Targeting Hedgehog (Hh) pathway components, such as Smoothened (SMO), is a developing strategy for the treatment of acute myeloid leukemia (AML) and for overcoming relapsed/refractory forms of this disease. Several SMO inhibitors are in clinical development for the treatment of various tumor types and the results from some clinical trials in AML have been reported. This review will discuss the role of Hh signaling in AML pathogenesis, describe the preclinical and clinical development of Hh pathway inhibitors for the treatment of AML, and examine the current evidence on Hh pathway inhibitor resistance and the implications for treatment selection in AML.

**Significance:** In acute myeloid leukemia (AML), components of the Hedgehog (Hh) signaling pathway, such as Smoothened (SMO), have been implicated in the development, maintenance, and expansion of leukemic stem cells (LSC), as well as sensitization to chemotherapy and the development of drug resistance in AML. Observations in preclinical studies of AML, as well as from samples of patients with AML, demonstrate that Hh pathway inhibitors act primarily on the stem cell pathway as differentiation agents. The current data for hematologic malignancies indicate the potential for a synergistic effect when a Hh pathway inhibitor is administered in combination with chemotherapy or investigational agents. It is thought that Hh pathway inhibitors act as agents that reduce LSC dormancy and promote LSC differentiation, thus the newly dividing LSCs can then be targeted by other chemotherapeutic drugs.

**INTRODUCTION**

Acute myeloid leukemia (AML) is heterogeneous at the cytogenetic and molecular level (1). Novel therapies targeting specific mutations such as FMS-like tyrosine kinase 3 (FLT3) and isocitrate dehydrogenase have recently emerged, representing a significant advance in the therapeutic armamentarium for AML. However, multiple clones frequently coexist, and resistant clones frequently emerge after specific targeted therapies, leading to clinical disease recurrence (1).

Leukemic stem cells (LSC) play a critical role in AML pathogenesis, progression, and persistence after standard therapy, being largely responsible for disease relapse and the development of chemotherapeutic resistance (2–4). LSCs are primitive progenitors that have the capacity to become quiescent, and are protected from apoptosis within the bone marrow microenvironment where they can self-renew (divide without differentiating). Therefore, LSCs are less affected by standard chemotherapeutic drugs that target rapidly dividing cells (both normal and malignant; refs. 2–4).

The Hedgehog (Hh) signaling pathway is a highly conserved and regulated pathway that plays a key role in embryogenesis, and stem cell maintenance, among other critical functions (5–8). In addition, dysregulation of components of the Hh pathway results in the development, maintenance, and expansion of LSCs (5–9).

The recognition of the role of LSCs in AML pathogenesis, treatment resistance, and clinical relapse has led to a search for agents that can eradicate the LSC population. The objectives of this review are to discuss the role of the Hh pathway in the development of the LSC population and AML pathogenesis, and of Hh pathway inhibitors as first-in-class LSC-targeting agents showing therapeutic effects for the treatment of AML.
Hedgehog Pathway Inhibitors for Acute Myeloid Leukemia

THE HEDGEHOG PATHWAY IN NORMAL AND MALIGNANT CELLS

In mammals, activation of the classical, canonical Hh pathway firstly occurs via binding of one of three Hh ligands (Sonic Hh, Indian Hh, and Desert Hh) to the Patched-1 (PTCH1) 12-transmembrane receptor protein at the cell surface (Fig. 1; refs. 5–8, 10). In its quiescent state, PTCH1 inhibits the activity of SMO. Upon ligand binding, the ligand–PTCH1 complex is internalized and degraded, releasing the PTCH1-mediated suppression of the 7-transmembrane receptor protein SMO (5–8, 10). SMO then translocates to the primary cilium, where its accumulation leads to the stabilization and activation of the glioma-associated oncogene homolog (GLI) family of zinc-finger transcription factors (TF; refs. 5–8, 10). GLI TFs then translocate to the nucleus, where they can activate or repress the expression of Hh target genes, for example, those involved in apoptosis, cell cycle, and differentiation (5–8, 10). In the absence of Hh pathway activation, the GLI TFs are held in an inactive state in the cytoplasm by a protein complex that includes suppressor of fused (SUFU), and through nonspecific kinase phosphorylation mediated by casein kinase, glycogen synthase kinase-3, and protein kinase A (5–8, 10).

There are also a number of regulatory steps and cellular components that are integral to Hh pathway signal transduction (5–8, 10). The Hh ligands can bind to a number of other transmembrane glycoproteins: PTCH2, which has different functionality and distribution from PTCH1; and Hh-interacting protein and Dispatched, both of which can modulate Hh signaling (5–8, 10). SMO translocation to the primary cilium is also mediated by β-arrestin 2 and G-protein-coupled receptor kinase 2 (5–8, 10).

The link between Hh signaling and malignancies was first reported in patients with nevoid basal cell carcinoma (BCC) syndrome (Gorlin syndrome). Patients with this entity have increased Hh signaling due to genetic mutations in PTCH1. This leads to the development of BCC and a predisposition to acute myeloid leukemia (AML). AML is a type of cancer that affects the blood and bone marrow, and it is characterized by the presence of abnormal white blood cells (leukemia). The Hedgehog pathway is an important signaling pathway in normal and malignant cells, and its activation can lead to the development of cancer.

