------|-----|
| Nuclear \( \beta \)-catenin | GAB1 |     |         |         | FFPE   | Least robust: nuclear \( \beta \)-catenin present in other subgroups | 25  |
| Gene expression | DKK1, DKK2, TNC, CCDC46, PYGL | SFRP1, BCHE, GLI1, ITIH2, MICAL1, PDLM3, PTCH2, RAB33A, SFRP1 | NPR3 | KCNA1 | FFPE | Frozen 13-gene multiplex mRNA expression assay | 53  |
| n/a | GLI1, SPHK1, SHROOM2, PDLM3, OTX2 | n/a | n/a | n/a | Frozen and FFPE | 54  |
| Mutation | CTNNB1 exon 3 | | | | FFPE | | 52  |
| Structural aberrations | Chromosome 6 monosomy (FISH or DNA copy number array) | | | | | 52  |
| Methylation | Illumina Infinium HumanMethylation 450 BeadChip array | | | | FFPE | Fresh frozen and FFPE | 121 |

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for the iSHH-II-subtype characterized by SMO mutations and chr9q deletions. Patients in the latter subtype profited from a radiation-free therapy, supporting the validity of a molecularly driven treatment approach. However, inspecting subtype-specific biomarkers reveals that iSHH-I and iSHH-II likely correspond to the already described SHHb and SHHC subtypes. Additional discrepancy across subclassifications stems from diverse patient populations (only children in20) utilized data types, and clustering methods. Prospective clinical trials are in great demand to identify biomarkers suitable for effective patient stratification.

Proteomics reveal novel stratifications and translational opportunities

Genomic studies provide invaluable insight into differences in cancer biology and outcome across MB subgroups. Nevertheless, translation of the proposed findings to subgroup-specific therapies remains difficult. Recent proteomic analyses recapitulated genomic subgrouping, what is more uncovered posttranscriptional MB heterogeneity not evident in the genome or the transcriptome. Since proteomics are more representative of cellular biology, the approach is well suited to identify functionally relevant therapeutic targets.

Figure 2. Risk stratification of SHH-activated medulloblastomas including prognostic biomarker candidates across all age groups as defined by Ramaswamy et al. (2016) (A), Schwalbe et al. (2017) (B) and Cavalli et al. (2017) (C).
Heterogeneous transcriptional patterns from untreated SHH, Group 3, and Group 4 MB samples converged only on two distinct protein-signaling profiles that partially overlap with molecular subgroups. The profile with MYC-like signaling encompasses all of SHH-activated and the majority of Group 3 samples, and is associated with a rapid death postrecurrence. The rest of Group 3 and the bulk of Group 4 tumors show enrichment of DNA damage/apoptosis/neuronal signaling. Protein profiling uncovered putative targets for MBs with MYC-like profile, including the inhibition of cell cycle progression and protein synthesis.

Protein analysis resulted in an alternative subdivision of SHH-MBs compared to the age-based split according to expression- and methylation-based studies. The majority of adult samples clustered in the SHHa, while pediatric samples were split between SHHa and SHHb subtypes. SHHa signatures contained elevated SOX2, a regulator of neural progenitors, and mutations of PTCH1, TERT, and PRKAR1A consistent with canonical SHH-pathway activation. Proteins in SHHb presented upregulation of calcium, glutamate, and RAS-signaling pathways and elevated CD47, more characteristic to Group 4 than to SHH-MBs. CD47 is a membrane protein that blocks macrophages from destroying tumor cells, and the anti-CD47 antibody, Hu5F9-G4, demonstrated therapeutic efficacy in PDX models of Group 3 MBs. Hence, focusing on posttranscriptional alterations reveals functionally relevant novel mechanisms of tumorigenesis with translational potential. An integrative approach incorporating data from various “omics” – including protein profiling – would yield a more complete understanding of cancer biology, therefore collaborative initiatives facilitating data sharing are much desired.

