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Hedgehog signaling inhibitors in solid and hematological cancers

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ABSTRACT

Background: The hedgehog signaling pathway is normally tightly regulated. Mutations in hedgehog pathway components may lead to abnormal activation. Aberrantly activated hedgehog signaling plays a major role in the development of solid and hematological cancer. In recent years, inhibitors have been developed that attenuate hedgehog signaling; 2 have been approved for use in basal cell carcinoma (BCC), while others are under development or in clinical trials. The aim of this review is to provide an overview of known hedgehog inhibitors (HHIs) and their potential for the treatment of hematological cancers and solid tumors beyond BCC.

Design: Published literature was searched to identify articles relating to HHIs in noncutaneous cancer. Both preclinical and clinical research articles were included. In addition, relevant clinical trial results were identified from www.clinicaltrials.gov. Information on the pharmacology of HHIs is also included.

Results: HHIs show activity in a variety of solid and hematological cancers. In preclinical studies, HHIs demonstrated efficacy in pancreatic cancer, rhabdomyosarcoma, breast cancer, and acute myeloid leukemia (AML). In clinical studies, HHIs showed activity in medulloblastoma, as well as prostate, pancreatic, and hematological cancers. Current clinical trials testing the efficacy of HHIs are underway for prostate, pancreatic, and breast cancers, as well as multiple myeloma and AML.

Conclusions: As clinical trial results become available, it will be possible to discern which additional tumor types are suited to HHI mono- or combination therapy with other anticancer agents. The latter strategy may be useful for delaying or overcoming drug resistance.

Introduction

Hedgehog (HH) signaling regulates development, cell proliferation, and tissue repair [1–3]. In mammals, HH signaling is activated by 3 ligands: Sonic hedgehog (SHH), Indian hedgehog (IHH), or Desert hedgehog; SHH is the most widely expressed in adult tissues [3]. The primary receptor for these ligands is Patched-1 (Ptch1). In the absence of ligand, Ptch1 inhibits Smoothened (Smo) (Fig. 1A); and upon ligand binding, Ptch1 inhibition is released, activating Smo, leading to activation of the glioma-associated oncogene (Gli) transcription factors Gli1, Gli2, and Gli3 (Fig. 1B) [2]. Gli1 activates target genes related to tumorigenesis (eg, *cyclin-D1*, *Myc*, *Bcl-2*), and angiogenesis factor genes [3].

Normal HH signaling is important for morphogenesis, from embryonic development to adult tissue homeostasis [4]; and plays a specific role in brain development, especially that of the cerebellum [5,6]. In postnatal bone remodeling [7], IHH is expressed at bone growth plates by chondrocytes and osteoblasts, and HH signaling is active [8]. After embryogenesis, HH signaling is quiescent, although SHH-mediated signaling is active in hair follicles, reprograms energy metabolism in adipocytes, and plays a role in multiple organ homeostasis [4,9–12]. This review discusses the pharmacology of HHIs and the preclinical and clinical evidence for HHI therapies for hematological cancer and solid tumors beyond advanced basal cell carcinoma (BCC).

Dysregulated hedgehog signaling and the development of cancer

The HH signaling pathway is normally tightly regulated; however, dysregulation due to mutations in *Smo* or *Ptch1* plays a major role in

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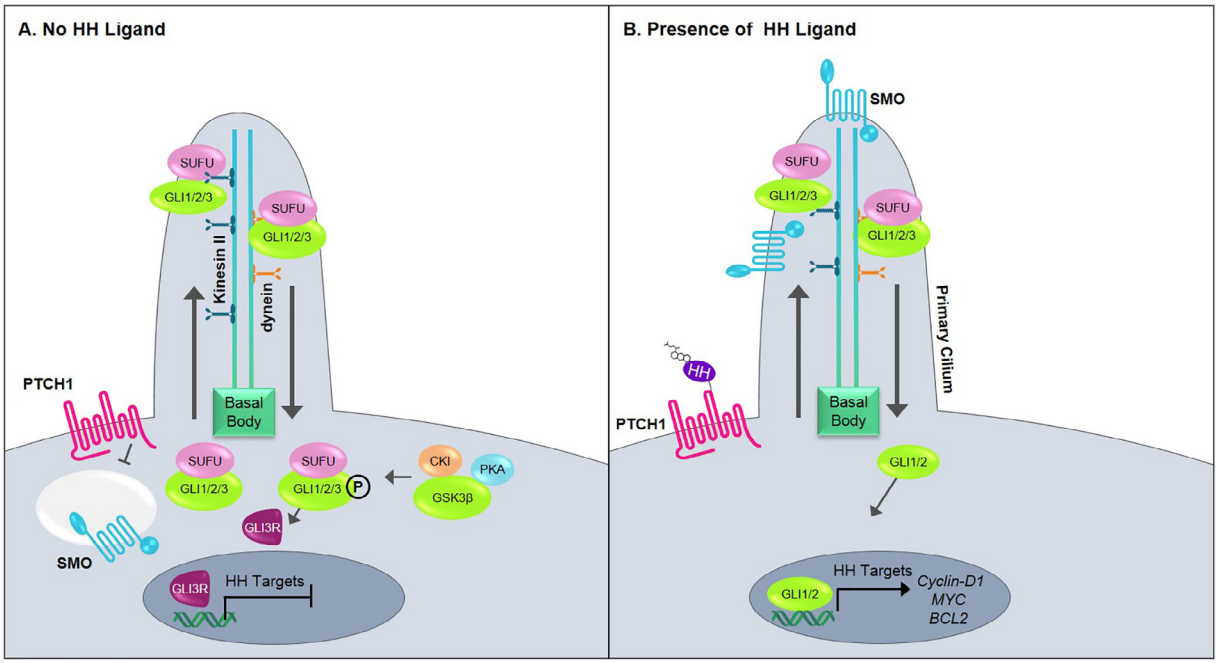

