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The Utility of Hedgehog Signaling Pathway Inhibition for Cancer

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Abstract

The Hedgehog (Hh) signaling pathway has been implicated in tumor initiation and metastasis across different malignancies. Major mechanisms by which the Hh pathway is aberrantly activated can be attributed to mutations of members of Hh pathway or excessive/inappropriate expression of Hh pathway ligands. The Hh signaling pathway also affects the regulation of cancer stem cells, leading to their capabilities in tumor formation, disease progression, and metastasis. Preliminary results of early phase clinical trials of Hh inhibitors administered as monotherapy demonstrated promising results in patients with basal cell carcinoma and medulloblastoma, but clinically meaningful anticancer efficacy across other tumor types seems to be lacking. Additionally, cases of resistance have been already observed. Mutations of *SMO*, activation of Hh pathway components downstream to SMO, and upregulation of alternative signaling pathways are possible mechanisms of resistance development. Determination of effective Hh inhibitor-based combination regimens and development of correlative biomarkers relevant to this pathway should remain as clear priorities for future research. *The Oncologist* 2012;17:1090–1099

Introduction

Hedgehog (Hh) proteins were initially discovered in *Drosophilia melanogaster*, together with their signaling transduction pathway [1, 2]. This signaling pathway plays a critical role in embryonic development but is generally silenced in adults [3, 4]. However, this pathway is reactivated during tissue repair and regeneration [5–8]. In the last decade, there has been increasing evidence that the Hh pathway plays an important role in carcinogenesis; this knowledge has provided an attractive platform for the development of novel anticancer agents [9, 10]. In this review, we discuss the relevance of the Hh pathway in cancers and summarize the clinical experience thus far with Hh inhibitors.

Hedgehog Proteins and Signaling Transduction Pathway

Three mammalian Hh proteins have been identified in humans; they are denoted by the prefixes Sonic, Indian, and Desert. Briefly, these proteins act as a ligand and activate the Hh signaling pathway by binding to Patched (PTCH1), a transmembrane protein present on the primary cilium of target cells. In the absence of an Hh ligand, PTCH1 represses the activity of Smoothened (SMO), which is now recognized to play an important role in the Hh signaling transduction pathway. Following Hh ligand binding to PTCH1, SMO repression is released and this subsequently results in the modulation of GLI transcription factors. There are three forms of GLI transcription factors: GLI1, GLI2, and GLI3. GLI1 has a transcriptional activator function, whereas GLI2 can either activate or repress gene expression depending on posttranscriptional/translational modifications. GLI3, converse to GLI1, yields a strong repressor effect on transcriptional activities [10, 11]. Primary cilia also play crucial roles in mammalian Hh signal transduction, as most of the key components for the Hh pathway (e.g., PTCH1, SMO and GLI transcription factors) are enriched

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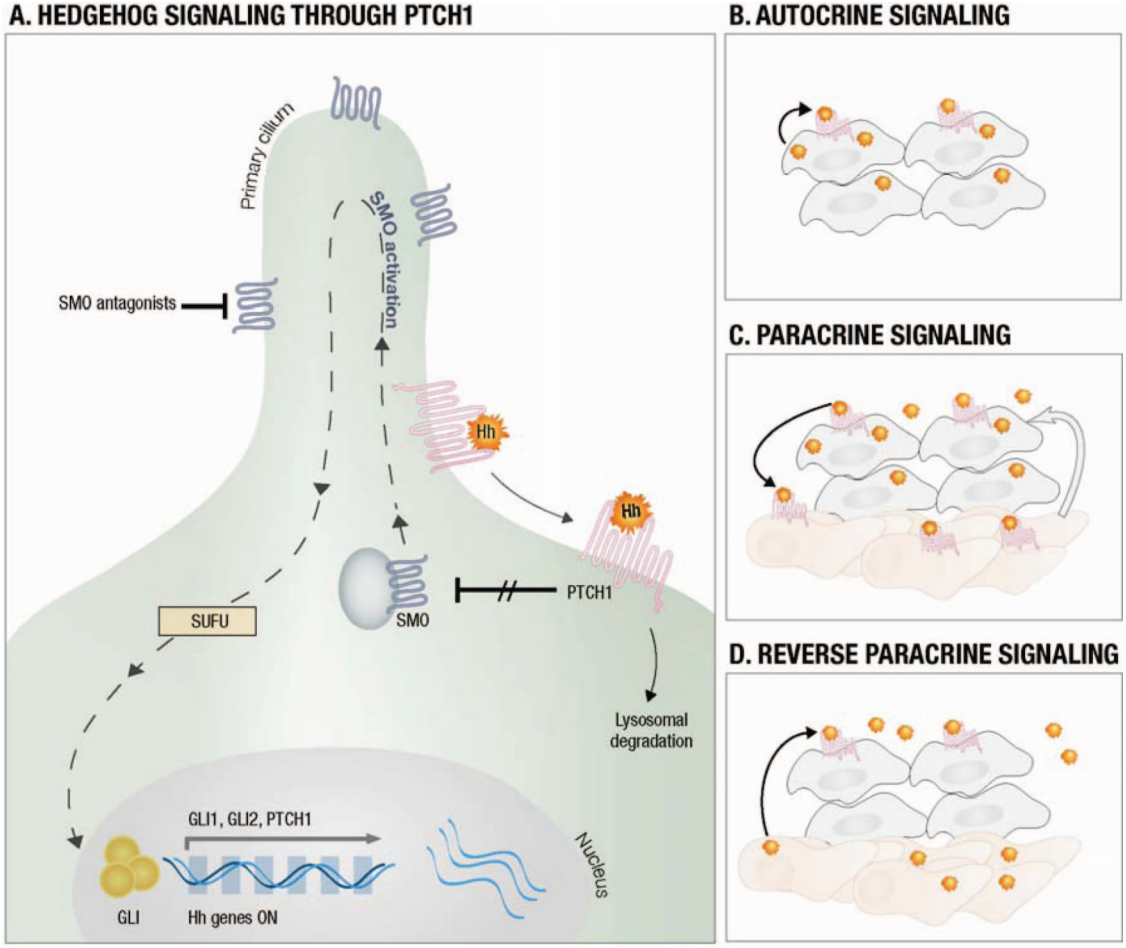

