CLINICAL INVESTIGATION

Therapeutic potential of Hedgehog signaling inhibitors in cancer: rationale and clinical data

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The Hedgehog (Hh)-signaling pathway has become recognized as a key therapeutic target in cancer. In addition to a recently approved first-in-class Hh-pathway inhibitor, several other small-molecule Hh inhibitors are currently under clinical investigation in a variety of cancer settings. Hh signaling occurs through both ligand-dependent and -independent signaling mechanisms and has been implicated in tumor propagation, maintenance of cancer cell stem niches, differentiation, metastatic potential and tumor-microenvironment interactions. Hh-pathway inhibitors have shown robust clinical efficacy in diseases driven by ligand-independent pathway activation. However, use of Hh antagonists in this setting may result in acquired drug-resistance. This reality poses interesting challenges for treating drug-resistant neoplasia. The rationales for therapeutic application of Hh-targeting agents beyond ligand-independent diseases are complex, and the positioning of Hh-targeted agents must consider context-dependent contributions to primary determinants of tumorigenesis and secondary contributions to tumor homeostasis in individual disease settings.

Keywords: basal-cell carcinoma • cancer stem cells • cyclopamine • drug resistance • Hedgehog • IPI-926 • medulloblastoma • NVP-LDE225 • vismodegib (GDC-0449) • XL-139 (BMS-833923)

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Introduction to the Hedgehog signaling pathway

The Hedgehog (Hh)-signaling pathway is a crucial regulator of normal embryonic development. Hh signaling is required for spatial and temporal control of cell differentiation, proliferation and survival, necessary for proper embryonic polarity and patterning during early development [1]. Appropriate embryogenesis relies on a fine balance and control of developmental pathways, created by gradients of activating factors and spatial and temporal regulation of Hh signaling through feedback-inhibition loops. As such, exogenous modulators of Hh signaling function as morphogens by augmenting activation of Hh signaling required for viable embryogenesis and formation of the vertebrate body plan. The name of this pathway is derived from the virtually contiguous, nonsegmented organization of spiny cuticles on *Drosophila* larvae, resembling the appearance of a Hh and phenotypically associated with mutations of the *Hh* gene [2]. Secreted Hh protein is responsible for initiating Hh-pathway activation and controlling aspects of embryonic patterning and segmentation in a dose-dependent fashion [3]. In humans, the Hh family of secreted proteins comprises three Hh homologues: Sonic Hh (Shh), Indian Hh (Ihh) and Desert Hh (Dhh). Expression of Ihh and Dhh are tissue specific, whereas Shh is more ubiquitously expressed. In a growing embryo, lack of Ihh produces developmental defects primarily restricted to aspects of bone development, and Dhh deficiencies result in flawed neuronal development

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and spermatogenesis. In contrast, Shh deficiencies result in extensive defects during embryogenesis and are most famously associated with embryos exhibiting a prominent proboscis, cyclopic eye and holoproscencephaly (failure to completely divide forebrain), as well as numerous other morphological defects. Hh signaling is a core competency during organogenesis and development of complex tissue structures. Hh signaling has been shown to be crucial for early lung development, proper gastrointestinal tract development and assembly of the blood–brain barrier [4–6].

The activity and signaling behavior of secreted Hh protein is determined by multiple evolutionarily conserved post-translational modifications [7]. Hh ligands are the only known secreted proteins modified through a C-terminal covalent linkage to cholesterol. Modification of Hh through cholesterol linkage is thought to favor localization of secreted Hh to the plasma membrane. As a result, Hh signaling is commonly restricted to cells near or adjacent to the cell of origin. In addition to cholesterol linkage, Hh protein is also palmitoylated. Palmitoylation at the N-terminus increases the ligand potency of secreted Hh protein and improves Hh-signaling potential. Most secreted Hh carries both cholesterol and palmitoyl modifications.