Fig. 1. Model of hedgehog signaling. A. In the absence of HH ligand, PTCH1 inhibits the surface localization of SMO, and GLI1 proteins are phosphorylated by protein kinases, leading to the production of an NH_2-terminal truncated form (GLI3) that represses HH target-gene transcription. SUFU also regulates the pathway by binding to GLI in both the cytoplasm and nucleus, thereby preventing HH target-gene transcription. B. In the presence of HH ligand, PTCH1 inactivation allows SMO relocation to the tip of the cilia, initiating downstream signaling events and GLI protein activation and increased HH target-gene. Adapted from [99], with permission. BCL2, B-cell chronic lymphocytic leukemia/lymphoma 2; CKI, casein kinase I; GLI, glioma-associated oncogene; GSK3β, glycogen synthase kinase 3 beta; HH, hedgehog; MYC, v-myc avian myelocytomatosis viral oncogene homolog; PKA, protein kiase A; PTCH1, Patched-1; SMO, Smoothened; SUFU, Suppressor of fused.

oncogenesis [3]. In normal tissue, SHH activation of the HH pathway increases DNA synthesis by stem cells [13]. In human cancer, the role of HH signaling was first described in patients with basal cell carcinoma nevus syndrome (BCCNS) and sporadic BCC [14]. Evidence shows that HH ligand binding to Ptch1 controls the cell cycle by promoting transition from Gap 2 to mitosis via binding to a complex of cyclin B1 and cyclin-dependent kinase 1. In BCCNS, Ptch1 acts like a tumor suppressor [1].

Aberrant activation of HH signaling underlies the pathogenesis of a variety of cancers in addition to BCC, including medulloblastoma and breast, lung, pancreatic, prostate, and hematological malignancies [15–19]. Unlike BCC and medulloblastoma, these solid tumors demonstrate ligand-dependent HH pathway activation [20] and a variety of mechanisms of aberrant HH signaling have been implicated in the development of these different cancer types [4] (Fig. 2). Ligand-independent signaling (Fig. 2A) occurs in BCC and medulloblastoma [15,16], and results from loss-of-function mutations in *Ptch* or Suppressor of fused (*Sufu*), or gain-of-function mutations in *Smo* [4,21–23]. Autocrine ligand-dependent activation (eg, in breast, lung, pancreatic, and prostate cancer), occurs when the tumor cell produces HH ligand, leading to activation of HH signaling within the same tumor cell (Fig. 2B) [4,15]. Paracrine ligand-dependent activation (eg, in colon, pancreas, and prostate) occurs when the tumor cell produces HH ligand, which then activates HH signaling (eg, vascular endothelial growth factor, interleukin 6, insulin-like growth factor, and wingless/integrated) in a nearby stromal cell, leading to stimulation of tumor growth (Fig. 2C) [4,15]. Inverse paracrine (reverse paracrine), ligand-dependent activation (eg, in lymphoma and multiple myeloma) occurs when HH ligand produced by the stroma cell activates tumor HH signaling (Fig. 2D) [4].

In prostate cancer, *SHH*, *PTCH1*, *GLI1*, *GLI2*, and *GLI3* expression increased (range, 1.5- to 300-fold) in microdissected tumor samples compared with normal tissue [24]. Intratumor expression was variable, likely relating to the heterogeneous nature of prostate cancer [24]. *GLI1* expression was consistently observed in prostate tumor cell lines and primary tumors, indicating an important role in prostate tumor cell proliferation and tumorigenesis [24]. Additionally, GLI1 gene and protein were also overexpressed in patient-derived human breast cancer tissue compared with normal breast tissue [25]. A positive association was noted between increased GLI1 expression, tumor stage, and lymph node status, correlating with an unfavorable overall survival (OS) [25]. A subsequent meta-analysis confirmed that GLI1 overexpression in breast cancer is associated with reduced disease-free survival, 3- and 5-year survival, and OS [26].

Immunohistochemical evaluation of gastric cancer tumor samples revealed that 57.8% (59/101) were positive for GLI1, and 71.3% (72/101) stained positive for SHH [27]. Overexpression of SHH and GLI1 protein were significantly associated with larger tumor size, tumor aggressiveness, and less differentiation; correlating with a high metastatic potential [27]. Moreover, reduced OS and recurrence-free survival were associated with increased SHH and GLI1 staining [27]; and GLI1 expression was identified as an independent prognostic factor for OS and recurrence-free survival in gastric cancer patients [27].

SHH overexpression has also been reported in 70% of primary pancreatic ductal adenocarcinoma (PDAC) patients, in which paracrine signaling occurs [28]. Immunohistochemical analysis showed that SHH and GLI1 expression were independent prognostic factors in PDAC patients undergoing curative-intent surgery; wherein low expression was associated with improved OS and disease-free survival (DFS) [28]. Logically, therefore, high SHH and GLI1 expression in resected PDAC patients was associated with a poorer prognosis [28].

A meta-analysis determined the prognostic value of GLI1 expression in solid malignancies (ie, breast, digestive tract, liver, pancreatic, and ovarian cancers), which showed an unfavorable impact of increased GLI1 expression on 3-, 5- and 10-year OS and DFS [29]. Furthermore, increased nuclear, but not cytoplasmic, GLI1 expression correlated with a worse prognosis [29].