**Figure 1.** Simplified overview of the Hh signaling pathway in the (A) inactive state and (B) active state. A, in the absence of ligand, PTCH1 prevents SMO from interacting with the SUFU-GLI complex within the cytoplasm. B, upon ligand binding, PTCH1 dissociates from SMO, leading to SMO accumulation at the primary cilium. SMO accumulation results in the stabilization and activation of GLI TFs, which then translocate to the nucleus and activate or repress transcription. Abbreviations: βA2, beta-arrestin 2; CK, casein kinase; DHh, Desert Hedgehog; GLI, glioma-associated oncogene homolog; GRK2, G-protein-coupled receptor kinase 2; GSK3, glycogen synthase kinase 3; Hh, Hedgehog; IHh, Indian Hedgehog; PKA, protein kinase A; PTCH1, Patched-1; SHh, Sonic Hedgehog; SMO, Smoothened; SUFU, suppressor of fused; TF, transcription factor.
medulloblastoma and rhabdomyosarcoma (6–8). Other associations between the Hh pathway and cancer include: genetic mutations in other Hh pathway components, such as SMO and SUFU in some instances of BCC and medulloblastoma; overexpression of Hh ligands in the tumor microenvironment of colorectal, pancreatic, and epithelial cancers; and Hh pathway regulation of cancer stem cells in breast cancer and glioblastoma (6–8, 10).

Abnormal activation of Hh signaling is critical to the development of a number of hematologic malignancies, one of which is chronic myeloid leukemia (CML; refs. 5–9). The constitutive activation of the BCR-ABL tyrosine kinase leads to dysregulation of critical hematopoietic stem cell processes and the persistence of LSCs (5, 8, 9). In a mouse model, as well as patient samples, constitutive activation of SMO resulted in the persistence of dormant BCR-ABL+ LSCs, with loss of SMO leading to depletion of BCR-ABL+ LSCs (11, 12). Components of the Hh pathway have also been shown to be upregulated in CD34+ CML stem/progenitor cells and patients with CML (6, 13, 14). Furthermore, pharmacologic inhibition of Hh signaling using SMO inhibitors in both mouse models and human CML cell lines repressed the growth of CML and reduced the self-renewal capacity of BCR-ABL+ LSCs, including in CML cells resistant to imatinib (6, 11, 12, 14). In summary, aberrant Hh signaling promotes BCR-ABL+ LSC self-renewal and proliferation; these BCR-ABL+ LSCs can then eventually proliferate, leading to transformation, and may be involved in the development of drug resistance and progression in CML.

Hh SIGNALING IN ACUTE MYELOID LEUKEMIA

The Hh pathway plays a prominent role in AML (2–8). Increased expression of Sonic Hh and GLI1 was observed in AML and other hematologic malignancy cell lines (15), with increased Hh pathway activity in both primary and cytokine-responsive CD34+ AML cells (16). In cytarabine-resistant CD34+ AML cells, apoptosis was induced using cyclopamine, an endogenous Hh inhibitor, Hh-interacting protein, or an anti-Hh–neutralizing antibody. Coadministration of cyclopamine and cytarabine reduced drug resistance in these CD34+ AML cell lines (16). Another study demonstrated that Indian Hh and SMO were expressed in CD34+ AML-derived cells (17). Hh-interacting protein was lower in AML-derived stromal cells versus healthy donor-derived stromal cells, and their expression levels correlated with activity in SMO+ leukemic cells. Decitabine treatment partially restored Hh-interacting protein expression and led to a reduction in cell proliferation of leukemic cells (17). GLI1 overexpression was reported in CD34+ AML cells and in samples from patients with AML (9). Treatment of the AML cells with GANT61, a GLI1 inhibitor, induced apoptosis and had a synergistic effect when coadministered with cytarabine (9). Similarly, more pronounced Hh signaling was shown in CD34+ cells versus CD34+ cells (18). Treatment with glasdegib, a potent SMO inhibitor, sensitized CD34+ cells to cytarabine. In a mouse model, glasdegib reduced tumor burden and leukemia initiation potential (18). An RNA interference screening study in AML cell lines identified several Hh pathway genes as 5-azacytidine-sensitizing hits, for example, Sonic Hh, SMO, and GLI3 (19). The SMO inhibitors erismodegib and vismodegib demonstrated moderate single-agent activity in these AML cell lines, whereas the combination of erismodegib and 5-azacytidine showed synergistic cytotoxicity (19). In addition, a SMO inhibitor, cyclopamine, or SANT-1, when combined with lipopolysaccharide, led to enhanced cytotoxic effects in AML cell lines and enhanced apoptosis in a mouse model (20). Similarly, cyclopamine plus TNFα or IFNs resulted in apoptosis in AML cell lines (20). Enhanced Hh pathway activation has also been reported in refractory AML cell lines. Sonidegib treatment sensitized these cell lines to radiation (21). Constitutively active SMO can act in conjunction with aberrant FLT3 activity to promote AML pathogenesis (22). Treatment with FLT3 and the SMO inhibitor IPI-926 (sarinegib, patidegib) resulted in a synergistic decrease in LSC growth compared with either agent alone (22). The Hh pathway also plays a critical role in the maintenance of multidrug resistance in myeloid leukemia cell lines (23).