**Preclinical Models of SHH-MB Reveal the Mechanisms of Tumorigenesis**

Preclinical models provide invaluable tools to study biological mechanisms underlying MB development and for evaluating new therapies. Several SHH cell lines have been established, such as DAOY, UW228, UW426, ONS-76, with confirmed subgroup identity based on transcriptional profiling. Out of the four SHH-MB cell lines, two are TP53-mutant, DAOY and UW228, reflecting that more aggressive subtypes are either easier to grow in cultures or more aggressive cells are selected and enriched in vitro.

Cell cultures and culture-derived allografts do not inevitably replicate the phenotypes of the original disease. Compared to allografts derived from original tumors with activated SHH-signaling, dependence on SHH-pathway activation is rapidly lost in cultured tumor cells and would not be restored when these cells are transplanted back to nude mice. The tumor microenvironment, particularly tumor-associated astrocytes are starting to emerge as key components promoting SHH-pathway activation in vivo. Altered in vitro signaling activity has therapeutic consequences, therefore preclinical models should be tested for how well they represent the original disease. Transplantation cancer models utilizing direct allografts maintain the genetic and histological profiles of original tumors, presumably better modeling patient responses to rational therapies.

Several preclinical mouse models of MB recapitulate the development of SHH-activated tumors, essentially through modeling SHH-pathway dysregulation. SHH-MBs arise from granule cells, yet they also develop in mice from granule neuron precursors of the cochlear nuclei. Approximately 10–20% of mice with a single-allele knockout for the Ptc1 gene (Ptc1+/−), a negative inhibitor of the Smo pathway (Fig. 3), develop cerebellar MBs. However the majority of CGNPs with Ptc1 loss will eventually differentiate into mature neurons, and the relatively low proportion of developing tumors indicates that, besides mutational activation of the SHH pathway, additional events are necessary for MB formation. For example, external beam irradiation considerably increases the tumor incidence rate (~80%). Following this first attempt, various models emerged that crossed Ptc1+/− with other aberrations, including disruption of DNA repair (DNA ligase IV loss) or cell cycle regulation (KiP1, INK4C, or INK4D inactivation) in conjunction with TP53 dysfunction. Nevertheless, simultaneous mutations in these pathways are rare in human SHH-MBs. Constitutive activation of Smoothened (SmoA1) in mouse CGNPs resulted in highly penetrant tumors with leptomeningeal metastasis with 48% incidence rates, along with increased expression of both Sonic hedgehog (Gli1, NMyc) and Notch (Notch2 and Hes5) target genes.

Mouse models of MB utilizing the Sleeping Beauty (SB) transposon system provide excellent tools to discern driver events of tumorigenesis. An unbiased SB transposon-based model, whole-body SB mutagenesis activated a gene network of neuronal transcription factors associated with metastatic disease. Activity of this network was mostly driven by Pten and Myt1 expression and associated with metastatic disease.
Figure 3. Sonic hedgehog signaling is crucial during normal development of cerebellum, and its dysregulation leads to medulloblastoma development. Mutations of PTCH1, SMO, and SUFU, or amplification of GLI2 contribute to downstream activation of Hedgehog signaling targets. Several small molecule inhibitors of SMO, such as sonidegib (LDE-225) or vismodegib (GDC-0449), are being investigated as potential targeted therapies in clinical trials. Mutations downstream of SMO render such inhibitors ineffective. Itraconazole has the ability to inhibit SMO activation including some SMO mutations that confer resistance to SMO inhibitors, and arsenic trioxide inhibits GLI2 ciliary accumulation.
and poor survival outcome in human subjects, especially in SHH-MBs. Network mutations converged on and increased the Igf2 expression critical for CGNP proliferation and tumor formation.80