In BCC, aberrant HH signaling is associated with sporadic mutations

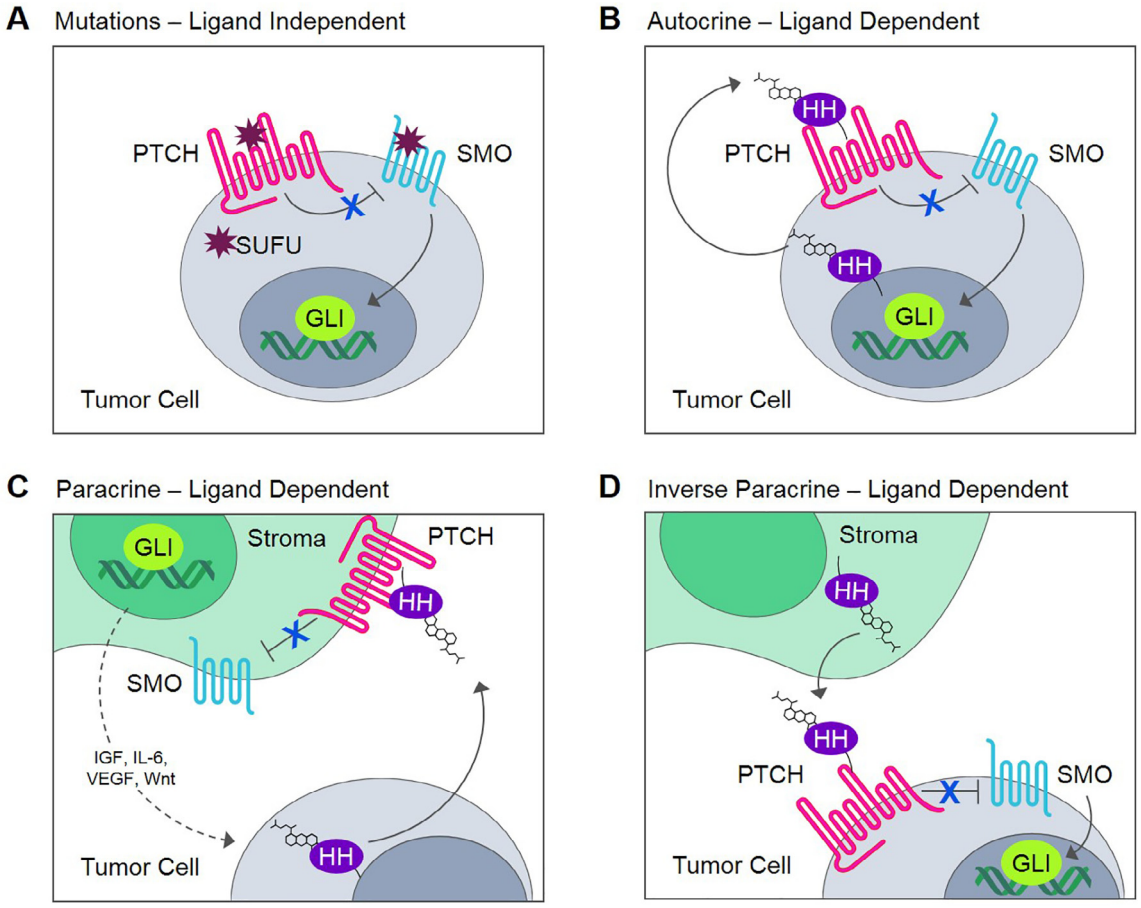

Fig. 2. Underlying mechanisms of Hedgehog pathway activation in cancer. A. Ligand-independent activation occurs due to inactivating mutations in the negative regulators PTCH or SUFU or activating mutations in the positive regulator SMO [21–23]. B. Autocrine ligand-dependent activation occurs when HH ligand, which is produced by the tumor cell, activates HH signaling within the same cell. C. Paracrine ligand-dependent activation occurs when HH ligand is secreted by tumor cells which then turns on HH signaling in the surrounding stroma. The stroma then stimulates tumor growth. D. Inverse paracrine ligand-dependent signaling occurs when stroma-derived HH ligand activates HH signaling within the tumor. GLI, glioma-associated oncogene; HH, hedgehog; IGF, insulin-like growth factor; IL-6, interleukin 6; PTCH, Patched; SMO, Smoothened; SUFU, Suppressor of fused; VEGF, vascular endothelial growth factor; Wnt, Wingless/integrated. Modified from reference [4].

in *PTCH* in > 85% of cases and in *SMO* in approximately 10% of cases [30]. Furthermore, *GLI1* is overexpressed in BCC and was reduced by at least 90% following 9 and 17 weeks of HHI therapy [31]. Additionally, newly diagnosed acute promyelocytic leukemia patients showed a significant increase in the expression of several HH signaling pathway components compared with normal controls, including *GLI1* (3.6-fold change, $P < 0.01$), *GLI2* (2.1-fold change, $P < 0.05$), *PTCH* (7.8-fold change, $P < 0.01$), and *SMO* (2.0-fold change, $P < 0.001$) [32]. Understanding the role aberrant HH signaling plays in various cancer types, therefore, provided a rationale for investigation into the potential of hedgehog inhibitor (HHI) therapy in their treatment.

Preclinical development of hedgehog inhibitors for the treatment of cancer

The discovery of the importance of HH signaling in oncogenesis led to the development of HHIs. Most HHIs inhibit HH signaling by binding to Smo (Fig. 3) [3,33]. Currently, 2 HHIs, vismodegib (Genentech Inc, South San Francisco, CA) and sonidegib (Sun Pharmaceutical Industries, Inc, Cranbury, NJ), are approved by the US Food and Drug Administration (FDA) for use in advanced BCC [34,35]. Itraconazole (Janssen Pharmaceuticals, Inc., Titusville, NJ) [36], an antifungal agent, inhibits HH signaling in cancer and is under investigation for clinical efficacy in several tumor types [37,38]. Patidegib was granted Orphan Drug and Breakthrough Therapy Designation by the FDA as a topical agent for the treatment of BCCNS [39,40]. Other HHIs under study for use in cancer are glasdegib and taladegib [41–44]. A summary of available pharmacokinetic data for HHIs is presented in Table 1. This section provides an overview of the preclinical development of HHIs in various cancers.

Pancreatic cancer

Pancreatic cancer has a poor prognosis and is usually refractory to standard chemotherapy agents [45]. In a mouse model of pancreatic adenocarcinoma, chemoresistance was associated with HH activation, which was partially overcome upon administration with patidegib [45]. Furthermore, mice with islet neoplasms showed 95% reduction in tumor volume with concomitant prolongation of median survival when treated with sonidegib [46]. Studies are ongoing to explore utilization of mouse pancreatic adenocarcinoma models for the development of new HHI analogs of vismodegib with enhanced antitumor activity [47].