Hh signal is transduced through the modulation of multiple inhibitory interactions between members of the Hh pathway. Ligand reception occurs within the primary cilia, a protrusion of the cell membrane that functions as a sensory organelle [8,9], and results in the nuclear translocation of transcription factors, culminating with expression of Hh-target genes (Figure 1). In the inactive state, the 12-pass transmembrane protein PTCH catalytically inhibits accumulation of the G-protein coupled receptor-like receptor, SMO in primary cilia, presumably through transport of an endogenous modulator of SMO [10–13]. In the absence of Hh ligand and cilial accumulation of SMO, the activating members of the GLI-family of zinc-finger transcription factors (GLI1 & GLI2) are also inhibited from co-localizing to the cilia by interaction with cytoplasmic SUFU [14]. Additionally, in the absence of Hh stimulation, an abundance of a truncated form of GLI3 acts as a transcriptional repressor [15]. Pathway activation is initiated when Hh ligand binds to PTCH, triggering PTCH internalization and degradation. Elimination of PTCH from primary cilia relieves PTCH-mediated inhibition of SMO and triggers activation of the intracellular components of the pathway. Downstream of cilial accumulation of SMO, active GLI transcription factors are liberated from SUFU, permitting GLI nuclear translocation and expression of Hh-target genes [14]. Additionally, SMO activation inhibits phosphorylation-dependent truncation of the GLI3 and formation of the transcriptional repressor form, while full-length GLI3 is targeted for rapid proteosomal degradation [16]. Expression of GLI-target genes directly contributes to cell proliferation and cell survival through the expression of cyclin D, cyclin E and BCL-2 [17,18]. Genes for PTCH and GLI1 are also transcriptional targets of the pathway and exemplify the presence of both negative and positive feedback within this system, respectively. Control of progenitor cell maintenance and differentiation involves additional levels of complexity and may involve expression of growth factors, chemokines, and interactions with other developmental pathways that control aspects of self-renewal and differentiation.

Hh-pathway inhibitors

The archetypical inhibitor of Hh signaling is cyclopamine, a natural product derived from *Veratrum californicum,* the California corn lily. In 1957, a group led by Wayne Binns at the US Department of Agriculture identified the wild corn lily as the teratogenic source associated with an epidemic of still-born cyclopic lambs, born to sheep that grazed on this flower on a farm in Idaho [19,20]. The characterization of phenotypic developmental abnormalities induced by consumption of this flower include a single midline cyclopic eye, pronounced proboscis and holoproscencephaly [21]. Although multiple teratogenic compounds were isolated from this flower, the steroidal alkaloid, cyclopamine, was among the most potent [22]. The link between cyclopamine and inhibition of the Hh pathway came 30 years after the isolation of cyclopamine, when it was realized that a similar embryonic phenotype was linked to $Shh^{-/-}$ mice [23]. It was later shown that cyclopamine inhibited Hh signaling through direct inhibition of SMO and could reverse the oncogenic effects of PTCH and SMO mutation [24–26].

Cyclopamine has proved to be an important tool in elucidating the therapeutic potential of SMO inhibitors in cancer. However, limitations to the potency, specificity, solubility and bioavailability of cyclopamine undermine therapeutic utility of this specific agent. Multiple discovery programs, aiming to identify inhibitors of SMO with binding modes that overlap that of cyclopamine, have generated numerous investigational new drugs representing the first class of Hh-pathway targeting agents. As a result, there are many Hh-pathway inhibitors currently undergoing clinical evaluation, all of which possess a cyclopamine-competitive mechanism of action [27–30].

Vismodegib (GDC-0449; Genentech/Roche), NVP-LDE225 (LDE225; Novartis), BMS-833923/XL-139 (XL-139; Bristol Meyer-Squibb/Exelixis) and IPI-926 (Infinity Pharmaceuticals) are the most clinically advanced inhibitors of SMO. Although vismodegib, XL-139 and NVP-LDE225 compete at the cyclopamine binding pocket, they are structurally distinct from the steroidal alkaloid scaffold of cyclopamine and are the result of development activities around structures identified through screens of novel compound libraries. Conversely, the structure of IPI-926 is the result of cyclopamine derivatization intended to yield a more favorable pharmacological profile. Vismodegib is the most clinically advanced of these inhibitors, as the US FDA recently granted approval for use of vismodegib as a first-line therapy in advanced basal-cell carcinoma (BCC), making this the first drug to be approved in this class. This landmark approval was based on preliminary results from a recently completed pivotal Phase II trial involving patients with BCC. Other inhibitors have been described to inhibit the pathway through alternative mechanisms, including inhibition of GLI-mediated transcription, although the utility of these inhibitors currently remains preclinical in nature [31–33].

Clinical development & therapeutic rationales

Tolerability

Hh signaling is an important regulator of maintenance and self-renewal in adult neural stem cells [34]. Hh signaling is also maintained within adult colonic epithelium, where it contributes to the homeostasis of the epithelial precursors [35,36]. Additionally, it is becoming increasingly evident that reactivation of Hh-pathway signaling is a core component to the tissue-damage response, tissue repair and related inflammatory processes [37–40]. Although Hh signaling has been implicated in maintenance and homeostasis of adult stem cell populations, perturbation of Hh signaling does not result in hematopoietic defects, as the signaling pathway is not essential for normal hematopoiesis and maintenance of adult hematopoietic progenitors *in vivo* [41,42]. Hh signaling is also a critical component to hair follicle and lingual taste papillae development [43–46]. As such, inhibition of Hh signaling in adults is commonly associated with alopecia and decreased appetite.