Figure 1. Hedgehog signalling. **(A)** Hedgehog ligands (Hhl) bind to PTCH1 and unrepress SMO with activation of GLI and target genes. **(B)** The tumor produces Hhl and stimulates itself. **(C)** Tumor cells produce Hhl and activate signaling in nonmalignant cells. In turn, other signaling pathways are activated and stimulate tumor growth (arrow). **(D)** Stromal cells produce the Hhl required for tumor growth/survival.

within this structure [12]. Additionally, cilia play an important role for the trafficking of Hh pathway proteins, which is crucial for pathway activation [12]. Ultimately, the balance between the activating and repressive functions of GLIs results in the expression of target genes, among others *GLI1, PTCH1, MYC, BCL-2,* and *Cyclin D1* [13–15].

Within this simplified description (Fig. 1A), there are numerous other cellular components engaged in the activation of Hh pathway, especially in the steps regulating GLI activity downstream from SMO. These components include suppressor of fused (SUFU), KIF7, protein kinase A (PKA), glycogen synthase kinase 3ß (GSK3ß), and casein kinase 1 (CK1) [13, 16–18]. SUFU is a negative regulator of this pathway; it achieves this effect via several mechanisms. Physically, SUFU sequesters GLI transcription factors, whereas functionally SUFU affects GLI transcription ability [19–21]. The kinase protein KIF7 acts as both a positive and negative regulator of Hh pathway [22, 23]. It interacts with GLI proteins and inhibits GLI-dependent transcriptional activation [22, 23]. Conversely, KIF7 may assume a positive role via its movement to cilia tip after pathway activation where it antagonizes the activity of SUFU [15]. However, the actual functions of most of these proteins are still subject to intensive studies and not fully understood [9, 10].

Dysregulation of Hedgehog Pathway in Solid Tumors

Aberrant activations of Hh pathway have been observed across a number of different malignancies (Table 1). The mechanisms by which aberrant activations of Hh signaling can lead to cancer are complex, but in general they include activating mutations of members in the Hh pathway (ligand-independent) and excessive/inappropriate expression of Hh ligands (ligand-dependent) [4, 10, 24].

Activating Mutations of Members in Hedgehog Pathway

Loss-of-function mutations in *PTCH1* were initially identified in patients with basal cell nevus syndrome (BCNS; also known as Gorlin syndrome). These mutations lead to constitutive upregulation of the Hh pathway and patients are highly predis-