Rhabdomyosarcoma

Rhabdomyosarcoma is the most common pediatric soft-tissue sarcoma. Although HH activation is thought to play a role in its development, it is unclear how HH becomes activated in this disease. A series of studies examining the expression of HH ligands in specimens from children with rhabdomyosarcoma and in animal models showed that autocrine ligand-dependent activation of HH may drive the disease, therefore, the HH pathway may be a useful target for therapy [48].

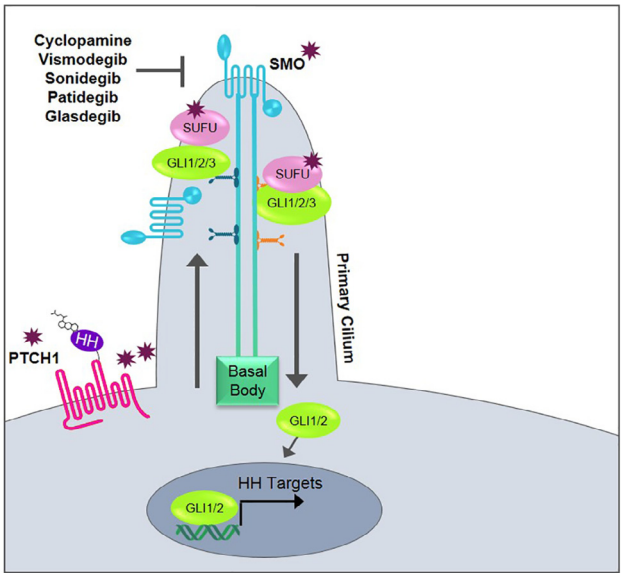

Fig. 3. Model of hedgehog signaling inhibition by SMO inhibitors. HH-mediated inactivation of PTCH1 allows translocation of SMO to the primary cilium, leading to downstream signaling and the activation of GLI1. Mutations in the pathway (*) or overexpression of the HH ligand are highlighted (**) along with possible avenues of treatment using HH inhibitors, as indicated. Adapted from [99], with permission. GLI, glioma-associated oncogene; HH, hedgehog; PTCH1, Patched-1; SMO, Smoothened; SUFU, Suppressor of fused.

Glioblastoma and medulloblastoma

Glioblastoma multiforme is the most common primary brain malignancy. Phosphoinositide-3 kinase (PI3K) is frequently activated in this tumor by loss of the tumor suppressor phosphatase and tensin homolog (PTEN). PTEN-deficient glioblastoma was found to be accompanied by SHH signaling. Furthermore, PI3K and HH pathways synergize to promote tumor growth and development. Combining PI3K inhibitors with HHIs suppressed activation of both pathways, resulting in increased tumor apoptosis [49]. In a mouse model of medulloblastoma, a combination of statins plus vismodegib synergistically inhibited tumor growth in vivo; the authors concluded that cholesterol biosynthesis was required for Smo activity, which is necessary for HH activity in medulloblastoma [50].

Breast cancer

The development of breast cancer may be supported by interactions between cancer-associated fibroblasts and stem cells, the self-renewal of which is mediated, at least in part, by the HH pathway. Targeting this interaction with vismodegib prevented cancer stem cell expansion and significantly decreased tumor volume [51].

Hematological cancers

In hematological cancers, HH activation may play a role in maintaining leukemia stem cells. Treatment with glasdegib reduced the potential of stem cells to initiate leukemia in vivo in mice [52]. Furthermore, in a mouse model of chronic myeloid leukemia (CML), addition of sonidegib to nilotinib enhanced survival and reduced the development of leukemia in secondary transplants [53]. Sonidegib also potentiated the effect of total body radiation and helped overcome resistance to radiation mediated by phosphorylated protein kinase B and nuclear factor kappa B in a model of acute myeloid leukemia (AML) [54]. Whereas cells from patients with AML showed moderate sensitivity to vismodegib or sonidegib, and using either agent in combination with 5-azacytidine showed synergistic efficacy in vitro and inhibited long-term repopulation in an in vivo model [55].

Clinical evidence for the efficacy of hedgehog inhibitors in various cancer types

Medulloblastoma

Medulloblastoma, whether occurring with BCCNS or sporadic BCC, can be associated with mutations in HH signaling components such as *Ptch* [16]. In advanced BCC and relapsed medulloblastoma, sonidegib reduced HH-dependent GLI1 expression and showed efficacy in its first-in-human phase 1 trial [56]. In a trial of adult and pediatric patients with medulloblastoma (and other tumors of the central nervous system [CNS]; NCT01125800; Table 2), a subset of patients without HH-driven tumors (determined by upregulation of GLI1, SHROOM2, sphingosine kinase-1, and PDLIM3, and the downregulation of orthodenticle homeobox 2) failed to respond to sonidegib. In total, 50% (5/10) of patients with HH-driven tumors had complete or partial responses to sonidegib; however, the prepubertal patients showed signs of premature growth plate closure [57]. In another trial of 31 patients with recurrent medulloblastoma, vismodegib was found to have activity only if the medulloblastoma was HH-driven (NCT00939484; Table 2) [58]. Taken together, these studies characterize which patients may benefit from HHIs by identifying those whose tumors are driven by aberrant HH signaling. Differential expression of *GLI1, SPHK1, SHROOM2, PDLIM3, OTX2* predicted response to HHIs in patients with medulloblastoma [59]. A cohort of HH-driven medulloblastomas were sequenced across all age groups revealing that children aged 3 years and older had many downstream *MYCN* and *GLI2* amplifications and frequent *TP53* mutations; which were rare in infants and adults [60]. Using in vivo xenograft models, tumors with mutations in *SUFU* or *MYCN* amplification were often unresponsive to HHIs [60] as expected, because these mutations signal downstream of the HH pathway.