Activation of SHH releases inhibition of Smo by the transmembrane receptor Ptch1 to activate Gli1/2 transcription activators (GLIA), Gli3, a transcriptional repressor, and downstream mitogenic genes such as Ccnd1 and Mycn (Fig. 3). In mice, Gli1 functions as an oncogene during MB development: inactivation of both Gli1 alleles significantly reduces the incidence of spontaneous MB in Ptch1+/− mice.81 Hdac-mediated deacetylation of Gli1 via Hdac1 enzyme promotes transcriptional activation and enhances SHH-signaling. Members of the Kcash (Kctd containing, Cullin3 adaptor, suppressor of Hedgehog) gene family, coding for potassium channel tetramerization containing, Cullin3 adaptor, suppressor of Hedgehog (Smo) inhibitors

Prospective Therapeutic Opportunities

SMO inhibitors

Smoothed (SMO) regulates the suppression of SUFU81; thus, potential SMO inhibitors could prevent SUFU activation and translocation of GLI proteins to the nucleus (Fig. 3). Several small molecule inhibitors of SMO, such as sonidegib (LDE-225) or vismodegib (GDC-0449), are being investigated as potential agents in clinical trials,92,93 including ongoing trial NCT01878617, and demonstrate particular efficacy in relapsed adult SHH-MBs.93,94 Approximately 80% of adult patients carry either PTC1 or SMO mutations, rendering them to be likely responders to SMO inhibition. However, infants and children frequently harbor mutations downstream of SMO and thus may be resistant.26 Gli2 and MYCN amplification or SUFU-driven SHH-signaling have consequences for
targeted therapy. SMO inhibitors act upstream of these genes; thus, GLI2- and MYCN-amplified tumors might be refractory to agents targeting SMO.\textsuperscript{26,95,96} Resistance against SMO inhibitors rapidly develops, and its mechanisms are under intense research.\textsuperscript{97} De novo resistance is linked to SMO mutations (such as D473H or E518), but resistance can also develop downstream of SMO.\textsuperscript{94,96} Finally, SMO inhibitions have serious side effects in pediatric patients because they inhibit bone and teeth development: vismodegib-treated children developed widespread growth plate fusions that persisted long after therapy cessation.\textsuperscript{98} Drug-resistant SMO mutations highlight the need for new therapies in SHH-MB. Itraconazole has the ability to inhibit SMO activation, including some SMO mutations that confer resistance to SMO inhibitors. Arsenic trioxide inhibits GLI2 ciliary accumulation (Fig. 3). These two drugs alone or in combination inhibit in vitro cell growth and in vivo MB development in mice bearing wild-type or SMO mutations and prolong the survival of mice with SMO inhibitor-resistant medulloblastomas. Their combined administration seems to be effective against all known SMO mutations.\textsuperscript{99}

**Statins**

Cholesterol is an essential component of plasma membranes, and its homeostasis is regulated by a tight network of proteins. Enhanced expression of genes related to cholesterol biosynthesis has been identified in SHH-MBs.\textsuperscript{100} Statin treatment promotes differentiation and inhibited proliferation in MB cells isolated from \textit{Ptc}h\textsubscript{1}+/− mice indicating that cholesterol is an essential component of MB progression. Cholesterol and vismodegib bind SMO at different binding sites, leading to synergistic effects on tumor cells: inhibition of cholesterol biosynthesis by statins alone or in combination with vismodegib repressed in vivo SHH-MB proliferation and growths in subcutaneous allograft tumors of \textit{Ptc}h\textsubscript{1}+/− mice.\textsuperscript{100} The efficacy of statins depends on SHH-pathway mutations: MBs with mutations in \textit{Smo}M2 or downstream of SMO, such as in \textit{SUFU} or GLI2 are intrinsically resistant to SMO- and cholesterol inhibitors. Potential combination of statins with vismodegib may lead to reduced dosing of the latter to prevent serious side effects in pediatric populations. Targeting cholesterol biosynthesis characterizes a promising alternative treatment strategy for a subset of SHH-MBs, nevertheless additional preclinical studies are required prior to their clinical use.\textsuperscript{100}