Table 1. Cancers associated with aberrant activation of Hedgehog pathway

Tumor type	Mechanism of Hedgehog pathway activation	References
Basal cell carcinoma	Ligand independent (mutations in *PTCH1* and *SMO*)	[4, 25–28, 34–36]
Breast	Ligand dependent (autocrine)	[43–45, 93]
Colorectal	Ligand dependent (autocrine and paracrine)	[11, 53]
Gliomas	Ligand dependent (exogenous ligand)	[94–96]
Hepatocellular	Ligand dependant/unclear mechanism	[85]
Leukemia	Ligand dependent/unclear mechanism	[97]
Lung	Ligand dependent (autocrine)	[8, 41,42, 98, 99]
Lymphoma	Ligand dependent (autocrine and reverse paracrine)	[54, 97, 100–103]
Medulloblastoma	Ligand-independent (mutations in *PTCH1, SMO, SUFU*)	[28–32, 35, 37, 38]
Multiple myeloma	Ligand dependent (reverse paracrine)	[54, 104]
Ovary	Ligand dependent/unclear mechanism Loss of heterozygosity at the *PTCH1* locus	[46, 47, 105]
Pancreatic	Ligand independent (mutations of *GLI1* and *GLI 3)*	[39, 53, 106, 107]
	Ligand dependent (autocrine and paracrine)	
Prostate	Ligand dependent (autocrine and paracrine)	[108–110]
Upper GI	Ligand dependent (autocrine)	[111]
Sarcoma	Mutations in *PTCH* and *SUFU* in rhabdomyosarcoma	[81, 112–115]
	Ligand dependent (autocrine) in osteosarcoma/chondrosarcoma	
	Direct activation of *GLI1* in Ewing sarcoma	

posed to the development of basal cell carcinomas (BCC) [4]. Further studies also showed that *PTCH1* mutations occur in sporadic cases of BCC and medulloblastoma [4, 25–28]. *PTCH1* mutations have been found in patients with central nervous system primitive neuroectodermal tumors or medulloblastomas [29–31]. More than 40 different *PTCH1* mutations have been reported, which mostly result in truncated protein and are scattered throughout the gene. Although no mutational hot spots have been identified, exon 17 mutations have been seen more frequently in sporadic cases of medulloblastoma than BCNS. These clinical findings were supported by several preclinical reports that elegantly demonstrated the role of these mutations in carcinogenesis [32, 33]. In one study, spontaneous development of BCCs occurred when Hh was overexpressed in a transgenic mouse model; in another report, mice with heterozygous *Ptch1* mutations went on to develop cerebellar medulloblastomas [32, 33].

Gain-of-function mutations in *SMO* are also present in some cases of sporadic BCCs [28, 34–36]. One mutation at base pair 1604 (G-to-T transversion) of exon 9 of the *SMO* gene changes codon 535 from tryptophan to leucine and has been reported in about 20% of sporadic BCCs [28, 35]. This mutation has resulted in constitutive SMO signaling and development of BCC-like tumors in transgenic mice [34, 36]. Additionally, the 1604 G-to-T mutation in *SMO* has also been described in medulloblastoma patients, albeit at much lesser frequency (1 out of 21 patients) [28]. Genetic alterations of other components of Hh pathway, such as *GLI and SUFU* mutations, have also been observed [37–39]. Inactivating germline mutations of *SUFU* have been reported in 3% of sporadic and >10% of desmoplastic medulloblastomas [37, 38]. Although alterations of *GLI1* and *GLI3* have been observed in global genomic analyses of pancreatic tumors, these are not thought to be activating, but rather are more likely to be passenger mutations [39].

Excessive/Inappropriate Expression of Hh Ligands

Aberrant activation of the Hh signaling pathway in cancers may also be ligand- dependent and has been reported in several malignancies [10, 40]. Ligand-dependent activation of the Hh pathway was initially described to occur in autocrine mode, but there is an increasing understanding that paracrine or reverse paracrine modes may also occur [10, 24].

Autocrine Stimulation

In the autocrine mode, tumor cells self-secrete Hh ligands to which they subsequently respond and culminate in activation of the signaling pathway. This mode has been previously described in a number of malignancies, as summarized in Table 1. In one study, 50% of small cell lung carcinomas (SCLCs) demonstrated overexpression of both Sonic hedgehog (SHH) and GLI1 in an autocrine fashion [8]. Moreover, the treatment of SCLC cell lines with cyclopamine (an SMO inhibitor) resulted in significant growth inhibition [8]. In another study, there was a marked increase in the expression of SHH in mice bearing human SCLC xenografts. Treatment of these mice with the SMO inhibitor LDE-225 following carboplatin and

etoposide chemotherapy was highly effective in preventing recurrence of residual tumors [41]. Overexpression of SHH, GLI1, GLI2, and SMO has also been reported in tumor tissues of 80 patients with non-small cell lung carcinoma. In this series, elevated expression level of SMO correlated with the presence of nodal disease, implicating its role in metastasis and disease progression [42].