Table 1
Pharmacokinetic Parameters for Hedgehog Inhibitors.

Drug Name	*f*, %	V_D, L	% Bound	$t_{1/2}$	Elimination	Reference(s)
Vismodegib	31.8	16.4–26.6	> 99	12 d (1 dose); 4 d (multiple doses)	feces	[100–102]
Sonidegib	69–102	2500	> 99	> 14 d	urine, feces	[80,103]
Patidegib	50–100	9.5–30	NA	20–40 h*	NA	[104,105]
Itraconazole	55	> 700	99.8	16–28 h (1 dose); 34–42 h (multiple doses)	urine, feces	[36]
Glasdegib	NA	165–225	NA	range 18.6–20.7 h[a]	urine, feces	[41,106]
Taladegib	94.4	235	NA	19 h	NA	[44]
Arsenic Trioxide	NA	562	NA	10–14 h	urine	[107]

% Bound, percentage bound to plasma proteins; *f*, %, (oral) bioavailability; NA, not available; $t_{1/2}$, time to reach half the original plasma concentration; V_D, L, volume of distribution, liters.
[a] Depending upon dose administered.

Table 2
Phase 2 and 3 Trials for HHIs in Oncology, Excluding Basal Cell Carcinoma.[a,b]

Name	Trial	N	Primary Endpoint(s)	Status
Vismodegib	NCT01267955. Vismodegib in Treating Patients with Advanced Chondrosarcomas	45	Clinical benefit	Active, not recruiting
	NCT00887159. A Randomized Phase II Study of Cisplatin and Etoposide in Combination with either HH Inhibitor GDC-0449 or IGF-1R MoAb IMC-A12 for Patients with Extensive Stage[c]	168	PFS	Completed
	NCT03052478. Study of Vismodegib in Advanced Gastric Adenocarcinoma Patients with SMO Overexpression	28	Objective response rate	Recruiting
	NCT02694224. Addition of Vismodegib to Neoadjuvant Chemotherapy in Triple Negative Breast Cancer Patients	40	Number with treatment-related adverse events	Recruiting
	NCT02523014. Vismodegib and FAK Inhibitor GSK2256098 in Treating Patients with Progressive Meningiomas	69	PFS; response rate (complete + partial response)	Suspended
	NCT02073838. Ribavirin and HH Inhibitor with or without Decitabine in AML	40	Overall response rate	Recruiting
	NCT00739661. A Study of Vismodegib as Maintenance Therapy in Patients with Ovarian Cancer in a Second or Third Complete Remission	104	PFS	Completed
	NCT01239316. Vismodegib in Treating Younger Patients with Recurrent or Refractory Medulloblastoma (MB)	12	Objective response; pharmacokinetics	Completed
	NCT00939484. Vismodegib in Treating Patients with Recurrent or Refractory MB	31	Objective response	Completed
	NCT00636610. A Study of Vismodegib with Concurrent Chemotherapy and Bevacizumab as First-Line Therapy for Metastatic Colorectal Cancer	199	PFS	Completed
	NCT00959647. A Study of Vismodegib in Patients Treated with Vismodegib in a Previous Genentech-sponsored Phase I or II Cancer Study	19	Percentage who experienced or discontinued due to ≥1 AE	Completed
	NCT01195415. Vismodegib and Gemcitabine Hydrochloride in Treating Patients with Advanced Pancreatic Cancer	25	Median percent at baseline and 3 weeks in CD44+/CD24+/ESA + cells from needle biopsy	Completed
	NCT00980343. GDC-0449 in Treating Patients with Recurrent Glioblastoma Multiforme That Can Be Removed by Surgery	44	6-month PFS	Completed
	NCT00982592. Combination Chemotherapy with or without Vismodegib in Treating Patients with Advanced Stomach Cancer or Gastroesophageal Junction Cancer	124	Median PFS	Completed
	NCT01154452. Vismodegib and Gamma-Secretase/Notch Signaling Pathway Inhibitor RO4929097 in Treating Patients with Advanced or Metastatic Sarcoma	78	MTD; PFS	Completed
	NCT01064622. Gemcitabine Hydrochloride with or without Vismodegib in Treating Patients with Recurrent or Metastatic Pancreatic Cancer	118	PFS	Completed
Sonidegib	NCT01431794. Gemcitabine + Nab-paclitaxel with LDE-225 (Hedgehog Inhibitor) as Neoadjuvant Therapy for Pancreatic Adenocarcinoma	52	Safety; MTD; resection rate	Active, not recruiting
	NCT01787552. A Phase Ib/II Dose-finding Study to Assess the Safety and Efficacy of LDE225 + INC424 in Patients With MF	50	DLT; proportion achieving spleen volume reduction ≥35%	Completed
	NCT02086552. Sonidegib and Lenalidomide After Stem Cell Transplant in Treating Patients with Multiple Myeloma	28	Rate of complete response	Active, not recruiting
	NCT01826214. Study of Efficacy and Safety of LDE225 in Adult Patients with Relapsed/Refractory Acute Leukemia	70	Rate of complete remission; complete remission with incomplete blood count recovery	Completed
	NCT01708174. A Phase II Study of Oral LDE225 in Patients with HH-Pathway Activated Relapsed MB	22	Percentage with overall response rate by independent review	Completed
	NCT01125800. A Phase I Dose Finding and Safety Study of Oral LDE225 in Children and a Phase II Portion to Assess Preliminary Efficacy in Recurrent or Refractory MB	76	Number with DLT; MTD of sonidegib; percentage with objective response rate	Completed
Taladegib	NCT02530437. Taladegib, Paclitaxel, Carboplatin, and Radiation Therapy in Treating Patients with Localized Esophageal or Gastroesophageal Junction Cancer	9	Toxicity of oral taladegib in combination with weekly paclitaxel, carboplatin, and radiation therapy; pathological complete response	Active, not recruiting
Glasdegib	NCT03466450. Glasdegib with Temozolomide Newly Diagnosed Glioblastoma	75	Recommended dose; OS	Recruiting
	NCT03416179. A Study Evaluating Intensive Chemotherapy with or without Glasdegib or Azacitidine with or without Glasdegib in Patients with Previously Untreated Acute Myeloid Leukemia (BRIGHT AML1019)	720	OS	Recruiting
	NCT03415867. Glasdegib in Refractory Patients with Sclerotic Chronic Graft-Versus-Host Disease	24	MTD	Recruiting
	NCT03390296. Pfizer Immunotherapy Combinations for AML Multi-Arm Study 1	138	AEs; composite complete response	Recruiting