**BET bromodomain inhibitors**

Genomic amplifications of zinc finger transcription factors GLI2 and GLI1 of the SHH-signaling pathway have been associated with more aggressive disease.\textsuperscript{95,101} BRD4 and other BET bromodomain proteins are critical regulators of GLI1 and GLI2 transcription downstream of SMO and SUFU. BET bromodomain inhibitors, such as JQ1, target BRD4 and significantly decrease tumor cell viability both in vitro and in vivo in genetically engineered mouse models, even when genetic lesions predispose tumors to resistance against SMO inhibitors.\textsuperscript{102}

**AURK or PLK inhibitors**

A population of cells in the \textit{Ptc}h\textsubscript{1} heterozygous mice model of MB that express surface carbohydrate antigen CD15/SSEA-1 augment tumor propagation following transplantation. These cells display increased expression of genes responsible for G2 and M phase regulation throughout mitosis. CD15 is expressed in a subset of human MBs associated with poor prognosis. Inhibition of G2/M regulators, such as Aurora kinases (AURK) or Polo-like kinases (PLK), reduces proliferation in vitro and tumor growth in vivo, and thus may represent a novel approach to treating CD15+/SHH-MBs.\textsuperscript{103} In addition, a class of Aurora kinase inhibitors may also disrupt the native conformation of Aurora A and, as a consequence, drive the proteolytic degradation of MYCN protein.\textsuperscript{104}

**cMET inhibitors**

Ablerrant MET-signaling is involved in metastasis development in various solid tumors and is upregulated in primary SHH-MBs,\textsuperscript{50} plus phosphorylated MET kinase-activity correlates with MB recurrence and poor survival.\textsuperscript{51} A MET inhibitor, foretinib, induced apoptosis and inhibited migration and invasion in SHH-MB cell lines, induced tumor regression, and prevented leptomeningeal metastases in mouse xenografts.\textsuperscript{51}

**Targeting stem-like cells**

Quiescent, therapy-resistant cells serve as a reservoir for relapse. In a model of SHH-MB, cells expressing the neural stem cell marker Sox2 comprised less than 5% of the tumor but drove tumor growth after antimitotic chemotherapy and SMO inhibition.\textsuperscript{68} Higher expression of stem cell genes is associated with worse outcome in numerous malignancies.\textsuperscript{105,106} Likewise, high SOX2 immunoreactivity is linked to worse survival in SHH patients.\textsuperscript{68} Mithramycin, an agent against SOX2+ cells, reduced the proliferation of human-derived primary SHH-MB cell lines and growth of subcutaneous allografts.\textsuperscript{68} Transducing NMyc in embryonic cerebellar cells induced SHH-activated MB formation with the contribution of...
transcription factor SOX9. SOX9 regulates stem cell properties, differentiation, proliferation, and survival and is commonly elevated in WNT- and SHH-MBs, particularly during early tumor initiation in a WNT/β-catenin-independent manner. FBW7, a protein participating in substrate recognition, regulates posttranslational regulation, particularly degradation of SOX9. FBW7 is frequently mutated in SHH-MBs, and its expression is downregulated across all MB subtypes. Lower levels of FBW7 are associated with increased quantity of SOX9 protein, metastasis, and poor survival. SOX9 abundance reduced the efficacy of cisplatin treatment both in vitro and in vivo. Activation of PI3K/AKT/mTOR signaling is associated with poor survival in adult SHH-MBs. Pharmacological inhibition of PI3K/AKT/mTOR pathway activity combined with cisplatin treatment stimulates FBW7 expression, degrades SOX9 protein levels, and increases apoptosis, providing a potential new opportunity to treat recurrent, chemoresistant MB patients. In an Ndg2:SmoA1 transgenic mouse model of MB, tumor-propagating CD15+ cells were regulated by Pten and PI3K signaling and displayed sensitivity to pan-FBW7 inhibitor both in vivo and in vitro but remained resistant to chemotherapy.