Autocrine activation of the Hh pathway has also been shown in breast cancer, both in cell lines and tumoral tissue studies [43–45]. In a study by Kubo et al., overexpression of SHH and GLI1 was detected in virtually all of the 52 breast cancer specimens examined [43]. In another series, the overexpression of SHH in breast cancers was shown to be secondary to hypomethylation of the ligand promoter as well as NF-κB upregulation [45]. Furthermore, akin to studies in lung cancers, inhibition of Hh pathway using cyclopamine led to suppression of proliferation in breast cancer cell lines in a dose-dependent manner [43]. Similar reports involving multiple ovarian cancer cell lines revealed upregulation in expression (more than fivefold) of several components of the Hh pathway, including *GLI1* (9 of 19 cell lines), *SMO* (9 of 19 cell lines) and *SHH* (10 of 19 cell lines) [46].

Furthermore, dysregulation of PTCH1 in ovarian cancers has also been described [46, 47]. The treatment of ovarian cancer cell lines with the SMO inhibitors cyclopamine or KAAD-cyclopamine prevented their growth and migration [47, 48]. In addition to cell line data, one study involving 80 ovarian cancer specimens reported that components of the Hh signaling pathway were significantly increased and overexpression of PTCH1 and GLI1 conferred poor survival [47].

Despite the encouraging data described in this section, cyclopamine-based studies have inherent limitations which affect their interpretation. In many of the cell-line studies, the doses of cyclopamine used are now recognized to be associated with antiproliferative effects that are independent of Hh signaling [49, 50]. It has been shown that cyclopamine in high concentrations can induce apoptosis by increasing N-SMase2/ceramide and via generation of nitric oxide [49]. Moreover, it is difficult to reach systemic levels of cyclopamine in vivo because of its toxicity and relatively short elimination half-life [51]. The preclinical studies are further limited by the fact that there is no agreed-upon standard method to measure the Hh pathway activity and none of commercial antibodies against PTCH, SMO, or GLI have ever been shown to work specifically on fixed tissues [52].

Paracrine Stimulation

In the paracrine mode, Hh ligands are secreted by tumor cells and induce activity within infiltrating stromal cells. This in turn results in the production of unknown factors within the tumor environment, which ultimately support tumor growth [10, 40]. In a study by Yauch et al., paracrine activation of the Hh pathway was found in patient-derived xenografts of pancreatic and colon adenocarcinoma. Furthermore, inhibition of Hh pathway in the stroma resulted in tumor growth retardation and thus supports a paracrine mode of stimulation [53]. The exact mechanism by which Hh pathway activation in stromal cells can enhance tumor progression is unclear, but insulin-like growth factor-1 receptor (IGF-1R) and Wnt signaling pathways may play a role [53].

Reverse Paracrine Stimulation

Reverse paracrine stimulation occurs when Hh ligands produced by surrounding stromal cells activate the tumoral cell Hh pathway. Dierks et al. demonstrated that Hh ligands secreted by bone marrow, nodal, and splenic stromal cells represented survival factors for malignant B-cell lymphoma and plasmacytoma cells derived from a transgenic Eμ-*Myc* mouse model or from patients with these malignancies [54]. However, such paracrine stimulation still needs further confirmation.

The different modes of Hh pathway signaling are summarized in Figure 1B–1D.

Hedgehog Signaling in Cancer Stem Cells

Cancer stem cells (CSCs) are a subpopulation of cancer cells that are self-sustaining with the exclusive ability to self-renew and give rise to heterogeneous cell lineages within the tumor. They have been identified in a variety of cancers, and evidence is accumulating that CSCs may be responsible for treatment resistance and relapse [24]. Preclinical data have suggested a possible regulatory role of Hh pathway in CSCs across a number of malignancies, such as glioblastoma, breast cancer, pancreatic adenocarcinoma, and hematological malignancies [54–58]. Activation of Hh signaling has been reported in CD133-positive glioma CSCs and treatment of these cells with siRNA or cyclopamine led to a loss of tumorigenic drive [55, 59]. Similarly, in breast cancers, Hh pathway activation (using Hh ligands or manipulation of GLI1/2) or suppression (using cyclopamine) in CSCs resulted in altered expression of BMI1, a central regulator of stem cells. This in turn led to an increase or loss of tumorigenic potential, respectively, in both in vitro and in vivo settings [57]. In chronic myeloid leukemia, loss of SMO caused depletion of stem cells and use of cyclopamine led to reduction of stem cells in mouse model [58].