(*continued on next page*)

Table 2 (*continued*)

Name	Trial	N	Primary Endpoint(s)	Status
Itraconazole	NCT01787331. Itraconazole in treating patients with biochemically relapsed cancer	21	Proportion of patients who achieve ≥50% decline in PSA	Completed
	NCT03458221. Signal Transduction Analysis in OVarian CancER	50	Survival benefit	Not yet recruiting
	NCT03081702. A Study of the Safety, Tolerability and Effectiveness of Hydroxychloroquine and Itraconazole in Platinum-resistant Epithelial Ovarian Cancer	52	MTD	Recruiting
	NCT02366884. Clinical Evaluation of a New Form of Cancer Therapy Based on the Principles of Atavistic Metamorphosis	250	Clinical benefit (tumor regression)	Enrolling by invitation

All trials were retrieved from www.clinicaltrials.gov on February 28, 2019. Phase 2 and 3 trials are listed; phase 1 trials were included if they were combined with the phase 2 trial.

AE, adverse event; AML, acute myeloid leukemia; BCCNS, basal cell carcinoma nevus syndrome; DLT, dose-limiting toxicity; ESA, epithelial specific antigen; FAK, focal adhesion kinase; GDC-0449, vismodegib; HH, hedgehog; HHI, hedgehog inhibitor; IGF-1R, insulin-like growth factor-1 receptor; LDE225, sonidegib; MB, medulloblastoma; MF, myelofibrosis; MoAb, monoclonal antibody; MTD, maximum tolerated dose; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen; SMO, Smoothened.

[a] Patidegib (IPI-926) is not listed since it was in trials for BCCNS only.

[b] Trials with status Withdrawn or Terminated are not listed. Seven rediscovery trials that include vismodegib along with other antineoplastic agents are not listed.

[c] Title was truncated on the clinicaltrials.gov website; cancer type is small-cell lung cancer.

Prostate cancer

To determine the pharmacodynamic effect of sonidegib in prostate cancer, tumor samples were obtained following sonidegib treatment from patients with high-risk localized prostate cancer undergoing radical prostatectomy. These samples were then compared with core specimens obtained posttreatment at prostatectomy. Of 7 men who received sonidegib, 6 achieved a ≥2-fold reduction in *GLI1* mRNA, a surrogate marker for HHI efficacy (NCT02111187) [61]. In contrast, in patients with castration-resistant prostate cancer, vismodegib failed to produce a clinical benefit (NCT02115828) [62]. Identifying the stages of prostate cancer at which HH inhibition may have the most clinical benefit may help clarify these results.

Pancreatic cancer

Addition of vismodegib to gemcitabine failed to improve overall response rate or OS in an unselected cohort of patients with metastatic pancreatic cancer (NCT01064622; Table 2) [63], contrasting with the results obtained with sonidegib in the preclinical model discussed above, wherein sonidegib reduced islet tumors significantly and prolonged median survival [46]. The preclinical model may not accurately reflect human metastatic disease; alternatively, differences in the unique molecular characteristics of the 2 compounds may affect their relative efficacy [63]. A phase 1 trial (NCT01383538) of patidegib added to FOLFIRINOX (the combination of 5-fluorouracil [5-FU], leucovorin, irinotecan, and oxaliplatin given biweekly) in advanced pancreatic adenocarcinoma showed a high objective response rate (67%), but tissue perfusion was inconsistent, as assessed by computed tomography imaging [64].

Hematological malignancies

Hematological malignancies were shown to be sensitive to HHI treatment [65,66]. In a clinical trial evaluating consolidation therapy (NCT00151242), pretreatment samples from AML patients demonstrated SMO, PTCH1, GLI1, GLI2, and GLI3 expression [67]. GLI2 expression correlated with inferior event-free survival, relapse-free survival, and OS [67]. Glasdegib showed a favorable pharmacokinetic and safety profile in a phase 1 trial (NCT00953758) with a modest decrease in blast count in some patients with heavily pretreated AML, and hematologic improvement in individual patients with myelodysplastic disease, CML, myelofibrosis, and AML [68]. This led to a 3-arm, phase 1 study assessing combination therapy following a standard 3 + 3 dose escalation: Patients who were not suitable for standard chemotherapy received either glasdegib plus low-dose cytarabine (LDAC) (arm 1), or glasdegib plus decitabine (arm 2); whereas patients considered fit for standard chemotherapy received combination therapy with glasdegib plus cytarabine/daunorubicin (arm 3) [69]. Therapy was found to be well tolerated [69]; and this led to a phase 2 trial for patients unfit for chemotherapy who were randomized 2:1 to receive LDAC alone or in combination with glasdegib (G + LDAC) [42]. Compared with LDAC alone, G + LDAC led to a higher rate of complete remission (17.0% vs 2.3%, $P < 0.05$) [42]. Median OS with G + LDAC was 8.3 months (range, 6.6–9.5 months) compared with 4.3 months (range, 2.9–4.9 months) with LDAC alone ($P = 0.0002$), demonstrating a significant clinical improvement [42]. Overall response rate was 26.9% with G + LDAC, compared with 5.3% with LDAC [42]. Combination therapy with glasdegib was expanded in a phase 2, single-arm study that enrolled 69 patients with a median age of 64 years [70]. The median OS was 14.9 months (17.7 months when patients were censored at the time of receiving stem cell transplant) [70]. Furthermore, sonidegib has been investigated to manage chronic graft-versus-host disease (GvHD) following allogeneic hematopoietic cell transplant. In a phase 1 study (NCT02086513), which included patients with steroid-refractory GvHD, 47% of patients (17 enrolled) had a partial response in skin

disease. Among the previous therapies given to patients were corticosteroids, rituximab, tacrolimus, and brentuximab vedotin. These results imply that inhibiting HH signaling deserves further investigation in this clinical setting, and that HHIs may be useful for treating chronic GvHD [71].