These findings have been corroborated in studies from samples of patients with AML (5–8, 24–30). Upregulation of PTCH1, SMO, and the GLI TFs has been reported in patients with AML (24), and were associated with reduced event-free and overall survival (OS), both in de novo and secondary AML (26). This was also demonstrated in relapsed AML, with GLI1 expression correlating with cytogenetic risk and reduced survival. In addition, GLI1 conferred drug resistance to cytarabine and ribavirin through elevated uridine 5′-diphospho-glucuronyltransferase-dependent glucuronidation (25). Genetic or pharmacologic inhibition of these GLI TFs resulted in reduced colony formation and proliferation, and increased apoptosis of AML cells (24). In contrast to GLI1 and GLI2, expression of GLI3 was downregulated in samples from patients with AML (27). GLI3 may be necessary for the antileukemic activity of SMO inhibitors, and restoration of GLI3 led to antiproliferative effects (27). In bone marrow samples derived from patients with AML, glasdegib treatment led to impaired self-renewal and cell-cycle regulation in LSCs (29). Furthermore, analysis of samples from pediatric patients with AML (both newly diagnosed and refractory) showed that epigenetic modifications of Hh pathway components were associated with AML diagnosis and disease relapse (28). In addition, it has also been suggested that Hh plays a role in early progenitors of myelodysplastic syndromes (MDS; ref. 30). Glasdegib led to a reduction in the self-renewal of NOD/SCID repopulating cells and a reduction in the population of CD34+CD38− cells. In addition, glasdegib plus 5-azacytidine increased apoptosis induction in MSD-L cells (30).

In summary, increased Hh signaling, upregulation of Hh pathway–activating proteins, and/or downregulation of Hh pathway–inhibitory molecules are associated with AML pathogenesis. Upregulation of several components of the Hh pathway correlated with worse outcomes and resistance to treatment; genetic or pharmacologic inhibition of the Hh pathway resulted in sensitization to chemotherapy and demonstrated antileukemic activity. Therefore, targeting Hh pathway components is a developing strategy for the treatment of AML.
Hedgehog Pathway Inhibitors for Acute Myeloid Leukemia

Hedgehog Pathway Inhibitors in Clinical Development

Observations in preclinical studies of AML and from samples of patients with AML demonstrated that pharmacologic inhibitors of SMO act primarily on stem cells as differentiation agents (16, 18–20, 29, 30). SMO inhibitors can reduce dormancy in LSCs and lead to differentiation of LSCs by prompting cell-cycle progression from dormancy. Once LSCs begin dividing, they can be targeted with other therapies. The combination of a SMO inhibitor with standard chemotherapy demonstrated synergistic cytotoxicity and/or a reduction in drug resistance, likely due to increased elimination of LSCs (16, 18–20, 29, 30). GLI1 and/or GLI2 TFs have also been shown to be upregulated in patients with AML, to confer drug resistance to cytarabine and ribavirin, and be associated with worse clinical outcomes (24–26). On the other hand, GLI3 has shown antileukemic activity and was downregulated in patients with AML (27).

Because of their critical role in Hh pathway signal transduction, and the maintenance and expansion of drug-resistant cancer stem cells, SMO and GLI are key targets for the development of newer agents for the treatment of malignancies, including AML. Other Hh pathway components are in the early preclinical development stage, but have not yet been assessed in relation to AML (31). Several inhibitors of SMO are currently in clinical development for the treatment of various tumor types (Table 1). The results from some clinical trials of SMO inhibitors for the treatment of AML have been reported (Table 2). Although preclinical results are promising, inhibitors of GLI have not yet been tested in clinical trials. GANT61 and GANT58 prevent GLI1 translocation to the nucleus and DNA binding (32). GANT61 demonstrated antitumor activity in AML cell lines, and worked synergistically with both cytarabine and rapamycin to enhance their cytotoxicity (9, 33). Arsenic trioxide is already approved for acute promyelocytic leukemia, and prevents GLI2 activation by preventing GLI2 trafficking in and out of the primary cilium (34). The antifibrotic drug, pirfenidone, has also been shown to inhibit GLI2 via SUFU (35). A number of other experimental GLI inhibitors are being assessed in other tumor models (36). Further investigation of these GLI inhibitors in AML and clinical trials is warranted.

The naturally occurring alkaloid cyclopamine was the first known inhibitor of the Hh pathway, and is one of the most widely studied pharmacologic inhibitors of SMO (37). Cyclopamine binds to the heptahelical transmembrane domain of SMO, thus preventing the conformational change necessary for the activation of SMO and its subsequent translocation to the primary cilium (38, 39). Cyclopamine was used extensively in preclinical models of various tumors to understand Hh signal transduction; however, it has poor solubility and stability, low potency, and off-target effects, making it unsuitable for clinical use (37). Cyclopamine derivatives with increased specificity, potency, stability, and solubility have been developed for clinical use (37). These agents exhibit dose-proportional pharmacokinetics, have high plasma protein binding, and undergo cytochrome P450 metabolism (Table 1). Within this class of drugs, there are significant differences, among them their pharmacokinetic properties. For example, the half-life ($t_{1/2}$) of vismodegib (4 days with continuous daily dosing), BMS-833923 (XL139; >7 days), and sonidegib (28 days), is considerably longer compared with taladegib (LY2940680; 3 hours), glasdegib (17.4 hours), and IPI-926 (20 to 40 hours; refs. 40–54).