In addition to having tumorigenic properties, CSCs are also implicated in cancer progression and metastasis [60]. Hh signaling has been one of proposed pathways in this process [58, 61, 62]. Higher expression of *PTCH1, GLI1, GLI2,* and the target gene *SNAIL1* has been reported in CD133-positive colon CSCs [63]. In vitro inhibition of Hh pathway activity in these cells using cyclopamine or siRNA resulted in decreased tumor cell proliferation and induced apoptosis [63]. Although these studies support the possible role of Hh pathway activation in CSCs, it remains pertinent to be reminded of the limitations posed by cyclopamine-based studies, as discussed previously.

Hh Inhibitors

The preclinical relevance of Hh signaling in cancers has resulted in the development of several targeted agents against this pathway. Most of these agents act by binding to and antagonizing SMO. At present, seven SMO inhibitors are being evaluated in the phase I or phase II clinical trial setting. These agents include vismodegib, BMS-833923, IPI-926, LDE-225,

PF-04449913, LEQ 506, and TAK-441. Currently, most data concerning the clinical utility of these agents are based on trials of vismodegib. Although these data suggest clinical benefit in cancers driven by mutational activation, targeting the Hh pathway may be less likely to be successful in tumors in which aberrant ligand overexpression or signaling are not the oncogenic drivers, but rather are secondary to genetic changes in other signaling pathways [64–67].

Hh Inhibitors Antagonizing SMO

Vismodegib (GDC-0449)

Vismodegib is a potent orally bioavailable small molecule inhibitor of the Hh pathway that acts by binding and inhibiting SMO [68]. Antitumor activity of this drug was initially shown in preclinical models of medulloblastoma, colon, and pancreatic tumors [69]. A phase I clinical trial of this agent involved 68 patients with refractory, locally advanced, or metastatic solid tumors; it demonstrated an acceptable safety profile with no dose-limiting toxicity (DLT). The most frequently reported adverse events (AEs) have been muscle spasms, dysgeusia, fatigue, alopecia, and nausea. Seven grade 4 AEs (hyponatremia, fatigue, pyelonephritis, presyncope, resectable pancreatic adenocarcinoma, and paranoia with hyperglycemia) were reported in six patients (9%). Grade 3 AEs were observed in 28% of patients; they most commonly included hyponatremia (10%), abdominal pain (7%), and fatigue (6%) [69].

A phase 1 clinical trial of vismodegib has shown significant clinical activity in tumors with driver mutations of the Hh pathway [64, 65, 69]. The overall response rate (defined as both complete and partial responses) in advanced BCC was achieved in 19 out of 33 patients (58%). Complete response was achieved in two patients. The authors reported a median response duration of 12.8 months (range: 3.7–26.4 months) among evaluable patients [69]. The clinical benefit of vismodegib has been also reported in medulloblastoma. Treatment of a patient with refractory widespread metastatic medulloblastoma with a somatic mutation in *PTCH1 (PTCH1-W844C*) and loss of heterozygosity resulted in a rapid, although transient, regression of disease at all tumor sites and improvement of symptoms [64].

As the maximum tolerated dose was not reached, the recommended phase II dose was chosen as 150 mg p.o. daily based on the pharmacokinetic data showing saturable plasma concentrations of vismodegib. An attempt was made to reduce the frequency to 150 mg three times per week or once weekly following a loading dose of 150 mg daily for 11 days, but this maneuver failed to achieve unbound plasma concentration associated with efficacy in patients with BCC and medulloblastoma [70].

Two recently published clinical trials have further demonstrated the remarkable clinical benefit of this agent in BCC [71, 72]. In a study by Sekulic et al., vismodegib at a dose of 150 mg daily was associated with objective response rates of 30% and 43% in patients with locally advanced and metastatic BCC, respectively. In the group of locally advanced BCC, 13 out of 63 patients (21%) had a complete response. Median duration of response for both groups was 7.6 months [71]. In addition, vismodegib had promising results in a randomized placebo-controlled trial in patients with BCNS [72]. The primary endpoint of this study was reduction in the incidence of new BCCs that were eligible for surgical resection. Vismodegib at a daily dose of 150 mg significantly reduced the per-patient rate of new surgically eligible BCCs (2 vs. 29 cases per group per year). Although no tumors progressed during treatment with vismodegib, BCCs and palmar-plantar pits associated with BCNS both recurred after stopping the treatment [72]. Both studies reported notable drug-related toxicity profiles. Muscle spasms, alopecia, dysgeusia, nausea, decreased appetite, diarrhea, fatigue, and weight loss were the most commonly reported adverse events [71, 72]. Although most of these toxicities were low grade in nature, they led to treatment discontinuation in 54% of patients in the BCNS study. Furthermore, in the study by Sekulic et al., seven fatal events were reported [71]. Although all seven patients had coexisting conditions at baseline, it is relevant to note that three of the deaths were due to unknown causes [71].