A phase 3 randomized study of glasdegib in combination with standard chemotherapy is currently under investigation for the treatment of AML (NCT03416179) in 2 cohorts [72]. One cohort comprises patients randomized to receive intensive chemotherapy with glasdegib or placebo ("7 + 3" regimen). The second cohort includes patients treated with nonintensive chemotherapy and glasdegib, or placebo [72].

Recent and ongoing phase 2 and 3 trials to assess the efficacy of vismodegib, sonidegib, taladegib, glasdegib, and itraconazole in solid and hematological cancers are presented in Table 2.

Toxicity profile associated with hedgehog inhibitor therapy

Patients receiving HHI therapy commonly experience at least 1 treatment-emergent adverse event (AE), including alopecia, muscle spasms, fatigue, vomiting, nausea, and dysgeusia [57,73], which are thought to be related to HH pathway inhibition in normal tissue [74].

Developmental toxicity was observed in a preclinical mouse model following Smo inhibitor administration [75]. This is consistent with grade 1 and 2 effects on bone and cartilage noted in 3 prepubertal patients treated with sonidegib for medulloblastoma (NCT01125800) ages 4, 8, and 11 years requiring discontinuation [57]. On the other hand, no drug-related bone or dental toxicities were observed in pediatric patients following vismodegib treatment [58]; possibly due to the short treatment duration in the majority of pediatric patients. Of the 4 patients who remained on study with responses to vismodegib, all were postpubertal and above the age of 17 years [57,58]. These data may limit the utility of targeting this pathway in young children with HH-activated tumors. Otherwise, the low toxicity profile associated with vismodegib and sonidegib treatment in the pediatric population was similar to that observed in adults. Furthermore, no patients withdrew due to unacceptable toxicity [58].

Sonidegib is currently under investigation for the treatment of relapsed/refractory acute leukemia (NCT01826214) [76]. All patients experienced at least 1 AE, including anemia, diarrhea, fatigue, nausea, muscle spasms, decreased appetite, and blood creatine phosphokinase increased.

The ongoing phase 2 trial in patients with AML (NCT01546038) evaluated the safety of G + LDAC [42]. The most frequently reported nonhematologic grade 3/4 AEs in the G + LDAC group included pneumonia, fatigue, dyspnea, hyponatremia, sepsis, and syncope; and pneumonia in the LDAC group [42]. Furthermore, the frequency of AEs commonly associated with SMO inhibitors (ie, alopecia, muscle spasms, and dysgeusia) was lower in the G + LDAC-treated patients [42]. The combination of G + LDAC was well tolerated and exhibited a manageable safety profile; results indicate that G + LDAC may be a suitable therapeutic strategy for intensive chemotherapy ineligible patients with AML [42].

Biomarkers of response to hedgehog inhibitors

It is important to determine which patients may be more responsive to HHI therapy. Previous studies aimed to identify biomarkers to pinpoint those more likely to exert a pharmacodynamic effect in response to HHI treatment [77]. Vismodegib exhibited activity in adult recurrent SHH-medulloblastoma patients but not in those with recurrent non–SHH-medulloblastoma, suggesting that HHI activity depends on genomic abnormality within the tumor [58]. Furthermore, a 5-gene HH signature assay was identified and clinically validated for use in medulloblastoma patients to identify those with HH-activated disease and, therefore, more likely to respond to sonidegib treatment [59]. The 5 differentially expressed genes (*GLI1*, *SPHK1*, *SHROOM2*, *PDL1M3*, *OXT2*) showed 100% correlation between HH activation status and gene expression profiling (n = 25). Moreover, of 50 patients treated with sonidegib, 6 of the 9 patients who had HH-activated tumors responded, while the remaining 3 had stable or progressive disease [59]. Logically, therefore, all 41 patients without HH-activated tumors failed to respond [59]. A phase 1 trial determining the efficacy and safety of sonidegib in advanced solid tumors showed that *GLI1* mRNA expression was reduced in tumor and skin posttreatment, suggesting *GLI1* expression as a pharmacodynamic marker for HH pathway activation [56]. Despite *GLI1* inhibition being dose- and exposure-dependent, the observed decrease did not coincide with tumor response due to a limited sample size [56]. Analysis of *GLI1* expression at week 17 in the phase 2 BOLT trial showed a decrease from baseline in patients with disease control [31]. In comparison, in a patient with progressive disease and evaluable tumor, *GLI1* expression increased by 10% [31]. HH inhibition was associated with GLI1 inhibition and tumor response, as evidenced by reduced *GLI1* expression in patients with disease control [31].

In the previously described phase 2 randomized trial in AML patients (NCT01546038), 21 genes, 38 circulating cytokine analytes, and 109 gene mutations were analyzed [43]. In the G + LDAC-treated patients, improved OS correlated with reduced expression of *FOXM1* and *MSI2*; and higher *BCL2* and *CCND2* [43]. Furthermore, macrophage inflammatory protein 1-alpha (MIP-1α) cytokine levels were reduced. Inhibition of HH signaling may override the pro-survival benefit of high *BCL2* expression; and reduced MIP-1α levels may facilitate glasdegib-induced cell cycle re-entry, coinciding with cell susceptibility to LDAC [43]. No mutations correlated with OS or overall response [43]. However, increased *PTCH1* and reduced *BAFF* expression did correlate with overall response in the G + LDAC-treated patients, suggesting increased HH pathway activity and, therefore, increased dependence on HH signaling. In the glasdegib plus chemotherapy cohort, OS correlated with low IL-8 and MIP-3β/CCL19 levels; as well as mutations in *CEP170* and *ANKRD26* [43]. Since this was a small study with a limited number of patients, further studies are warranted to determine if the biomarker differences observed between the cohorts are treatment related [43]. Additional information regarding biomarker analysis is expected from the ongoing phase 3 trial with glasdegib in patients with previously untreated AML (NCT03416179). Collectively, these studies indicate that molecular and genomic analyses are critical to identify patients that will benefit from targeted HHI therapy [58].