Approved SMO Inhibitors

Vismodegib (GDC-0449 or Erivedge) was the first cyclopamine derivative and Hh pathway–targeting drug approved for the treatment of cancer (53, 54). Vismodegib is currently approved for the treatment of patients with advanced or metastatic BCC (53, 54). A single-arm phase Ib study of vismodegib produced minimal clinical efficacy in patients with relapsed/refractory AML, with a median OS of 3.4 months and overall response rate of 6.1% (55). Of the 33 patients who died during the study, the majority ($n = 27$) died due to disease progression; no deaths were considered to be related to vismodegib (Table 2; ref. 55). There are a number of clinical trials ongoing for vismodegib (both monotherapy and combination) in hematologic malignancies as well as other solid tumors.

Sonidegib (LDE225, erismodegib, or ODOMZO) is another cyclopamine-derived SMO inhibitor discovered in an in vitro, high-throughput screen. Sonidegib is approved for the treatment of patients with advanced BCC that has recurred following surgery or radiotherapy, or those for whom surgery or radiotherapy is not an option (49, 50). Clinical trials are ongoing for sonidegib (both monotherapy and combination) in hematologic malignancies, including AML, and solid tumors.

The cyclopamine derivative glasdegib (PF-0449913 or DAURISMO), was the first SMO inhibitor approved by the FDA for the treatment of AML (Table 2). Glasdegib is approved for the treatment of newly diagnosed patients with AML who are ≥75 years or unsuitable for treatment with intensive induction chemotherapy (43). Glasdegib plus LDAC has been granted initial authorization by the European Medicines Agency (EMA) to treat newly diagnosed de novo or secondary AML in adult patients who are not candidates for standard induction chemotherapy (42). In a phase 1 study in patients with various leukemias (AML, MDS, CML, chronic myelomonocytic leukemia, and myelofibrosis), the MTD of glasdegib monotherapy was established as 400 mg once daily (36). Glasdegib exhibited dose-proportional pharmacokinetics, with a mean $t_{1/2}$ of 24 hours for the 400 mg once daily dose. The most common treatment-related AEs included alopecia, decreased appetite, and dysgeusia (56). A phase 1 trial of glasdegib monotherapy in Japanese patients with hematologic malignancies reported no dose-limiting toxicities up to 100 mg once daily, and no new safety signals were identified (57). A phase Ib study evaluated glasdegib 100 and 200 mg once daily in combination with low-dose cytarabine (LDAC), decitabine, or cytarabine plus daunorubicin, in patients with AML or high-risk MDS (58). Complete remission (CR)/CR with incomplete blood count recovery was achieved in 31.0% of patients. AEs of alopecia, dysgeusia, and muscle spasms were generally mild.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Approval status</th>
<th>Clinical trials in hemato-oncology</th>
<th>Most common TEAEs</th>
<th>Pharmacokinetic profile</th>
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</thead>
<tbody>
<tr>
<td>BMS-833923/XL139 (40, 41)</td>
<td>Not approved</td>
<td>Phase II trial in CML was terminated: NCT01357655 Phase I/II trial in CML: NCT01218477 Phase I trial in MM: NCT008884546</td>
<td>Reported in ≥30% of patients: dysgeusia, alopecia, anorexia, nausea, weight loss, muscle spasms, fatigue</td>
<td>$t_{1/2}$: &gt;7 days Dose proportionality over 30 to 240 mg</td>
</tr>
<tr>
<td>Glasdegib/DAURISMO/PF-04449913 (42, 43)</td>
<td>FDA: approved in combination with LDAC for newly diagnosed AML in patients ≥75 years or with comorbidities precluding intensive induction chemotherapy. EMA: initial authorization</td>
<td>Phase III trials: AML (NCT03416179, NCT04168502, NCT04093505) Phase II trials: AML, CMML, and MDS (NCT02367456), AML (NCT03390296), AML (NCT02226418), NCT04051996, NCT01841333), AML with MDS (NCT04231851), AML or MDS (NCT01546038), MDS (NCT01842646) Phase I trials in select hematological malignancies: NCT02038777, NCT00953758 Real-world AML cohort: NCT04230564</td>
<td>Reported in ≥20% of patients: anemia, fatigue, hemorrhage, febrile neutropenia, musculoskeletal pain, nausea, edema, thrombocytopenia, dyspnea, decreased appetite, dysgeusia, mucositis, constipation, rash, diarrhea</td>
<td>$t_{1/2}$: 17.4 hours Dose-proportional increases in AUC and $C_{\text{max}}$ over 5 to 600 mg Mean absolute bioavailability: 77% High-fat meal decreased exposure High plasma protein binding: &gt;90% Metabolism: mainly CYP3A4, with minor contribution from CYP2C8 and UGT1A9 Elimination: 49% eliminated in urine and 42% eliminated in feces</td>
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<tr>
<td>IPI-926/saridegib/patidegib (44–46)</td>
<td>EMA and FDA: granted orphan drug designation for nevoid BCC</td>
<td>None ongoing</td>
<td>In ≥30% of patients: fatigue, nausea, muscle spasms, vomiting, ALT and AST elevations, low hemoglobin</td>
<td>$t_{1/2}$: 20 to 40 hours $T_{\text{max}}$: 2 to 8 hours Dose-proportional increases in AUC and $C_{\text{max}}$ over 20 to 210 mg</td>
</tr>
<tr>
<td>Itraconazole/Sporanox (47, 48)</td>
<td>FDA: approved as an antifungal treatment. EMA: granted orphan drug designation for nevoid BCC syndrome</td>
<td>Phase IV trial in hematological malignancies was terminated: NCT02895529 Phase I/II AML or MDS trial: NCT00045942 Phase I trials: advanced solid tumors or relapsed/refractory lymphoma (NCT02259010), relapsed MM (NCT02401295)</td>
<td>In ≥5% of patients: nausea, rash, vomiting. Rare cases of liver failure and heart failure have occurred.</td>
<td>$t_{1/2}$: approximately 37 hours (multiple doses taken with food) High-fat meal increased bioavailability High plasma protein binding: &gt;99% Metabolism: mainly CYP3A4 Elimination: 54% eliminated in feces and 35% eliminated in urine</td>
</tr>
<tr>
<td>Sonidegib/LDE-225/erismodegib/ODOMZO (49, 50)</td>
<td>Approved by the FDA and the EMA for locally advanced BCC not targetable by, or recurring after surgery or radiotherapy</td>
<td>Phase II trials: acute leukemia (NCT01826214), MM (NCT02086552); relapsed/refractory MM (NCT02254551, terminated) Phase I trials: CML (NCT01456676), myeloid malignancies (NCT02129101)</td>
<td>In ≥10% of patients: muscle spasms, alopecia, pruritus, dysgeusia, fatigue, nausea, vomiting, diarrhea, decreased weight, decreased appetite, pain, abdominal pain, musculoskeletal pain, headache</td>
<td>$t_{1/2}$: approximately 28 days Single-dose $T_{\text{max}}$: 2 to 4 hours Dose-proportional increases in AUC and $C_{\text{max}}$ over 100 to 400 mg High-fat meal increased exposure High plasma protein binding: &gt;97% Metabolism: CYP3A Elimination: mainly hepatic, with &gt;70% eliminated in feces and &lt;30% in urine</td>
</tr>
</tbody>
</table>
### Table 1. SMO inhibitors in clinical development for the treatment of hematologic malignancies (Continued)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Approval status</th>
<th>Clinical trials in hematolo-oncology</th>
<th>Most common TEAEs</th>
<th>Pharmacokinetic profile</th>
</tr>
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<tbody>
<tr>
<td>Taladegib/LY2940680</td>
<td>Not approved</td>
<td>None ongoing</td>
<td>≥40% of patients: dysgeusia, fatigue, nausea, muscle spasms</td>
<td>t_{1/2}: approximately 19 hours, T_max: approximately 3 hours, Dose proportionality, High plasma protein binding: &gt;94% Metabolism: mainly CYP3A4</td>
</tr>
<tr>
<td>Vismodegib/GDC-0449/Erivedge</td>
<td>Approved by the FDA and the EMA for metastatic BCC and for recurrent, locally advanced BCC not targetable by surgery or radiotherapy</td>
<td>Phase II trial: AML (NCT02073838), high-risk or relapsed MM (NCT01330173) Two phase II trials in malignant solid tumors were terminated: NCT01880437, NCT01944943 Phase I trial in myelofibrosis: NCT02593760</td>
<td>≥10% of patients: muscle spasms, alopecia, dysgeusia, weight loss, fatigue, nausea, diarrhea, decreased appetite, arthralgia, vomiting, ageusia, constipation</td>
<td>t_{1/2}: 4 days with continuous daily dosing, or 12 days after a single dose Absorption is saturable Single-dose mean bioavailability: 31.8% High plasma protein binding: &gt;97% Metabolism: oxidation, glucuronidation, pyridine ring cleavage, as well as CYP2C9 and CYP3A4/5 Elimination: mainly hepatic, with 82% eliminated in feces and 4.4% in urine</td>
</tr>
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</table>