A phase II randomized trial of vismodegib or placebo in combination with oxaliplatin, 5-fluorouracil, and leucovorin or irinotecan, 5-fluorouracil, and leucovorin plus bevacizumab in 195 patients with previously untreated metastatic colorectal cancer did not meet the primary endpoint of extending the progression-free survival [66]. Another phase II randomized placebo-controlled trial investigating vismodegib as maintenance therapy in patients with ovarian cancer in second or third complete remission did not demonstrate any significant improvement in progression-free survival [67]. These results underline the complex challenges in attaining clinical benefit from Hh pathway inhibition and also highlight the lack of utility for this class of agents to be used in cancer management when aberrance of Hh pathway is not the oncogenic driver.

At present, there are several phase II trials investigating the role of this agent in specific tumor types and different combinations with chemotherapy regimens (Table 2).

BMS-833923 (XL139)

BMS-833923 is another potent, oral, small-molecule antagonist of SMO [73]. The inhibitory effect of this agent has been demonstrated in multiple cell lines, including engineered human medulloblastoma cell lines. A phase I trial of BMS-833923 has shown good clinical tolerance at doses up to 360 mg; evaluation of this agent in a phase I setting was still ongoing at the time of abstract reporting. The most common AEs include dysgeusia (44%), muscle spasms (44%), alopecia (15%), diarrhea (11%), myalgia (11%), dry mouth (11%), and nausea (11%). Grade 2 pancreatitis and lipase elevation occurred in one patient at a dose of 240 mg, whereas grade 3 hypophosphatemia was observed in one patient at 540 mg.

In this study, one patient with BCNS achieved complete response, one patient with non-small cell lung cancer had a partial response, and 21% (6 of 28 patients) remained on treatment longer than 100 days at the time of report [73]. Combination regimens of BMS-833923 with chemotherapy in several tumor types are currently underway; they are summarized in Table 2.

Table 2. Hedgehog pathway inhibitors currently in clinical trials in cancer

Drug	Phase	Tumor site	Combination	ClinicalTrials.gov identifier
GDC-0449 (Vismodegib; Genentech, Roche, Curis)	II	Colorectal	FOLFOX or FOLFIRI + bevacizumab	00636610
		Gastric	FOLFOX	00982592
		Glioblastoma		00980343
		Medulloblastoma		00939484
		Pancreas	Gemcitabine	01064622
			Gemcitabine + nab-paclitaxel	01088815
		Small cell lung cancer	Cisplatin + etoposide	00887159
BMS-833923 (XL 139; Bristol-Myers Squibb, Exelexis)	I	Solid tumors		00670189
		Gastric	Cisplatin + capecitabine	00909402
		Myeloma	Lenadolamide + dexamethasone + bortezomib	00884546
		Small Cell Lung Cancer	Carboplatin + etoposide	00927875
	I/II	Leukemia	Dasatinib	01218477
IPI-926 (Infinity)	I	Solid tumors		00670189
		Head and neck	Cetuximab	01255800
	I	Pancreas	FOLFIRINOX	01383538
	II	Chondrosarcoma		01310816
LDE225 (Novartis)	I	Solid tumors		00880308
		Sporadic BCC (topical preparation)		01033019
	II	Metastatic BCC		01327053
PF-04449913 (Pfizer)	I	Solid tumors		01286467
	I/II	Chronic myeloid leukemia	Dasatanib or bosutinib	00953758
LEQ 506 (Novartis)	I	Solid tumors		01106505
TAK-441 (Millennium)	I	Solid tumors		01204073

Abbreviations: BCC, basal cell carcinoma; FOLFIRI, 5-fluorouracil/leucovorin and irinotecan; FOLFORINOX, 5-fluorouracil/leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-fluorouracil/leucovorin and oxaliplatin.

IPI-926 (Saridegib)

IPI-926 is an orally bioavailable, semisynthetic derivative of cyclopamine, which targets the Hh pathway by inhibiting SMO [74, 75]. When combined with gemcitabine, IPI-926 increases survival in a gemcitabine-resistant pancreatic cancer mouse model [76]. The potent antitumor activity of IPI-926 has been shown in medulloblastoma mouse models that are compound heterozygous for *PTCH1* and *HIC1* mutations [75]. In a phase I dose escalation trial conducted by Rudin et al., IPI-926 was well tolerated up to a dose of 160 mg daily. The most common AEs were fatigue (29% total; 3% were grade 3), alanine aminotransferase elevation (20%; 8% were grade 3), aspartate aminotransferase elevation (19%; 4% were grade 3); and nausea (19%; none were grade 3). Dose-limiting toxicities in this study were asymptomatic grade 3 increased transaminases and/or bilirubin (one patient at 160 mg, four patients at >160 mg), all of which resolved when drug was held. Clinical activity was observed in three patients with locally advanced BCC who received IPI-926 for longer than 12 months at the time of report [77].