Mechanisms of resistance

As with other cancer treatments in development, resistance is a concern with HHIs. Most observations regarding HHI resistance originate from patients with BCC [78]. In the clinic, resistance to vismodegib and sonidegib were observed when used to treat advanced BCC [78,79]. Acquired mutations in *SMO* cause resistance [78,80], and neither vismodegib nor sonidegib are active against SMO D473H, a mutation reported in a number of HHI-resistant patients [79,81]. Although some clinical results suggest that resistance to 1 HHI implies resistance to another HHI in the same class [79], data are conflicting [37]. Importantly, in a preclinical model of medulloblastoma, patidegib was effective on tumors resistant to vismodegib [82], suggesting a lack of cross-resistance in all instances. Additionally, binding of HHIs to mutant forms of Smo revealed that a mutation at residue 518 increased affinity for sonidegib, while concomitantly decreasing affinity for vismodegib [83,84].

Amplification of downstream HH genes (eg, *Gli2*) as a mechanism of resistance has been reported for vismodegib and sonidegib in cultured cells and a mouse model of medulloblastoma. The amplification correlated with tumor growth in a Smo-independent manner [4,85,86].

Mutations in genes controlling ciliogenesis are another mechanism of HHI resistance. For example, mutations in the ciliogenesis oral-facial-

digital-syndrome-1 gene (*Ofd1*) lead to HHI resistance via the loss of primary cilia that regulate HH signaling [87]. The investigators noted that mutations in *Ofd1* or in the HH signaling component gene *Sufu* conferred resistance to chemotherapy agents [87].

Overcoming resistance

The mechanistic diversity of HHI resistance identified to date suggests that several tactics might be used to overcome such resistance. One strategy is the use of combination therapy. In a report of 5 patients with metastatic BCC resistant to HHI therapy, itraconazole plus arsenic trioxide (ATO) induced stable disease in 3 of 4 patients; the fifth patient died (mean follow-up 12.6 months from enrollment date) [88]. Another approach is to target a different downstream mediator, such as DYRK1B, which positively regulates HH signaling/Gli activity [89]. In preclinical studies, DYRK1B antagonism greatly decreased Gli1 expression in medulloblastoma cells regardless of whether they were sensitive or resistant to HHIs [89]. Furthermore, combination HHI therapy with itraconazole and sonidegib was used to overcome HHI resistance in a patient previously treated with vismodegib [37].

The role of multiple signaling pathways

It is possible that more than 1 signaling pathway is active in a particular cancer; therefore, simultaneously targeting these pathways may help prevent or postpone the development of resistance. For example, in vitro in prostate cancer cells, inhibition of HH and Sox2 signaling concomitantly led to reduced metastatic behavior, survival capacity and invasiveness, and increased apoptosis [90]. In a mouse model of medulloblastoma, blocking mammalian target of rapamycin (mTOR) and HH signaling prevented HHI resistance [86]. In addition, crosstalk between HH and other pathways (eg, mitogen-activated protein kinase/extracellular signal-regulated kinase [Ras/Raf/MEK/ERK], phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin [PI3K/AKT/mTOR]) may provide a basis for effective combination cancer therapy [91]. Evidence for crosstalk between HH and Ras/Raf/MEK/ERK has been observed in colon, lung, and pancreatic cancer; melanoma, and leukemia [91], while crosstalk between PI3K/AKT/mTOR and HH signaling has been observed in esophageal cancer models [91]. In the future, HHI therapy might be combined with other targeted therapies for effective cancer treatment.

Potentials and pitfalls of hedgehog inhibitors

HH signaling is complex and plays a role not only in tumorigenesis but also drug resistance. Individual response to HHIs varies and detailed biomarker analyses should help to identify which patients are more suited to HHI therapy, which has significant AEs associated with reduced quality of life, drug tolerance, and patient adherence often leading to discontinuation [80]. Vismodegib and sonidegib have comparable efficacy and tolerability in the treatment of BCC; and if side effects arise, it may be possible to switch from one to the other, although most of the side effects are class-related [30,74,92–94]. Based on differences in CNS penetration, sonidegib might be selected in preference to other treatment options based on the presence of CNS disease (ie, medulloblastoma or metastasis) [95]. ATO and itraconazole are less efficient but could be used following resistance to SMO inhibitors [96]. However, for patidegib and glasdegib, it is currently unclear. Phase 3 trials are ongoing for topical patidegib (NCT03703310), which might prove to be a good option for BCCNS, but certainly not for visceral tumors [97]. The role of other inhibitors in hematological malignancies and glasdegib in other diseases beyond AML should be explored. Furthermore, additional combinational therapies might prove valuable, such as azacytidine and venetoclax, which is the new standard treatment for newly diagnosed AML patients aged 75 or older with comorbidities that preclude the use of intensive induction chemotherapy [98].

Overall, the benefit of HHI therapy often outweighs the negative side effects, and increased patient education and management may help to extend treatment and improve clinical outcomes [80].

Summary and future directions

The potential of HHIs for the treatment of solid and hematological malignancies is an ongoing investigation. Clinical trial results will facilitate our ability to discern which additional tumor types are best suited for HHI mono- or combination therapy. New generation HHIs should provide valuable information regarding the range of cancers amenable to HH inhibition as a therapeutic modality, and insight into overcoming the development of resistance.

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