The chemokine ligand CXCL12 and its receptor CXCR4 are expressed in brain tumors (including medulloblastomas), and their expression is associated with poor prognosis. CXCL12 and CXCR4 are potentially important coactivators of SHH-signaling: SHH-CXCR4 coactivated tumors express higher levels of ATOH1 and cyclin D1 and exhibit maximal tumor growth. Targeting Cxcr4 alone with the infusion of small molecule antagonist AMD 3100 (plerixafor) inhibits growth of intracranial MB xenografts by decreasing cellular proliferation and increasing apoptosis. Combined Shh and Cxcr4 antagonism by vismodegib and plerixafor results in a synergistic antitumoral effect in xenografts injected with SmoA1-derived primary tumor cells by specifically targeting the MB stem-like cell pool, revealed by decreased expression of stem cell markers Bmi1 and Sox2.

**Immunotherapy**

Immunological differences within the tumor microenvironment across MB subtypes suggest different regulatory mechanisms and determine possible immunotherapeutic strategies. Increased expression of inflammation-related genes and elevated infiltration of tumor-associated macrophages (TAM) were described in SHH-MBs compared to Group 3 and Group 4 tumors. Likewise, increased frequencies of CD4+ and CD8+ T cells, myeloid cells, and dendritic cells were identified in Ptc1+/- SHH-MB-bearing mice compared to another model of MYC-amplified MBs, characterized by a higher proportion of CD8+ PD-1+ double positive T cells. The higher percentage of infiltrating myeloid-derived suppressor cells and TAMs in SHH-MBs suggests an immunologically suppressive tumor microenvironment. Accordingly, treatment with either anti-CTLA-4 alone, anti-PD-1 alone, or in combination was not effective in Ptc1+/- SHH-MB-bearing mice but showed survival benefits in MYC-amplified tumor bearing animals.

There is a pronounced urgency for alternative therapeutic modalities in pediatric brain malignancies. Identification of differentially expressed cell surface markers between tumor and normal cells may lead to novel immunotherapeutic strategies. The tumor-associated antigen, PRAME, is detected in the majority of MB samples but not in normal tissue, and high PRAME expression correlates with worse survival. Genetically modified T cells directed toward the PRAME antigen both in vitro and in vivo were effective against multiple HLA-A*02+ MB cell lines, including DAOY cells. Adaptive immunotherapy targeting PRAME represents a promising innovative approach for patients with HLA-A*02+ MBs. Comparing high-risk neuroblastomas and normal tissues identified differential expression of tumor-specific cell surface molecule, Glypican 2 (GPC2), an extracellular proteoglycan signaling coreceptor, required for cellular proliferation. A GPC2-directed antibody-drug conjugate, D3-GPC2-PBD, was effective against neuroblastoma cells in vitro in an antigen- and concentration-dependent manner, and in vivo in murine PDX models. GPC2 is also highly expressed both in primary and metastatic MBs, and similarly to neuroblastomas, MBs express the GPC2 transcript. GPC2 expression is highest among Group 4 MBs, but the relatively high expression across SHH- and Group 3 MBs suggests its potential relevance in other subtypes as well.

**Conclusions**

An enormous amount of data on the genetic background of SHH-activated MBs have accumulated in the past decade. While subgroup affiliation still does not provide reliable prediction of therapy response, emerging models offer more layered patient stratification. A more collective approach could accelerate translation of new insights into clinical practice. Collaborative efforts with improved communication, material, and data sharing could permit the development of better tailored preclinical models for basic biology studies and therapeutic development, and facilitate the integration of multilayer (genomic, epigenetic, proteomic, and metabolomic) molecular data to uncover novel disease biomarkers. Rational clinical trial design with the incorporation of available molecular
understanding, focusing especially on high-risk patients are inevitable and may bring the much-sought-after breakthrough in the stagnant survival rates of the past decades.

**Conflicts of Interest**

The authors have declared no conflicts of interest.

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

Table S1. Frequent genetic alterations in SHH-activated medulloblastomas according to [12, 22, 37, 38, 46, 48, 53, 54]. % patient values refer to within subgroup percentages.