Abbreviations: ALT, alanine transaminase; AML, acute myeloid leukemia; AST, aspartate transaminase; AUC, area under the curve; BCC, basal cell carcinoma; C_{max}, maximum plasma concentration; CYP, cytochrome P450; MDS, myelodysplastic syndrome; MM, multiple myeloma; t_{1/2}, half-life; TEAE, treatment-emergent adverse event; T_{max}, time to C_{max}; UTG1A9, UDP-glucuronosyltransferase 1-9.

### Table 2. Clinical efficacy and safety of SMO inhibitors in the treatment of AML: results from published trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patient profile</th>
<th>Treatment arms</th>
<th>Efficacy end points</th>
<th>Safety end points and common AEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasdegib (DAURISMO, PF-04449913)</td>
<td>Open-label, dose-finding, phase I (56)</td>
<td>Untreated AML (n = 28), MDS (n = 6), CML (n = 5), CMML (n = 1), or MF (n = 7)</td>
<td>Glasdegib 5 to 600 mg QD  Clinical activity in 23/47 (49%) patients</td>
<td>DLTs (n = 2) Most common treatment-related AEs: dysgeusia (28%), decreased appetite (19%), alopecia (15%)</td>
</tr>
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<td></td>
<td>Open-label, phase Ib (59)</td>
<td>Untreated AML (n = 30) or higher-risk MDS (n = 30)</td>
<td>Glasdegib 100 mg QD plus azacitidine 75 mg/m²  AML: 20% CR; 70% 6-month SP  MDS: 17% CR; 78.9% 6-month SP</td>
<td>Most common all-cause AEs in AML: nausea (60%), decreased appetite (53%), constipation (50%); in MDS: nausea (67%), constipation (47%), diarrhea (40%), muscle spasms (40%), neutrophil count decreased (40%) Most common serious TEAEs in AML: febrile neutropenia (20%), pyrexia (13%); in MDS: febrile neutropenia (17%), sepsis (17%)</td>
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</table>