The use of this agent in pancreatic cancer has also been evaluated. Initial preclinical studies demonstrated promising activity in a pancreatic cancer mouse model [76]. However, in a randomized, phase II, placebo-controlled study of gemcitabine plus IPI-926 versus gemcitabine plus placebo in patients with metastatic pancreatic cancer, the trial was stopped early due to a difference in survival favoring the placebo arm. Different combinations of IPI-926 with systemic agents as well as tumor specific trials are currently underway (Table 2).

LDE-225

LDE-225 is a selective orally bioavailable inhibitor of SMO. Phase I dose escalation of this agent in 72 patients with advanced solid tumors has shown an acceptable safety profile with a recommended phase II dose of 800 mg daily. The most frequently reported AEs were fatigue, nausea, vomiting, anorexia, muscle cramps, myalgia, and dysgeusia [78]. Topical preparation of LDE-225 has been also developed and studied in patients with BCNS. In a double-blind randomized study involving eight patients with 27 BCC tumors, of 13 BCCs treated with active compound, three showed complete responses, nine had partial responses, and one had no clinical response, in con-

trast to only one partial response out of 14 BCC tumors treated with the vehicle [79].

Clinical activity of LDE-225 was seen in medulloblastoma (partial response in one patient) and advanced BCC (complete response in one patient and partial responses in four patients). Disease stabilization (>4 months) was observed in five patients with lung adenocarcinoma, spindle cell sarcoma, breast cancer, and BCC [78].

PF-04449913, LEQ 506, and TAK-441

PF-04449913, LEQ 506, and TAK-441 are oral inhibitors of the Hh pathway that are currently undergoing evaluations in a phase I setting. A summary is detailed in Table 2.

Hh Inhibitors Antagonizing Components Downstream to SMO

Increased understanding of the Hh signaling pathway has led to new discoveries with previously known anticancer agents, such as arsenic. Recent data have shown that arsenicals antagonize the Hh pathway by targeting GLI transcriptional factors. Kim et al. [80] showed potent inhibitory effects of arsenic trioxide (ATO) and phenylarsine oxide on the Hh pathway in a cell line model (NIH 3T3). As arsenic treatment has been shown to affect the p38 MAPK and JNK pathways, the authors of this paper investigated whether the involvements of these two pathways are required for arsenic inhibition of Hh signaling. Interestingly, the inhibitory effect of the Hh pathway by arsenic was sustained even in the presence of a p38 MAP kinase (SB203580) or JNK (SP600125) inhibitors. This data suggest that the anti-Hh effect of arsenicals is independent of the p38 MAPK and JNK pathways.

In comparison with control specimens, ATO also significantly delayed the growth of medulloblastoma allografts derived from *Ptch +/− p53 −/−* mice [80]. Furthermore, ATO inhibited Hh activity mediated by *SMO-D477G*, a mutation that confers resistance to SMO antagonist. In another study by Beauchamp et al., ATO inhibited GLI1 activity in HepG2 cells co-transfected with GLI1 and pGL38×GLI binding element-driven luciferase reporter. This inhibitory effect was shown to be by direct interaction of ATO with GLI1 protein. These investigators also had previously established GLI1 as an important transcriptional target of the oncogenic fusion protein EWS/FLI1, an important driver of Ewing sarcomas [81]. Further observations corroborate this finding; for instance, treatment of Ewing sarcoma family of tumors (ESFT) cell lines with ATO led to marked cytotoxicity and the presence of higher GLI1/2 expression seems to indicate increased sensitivity to ATO. Additionally, tumor growth in ESFT xenografts was inhibited with ATO administration; this treatment was associated with an increased survival in constitutively activated SMO transgenic mouse model for medulloblastoma (ND2:SmoA1), with significantly decreased *GLI* target gene expression [82].

Novel inhibitors of GLI have also been evaluated in the preclinical setting, including GANT61, a small molecule which inhibits direct binding of GLI1 and GLI2 to the promoters of target genes *HIP1*, *BCL-2,* and the transcriptional activation of *BCL-2* [11]. The use of this agent resulted in significant cell death across five different human colon carcinoma cell lines and was found to be more potent than cyclopamine [83]. Significant anti-tumor activity of GANT61 has been also noted in prostate cancer human xenografts [84].