Treatment-related nonhematologic AEs were mostly grade 1 to 2, and glasdegib was generally well tolerated in all three combinations studied (58). Another phase Ib study evaluated glasdegib 100 mg once daily plus azacitidine in patients with AML, higher-risk MDS, or chronic myelomonocytic leukemia (59). Of patients with AML, the 6-month survival was 70%, and 20% achieved CR. Corresponding rates in patients with MDS were 78.9% and 17%. The CRs observed were favorable compared with the 15%-17% CR rate reported with azacitidine alone. The most common AEs were nausea and constipation (59). These studies were followed by expansion studies of glasdegib plus intensive or low-dose chemotherapy. In a phase II study in patients with untreated AML or...
### Table 2. Clinical efficacy and safety of SMO inhibitors in the treatment of AML: results from published trials (Continued)

<table>
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<tr>
<th>Trial</th>
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<tbody>
<tr>
<td>Open-label, multicenter, dose-finding, phase Ib (58)</td>
<td>Untreated AML or high-risk MDS (n = 52)</td>
<td>Glasdegib 100 or 200 mg QD plus LDAC (arm A; n = 23) or decitabine (arm B; n = 7) or cytarabine + daunorubicin (arm C; n = 22)</td>
<td>CR: arm A: 8.7%; arm B: 28.6%; arm C: 54.5%; Median OS: arm A: 4.4 months; arm B: 11.5 months; arm C: 34.7 months</td>
<td>DLT (grade 4 neuropathy), n = 1; Most frequent Grade 3 to 4 AEs were hematologic AEs (all arms); Most common all-causality AEs in arm A: constipation (43.5%), diarrhea (43.5%), nausea (43.5%); in arm B: nausea (71.4%), back pain (57.1%), neutropenia (57.1%); in arm C: nausea (77.3%), diarrhea (72.7%), constipation (59.1%); Treatment-related deaths in arm A (n = 1 each): acute respiratory distress syndrome; acute myocardial infarction with congestive heart failure; multiple organ dysfunction syndrome</td>
</tr>
<tr>
<td>Open-label, multi-center phase I (57)</td>
<td>AML (n = 7), MDS (n = 4), CMML (n = 1), MF (n = 1) refractory, resistant, or intolerant to prior therapies</td>
<td>Glasdegib 25, 50, or 100 mg QD</td>
<td>CR: one patient with AML and one with MDS; SD: four patients with AML and two with MDS</td>
<td>No DLTs; Most common all-causality AEs: dysgeusia (69.2%), constipation (46.2%), decreased appetite (46.2%), muscle spasms (46.2%), pyrexia (46.2%); Most frequent Grade 3 to 4 AEs were hematologic AEs (all arms); Most common treatment-related AEs: dysgeusia (69.2%), muscle spasms (38.5%), alopecia (30.8%), decreased appetite (30.8%); Most common all-causality AEs: diarrhea (71.0%), febrile neutropenia (63.8%), nausea (58.0%)</td>
</tr>
<tr>
<td>Ongoing, open-label, multi-center phase II (60)</td>
<td>Untreated AML or high-risk MDS (n = 69)</td>
<td>Glasdegib 100 mg QD plus cytarabine/daunorubicin</td>
<td>Investigator-reported CR: ≥55 years: 40.0%; &lt;55 years: 88.9%; Overall median OS: 14.9 months; 66.6% 12-month SP</td>
<td>Most common all-causality AEs: diarrhea (71.0%), febrile neutropenia (63.8%), nausea (58.0%); Most common all-causality grade ≥3 AEs: febrile neutropenia (63.8%), anemia (37.7%), thrombocytopenia (31.8%); Deaths: 41/69 (59.4%) patients. One case of sepsis was considered treatment-related by the investigator</td>
</tr>
<tr>
<td>Ongoing, open-label, multi-center phase II (61)</td>
<td>Untreated AML or high-risk MDS (n = 132)</td>
<td>Glasdegib 100 mg QD plus LDAC (n = 88) or LDAC alone (n = 44)</td>
<td>For glasdegib + LDAC versus LDAC alone, CR was 17% vs. 2.3%; median OS was 8.8 vs. 4.9 months; 6-month SP was 59.8% vs. 38.2%; 12-month SP was 39.5% vs. 9.5%</td>
<td>Most common all-causality AEs on glasdegib + LDAC: anemia (45.2%), febrile neutropenia (35.7%), nausea (35.7%); on LDAC alone: anemia (41.5%), thrombocytopenia (26.8%), dyspnea (26.8%); Most common all-causality grade ≥3 AEs on glasdegib + LDAC: anemia (41.7%), febrile neutropenia (35.7%), thrombocytopenia (31.0%); on LDAC alone: anemia (36.6%), thrombocytopenia (24.4%), and febrile neutropenia (24.4%)</td>
</tr>
<tr>
<td>Vismodegib (GDC-0449, Erivedge)</td>
<td>Relapsed/refractory AML (n = 47)</td>
<td>Vismodegib 150 mg QD</td>
<td>ORR: 6.1%; Median OS: 3.4 months</td>
<td>Most common all-causality AEs: pyrexia (39.5%), nausea (39.5%), dysgeusia (31.6%); Most common all-causality grade ≥3 AEs: febrile neutropenia (21.1%), anemia (15.8%), thrombocytopenia (15.8%)</td>
</tr>
</tbody>
</table>

Abbreviations: AE, adverse event; CAD, coronary artery disease; CMML, chronic myelomonocytic leukemia; DLT, dose-limiting toxicity; IWG, International Working Group; mCR, complete response as determined by magnetic resonance imaging; MF, myelofibrosis; ORR, objective response rate; SAE, serious adverse event; SD, stable disease; SP, survival probability; TEAE, treatment-emergent adverse event.
Hedgehog Pathway Inhibitors for Acute Myeloid Leukemia

Drug designation by the FDA and EMA for the treatment of nevoid BCC syndrome (44, 45). A number of clinical trials are now underway investigating IPI-926 in a variety of tumor types, for example, BCC, metastatic pancreatic cancer, and chondrosarcoma. Results of several phase I studies of BMS-833923 (XL139) have been reported (40, 41, 62). Taladegib (LY2940680) has also been investigated in phase I trials (51, 52). Clinical trials for BMS-833923 and taladegib are ongoing in CML and a range of solid tumors. In the future, and given the involvement of SMO in AML, it is possible that these agents may be investigated as a treatment for AML.

On the basis of the clinical evidence to date, SMO inhibitors are showing promise for the treatment of a variety of cancer types. Their role in BCC is established, and glasdegib plus low-dose chemotherapy has demonstrated clinical efficacy for the treatment of AML. Although several of the ongoing clinical trials are investigating SMO inhibitors as monotherapy, the current preclinical and clinical data for hematologic malignancies indicate the potential for a synergistic effect when a SMO inhibitor is administered in combination with standard chemotherapeutic and other investigational agents. These observations with chemotherapeutic agents support the mechanistic action of SMO inhibitors as agents that reduce LSC dormancy and promote LSC differentiation, thus allowing the newly dividing LSCs to be targeted by standard chemotherapy.

The emergence of tumors that are resistant to SMO inhibitor monotherapy lends further support to the continued need for investigation into the use of SMO inhibitors as part of a combination therapy regime (5, 10).

**MECHANISM OF RESISTANCE TO Hh PATHWAY INHIBITORS**

Use of inhibitors of the Hh pathway has led to significant improvements in the treatment of several malignancies; however, reports indicate that certain tumor types are primary-resistant to or have acquired resistance to SMO inhibitors.

**Nonapproved SMO Inhibitors**

Itraconazole (Sporanox) is a commonly used antifungal agent; however, emerging evidence has highlighted that it could be used as a noncyclopamine-derived SMO inhibitor. Indeed, itraconazole was recently granted orphan drug designation by the EMA for the treatment of nevoid BCC syndrome (44, 45). A number of clinical trials are now underway investigating IPI-926 in a variety of tumor types, for example, BCC, metastatic pancreatic cancer, and chondrosarcoma. Results of several phase I studies of BMS-833923 (XL139) have been reported (40, 41, 62). Taladegib (LY2940680) has also been investigated in phase I trials (51, 52). Clinical trials for BMS-833923 and taladegib are ongoing in CML and a range of solid tumors. In the future, and given the involvement of SMO in AML, it is possible that these agents may be investigated as a treatment for AML.

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inhibitors (5, 10, 63–67). Resistance mechanisms to other Hh pathway inhibitors, for example, GLI inhibitors, are currently unknown as these agents are still in early preclinical development. The main mechanisms of resistance to SMO inhibitors include: impaired inhibitor–SMO interactions due to mutations in the ligand-binding pocket of SMO; activation of Hh pathway components downstream of SMO; GLI1 activation independent of SMO through upregulation of noncanonical signaling pathways (Fig. 2; refs. 5, 10, 63–67).

The structure of SMO consists of two ligand-binding cavities, one in the transmembrane domain region and another in the extracellular cysteine-rich domain, and these serve as the interaction site for cyclopamine-derived SMO inhibitors (10, 64). SMO inhibitors interact with different residues within the ligand-binding pocket, and to varying degrees, for example, vismodegib binds to D473 and E518 residues, whereas taladegib binds weakly to D473 residues and instead interacts with Q477, W480, E481, and F484 residues (10, 64). Several SMO mutations have been discovered that lead to drug resistance (Table 3). SMO residue mutations include those located close to the ligand-binding sites that directly impair SMO–SMO inhibitor interactions, and those located outside of the drug-binding site that may have allosteric effects and/or promote constitutive activation of SMO that is desensitized to SMO inhibitors (64, 67, 68).

Another mechanism of resistance to SMO inhibitors is the activation of Hh components downstream to SMO. Depletion of SUFU, a negative regulator of the Hh pathway, led to sonidegib resistance in medulloblastoma cell lines (66). Editing of GLI1 transcripts by adenosine deaminase acting on RNA1 (ADAR1) rendered GLI1 impervious to SUFU inhibition, resulting in multiple myeloma propagation (69). Pediatric patients with medulloblastoma harboring inactivating SUFU mutations reported primary resistance to sonidegib (70). Upregulation of GLI2 expression is implicated in resistance to vismodegib and sonidegib in medulloblastoma (71, 72). Sensitivity to sonidegib was partially restored in these cell lines via suppression of GLI2 (72). The Hh pathway target gene \( \text{CCND1} \), which promotes cell-cycle progression, was also upregulated in a vismodegib-resistant medulloblastoma model (71).