A polymeric nanoparticle encapsulated formulation of a novel GLI1/GLI2 inhibitor, HPI-1 (NanoHHI), has also undergone early preclinical testing. This agent was shown to actively inhibit the proliferation and invasion of human HCC cell lines. NanoHHI also had a potent activity in HCC xenografts and resulted in decrease in the weight of the subcutaneous tumor xenografts. Additionally, it also significantly reduced the population of CD133-expressing HCC cells in orthotopic liver tumors [85].

Resistance to Hh Inhibitors Antagonizing SMO

The early data generated from a number of Hh inhibitors held promise for some tumor types, especially in patients with BCCs and medulloblastoma. However, akin to any targeted therapies that have been developed in cancer, the emergence of drug resistance is a distinct problem. Yauch et al. presented molecular profiling results at the time of resistance development from a patient with medulloblastoma taking vismodegib; the patient had an impressive clinical and radiological response at 2 months but relapsed at 3 months after treatment initiation. Although *PTCH1* mutation was found in the original tumor, a novel mutation in *SMO* was detected after treatment initiation, explaining the acquired resistance [86].

Indeed, mutations at multiple sites in *SMO* can confer resistance to vismodegib and other SMO antagonists. A *SMO* mutation, heterozygous G-to-C mis-sense mutation at position 1697, that resulted in a change of codon 473 from Asp to His (D473H) has been described in Hh inhibitor resistance. Lack of specific binding of 14C-labeled GDC-0449 to SMO-D473H suggested deficiency in drug binding as a mechanism for developing resistance. Interestingly, a mutation altering the same amino acid also arose in a vismodegib-resistant mouse model [86].

Amplifications of *GLI2* transcription factor and Hh target gene *Cyclin D1* have been identified as alternative mechanisms for the development of resistance to Hh inhibitors [87]. Focal amplification of *GLI2* conferred resistance to LDE225 in medulloblastoma mouse models. Additionally, a small number of resistant tumors also showed increased GLI2 mRNA expression in the absence of clear amplification, suggesting that upregulation may be secondary to alternative mechanisms [88].

Compensatory upregulation of the IGF-1R/PI3K pathway may also play a role in resistance development to SMO antagonists. This is based on the observation of increased upregulation of the IGF-1R/PI3K pathway in LDE225-resistant tumor samples. In keeping with this finding, the addition of the PI3K inhibitor BKM120 or the dual PI3K-mTOR inhibitor BEZ235 to the initial treatment with the SMO antagonist markedly delayed or even prevented the development of drug resistance in *Ptch+/− Hic+/−* mouse medulloblastomas [88].

Potential On-Target Adverse Effects of Inhibiting the Hh Pathway

The Hh pathway has a substantial role in endochondral ossification and bone homeostasis [89, 90]. This raises the possibility of adverse skeletal effects with Hh pathway inhibition. In one study using mouse models, the use of cyclopamine resulted in significantly lower bone mass and mineral density in comparison to the control group [91]. Moreover, transient inhibition of the Hh pathway in young mice has resulted in permanent bone defects and altered growth, which persisted after cessation of the Hh pathway inhibitor and restoration of pathway [92]. Given the fact that medulloblastomas and BCNS mostly present in pediatric patients, different Hh pathway inhibitors will be developed and studied in this patient population. Thus, special attention should be made to detect potential skeletal adverse effects, especially in pediatric patients.

Conclusion

Hh inhibitors currently represent an opportunity in the quest for novel anticancer therapies. However, the understanding of how this pathway affects different cancers is still under intense study and is not fully understood. This effort is further complicated by the limitations of earlier preclinical studies in which cyclopamine was used at a high dose.

For tumors in which Hh components are mutated, such as BCC, BCNS, and medulloblastoma, the proof of concept for this class of agents has been satisfied. Challenges still exist for the utility of these drugs in other tumor types. In such cancers, targeting Hh alone is unlikely to be effective and appropriate combinations with cytotoxic or other targeted agents need to be studied further. Reassuringly, a number of these initiatives are already in progress (Table 2). Moreover, the development of agents that target the Hh signaling pathway downstream to SMO will also provide further useful therapeutic strategies in this area.

Another challenging aspect in the development of Hh inhibitors is the development of acquired drug resistance, as it can substantially diminish the potential that these agents hold. Deeper understanding on the resistance mechanisms will enable the development of better strategies to overcome this problem. Currently, strategies may include concurrent combination with other targeted therapies such as PI3K-mTOR inhibitors or the development of second-generation Hh inhibitors that cotarget compensatory pathways conferring resistance. Finally, the development of correlative biomarker studies could also be informative in shedding light on the optimal patient populations to treat and should be undertaken with priority.

Author Contributions

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