Increasing evidence indicates that GLI1 can be activated independent of SMO in a number of malignancies (5, 10, 64). Instead of ligand-dependent canonical activation of the Hh pathway, the activation and modulation of GLI1 is reported to occur via other signal transduction pathways implicated in oncogenesis, such as the MAPK/ERK, mTOR, and TGF\( \beta \) signaling pathways (5, 10, 64). Proposed mechanisms of SMO-independent GLI1 activation include decreased degradation, the involvement of atypical protein kinases, and the regulation of transcriptional activity and nuclear localization (5, 10, 64). In medulloblastoma, gene expression analysis identified upregulation of PI3K signaling in resistant tumors. Sonidegib coadministration with a PI3K inhibitor or a PI3K/
mTOR inhibitor decreased the development of resistant tumors (72). In BCC, upregulation of MAPK signaling and atypical protein kinase C have been observed in vismodegib-resistant tumors (66, 73).

Given the complexities of Hh pathway signaling and the development of resistant tumors, predictive biomarkers may potentially be useful to identify patients most likely to respond to therapy with SMO inhibitors. Evaluation of SMO mutational status in patients could be performed to evaluate sensitivity to SMO inhibitors during a therapy plan (5, 64, 67, 68). GLI1 can be a negative prognostic marker for survival; therefore, assessment of GLI1 activation, either by canonical or noncanonical processes, could potentially help to identify patients who may become resistant to SMO-inhibitor treatment (5, 10, 24, 26, 64). GLI has also been assessed as a pharmacodynamic marker; however, it may not be optimal for evaluating tumor response during treatment (27, 74, 75). Challenges in establishing pharmacodynamic markers of the Hh pathway arise due to tumors being ligand-dependent or ligand-independent, and the impact of other signal transduction pathways and factors on Hh signaling (27, 74, 75). Finally, in a study of bone marrow samples derived from patients with AML, it was reported that the transcription factor NANOG, which interacts with GLI1, decreased during glasdegib treatment, and thus could act as a responsive biomarker (29). Further investigation of predictive biomarkers to optimize selection of patients for treatment with SMO inhibitors, as well as overcoming the challenges of pharmacodynamic markers during treatment with SMO inhibitors, is warranted.

**SELECTION OF Hh PATHWAY INHIBITORS FOR THE TREATMENT OF AML**

The SMO inhibitor glasdegib is currently the only Hh pathway inhibitor approved for the treatment of AML. Clinical trials are ongoing for glasdegib in AML and other hematologic malignancies, and for other SMO inhibitors (for example, sonidegib and vismodegib) in hematologic malignancies and solid tumors. Inhibitors of other Hh pathway components, such as GLI inhibitors, are still in early preclinical development. The results from these trials and preclinical studies will provide additional information to help establish the role of Hh pathway inhibition and the optimum therapy combinations for the treatment of AML, and will help identify patient subsets who would benefit the most from treatment with Hh pathway inhibitors.

Venetoclax in combination with hypomethylating agents or LDAC has also been approved for patients with newly diagnosed AML who are ≥75 years or who have comorbidities that preclude use of intensive induction chemotherapy (76). Interim results from a phase III study of venetoclax plus LDAC versus LDAC alone in newly diagnosed patients with AML demonstrated clinical activity of the combination regimen, although the primary endpoint of OS was not met (77). Because of the lack of direct comparisons, as well as the differing study designs and safety profiles, between glasdegib and venetoclax, limited comparisons can be made between these agents. Both drugs have shown promising efficacy in AML, therefore, further investigation is warranted to establish the role of these agents in the treatment of AML, as well as the patient subpopulations who would potentially derive the most benefit from either glasdegib or venetoclax, or as a combination treatment.

**SUMMARY**

Hh signal transduction and Hh pathway components, such as SMO and GLI1, play a critical role in the dormancy and maintenance of LSCs, and thus AML pathogenesis and progression. In addition, Hh signaling is implicated in the development of resistant and refractory disease. Pharmacologic inhibition of SMO, including in some instances as part of a combination regimen, has demonstrated efficacy in preclinical and clinical studies across a variety of tumor types. Inhibitors of other Hh pathway components are still in early preclinical development. Glasdegib is currently the only approved SMO inhibitor for the treatment of AML and targets LSCs; however, a number of clinical trials for other SMO inhibitors are ongoing in both solid tumors and hematologic malignancies. Treatment selection is dependent on a number of clinical factors, for example, mutational status, patient comorbidities, and drug resistance; however, the variety of targeted therapy options available allows physicians and patients to select the optimum therapy for a given patient.

**Disclosure of Potential Conflicts of Interest**

C. Jamieson reports having equity ownership in Impact Biomedicines and Wintherix LLC; receives research funding from Celgene and Johnson & Johnson; and patents and royalties from Forty Seven, Inc. G. Martinelli is a consultant for Amgen, Ariad Pharmaceuticals, Incyte, Pfizer, Roche, Celgene, Janssen, Jazz, Daiichi Sankyo, and AbbVie; speakers bureau honoraria from Pfizer, Celgene, Novartis; and reports receiving trial grants from Pfizer, and AbbVie. C. Papayannidis reports receiving honoraria from Pfizer, Amgen, and Novartis. J. E. Cortes is a consultant for Astellas Pharma, Bristol-Myers Squibb, Daiichi Sankyo, FORMA Therapeutics, Novartis, Pfizer, and Takeda; reports receiving research funding from Amphivena Therapeutics, Astellas Pharma, Bristol-Myers Squibb, Daiichi Sankyo, FORMA Therapeutics, ImmunoGen, Merus, Novartis, Pfizer, Sun Pharma, and Takeda. No other potential conflicts of interest were disclosed.

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