In addition to these relatively benign effects, the severe teratogenic nature of Hh inhibition precludes the use of pathway inhibitors in pregnant women and dictates that application in women of childbearing potential must proceed with extreme caution. In contrast to the severe abnormalities related to embryonic and neonatal development caused by xenobiotic

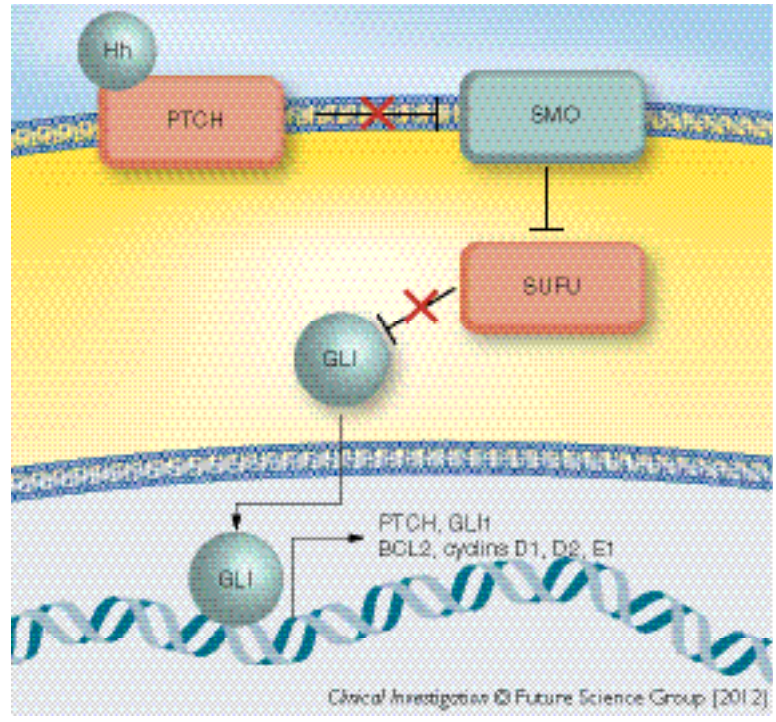

Figure 1. Hedgehog signaling pathway. Signaling proteins that activate the pathway are colored green, whereas signaling proteins that suppress pathway activity are colored red. Hh ligand binds to and inhibits PTCH. When PTCH-mediated inhibition of SMO is eliminated, SMO inhibits sequestration of GLI-transcription factors by SUFU. This allows GLI to translocate to the nucleus and activate transcription of pathway target genes.
Hh: Hedgehog.
Color figures can be found online.

alteration of Hh signaling, the roles of the Hh pathway in adult systems are more safely amenable to therapeutic modulation. While the Hh-signaling pathway retains functional relevance in adults, clinical toxicities associated with SMO antagonists include limited side effects and few instances of grade 3 or higher adverse events (AEs) [47–51]. In addition to alopecia, dysgeusia and decreased appetite, the most commonly reported AEs demonstrated across all inhibitors in this class include muscle spasms, nausea, fatigue and diarrhea (Table 1). Although the prevalence of drug-related muscle spasms demonstrated across these agents is highly suggestive of an on-target cause, the pathogenesis for these events in relation to SMO inhibition remains undefined. Grade 3 or higher AEs are rare with no clear unifying trends across this class of inhibitors. Due to the general tolerability of these agents and lack of high-grade dose-limiting AEs, determination of maximum tolerated doses has been difficult, and in some cases determined maximum tolerated doses are well above maximally effective

Table 1. Hedgehog inhibitors commonly reported adverse events.

Hedgehog inhibitors	Adverse events (n [%])					Half-life (days)	MTD as single agent (mg/day)	Ref.
	Muscle spasms	Dysgeusia	Fatigue	Alopecia	Nausea			
Vismodegib (n = 68)	32 (47)	28 (41)	28 (41)	25 (35)	23 (34)	12	ND	[47,110]
IPI-926 (n = 84)	10 (12)	15 (18)	29 (35)	16 (19)	21 (25)	1–2	160	[49]
XL-139 (n = 28)	12 (44)	12 (44)	2 (7)	4 (15)	3 (11)	>7	360	[48]
LDE225 (n = 35)	6 (17)	2 (6)	6 (14)	N/A	8 (23)	6	800	[50,51]

MTD: Maximum tolerated dose; N/A: Not available; ND: Not determined.

drug doses as determined by limits of pharmacodynamic and pharmacokinetic parameters [50,52]. Notably, these agents generally demonstrate remarkably durable pharmacokinetics, with terminal plasma half-life values exceeding 1 week in several instances (Table 1). Although this class of inhibitor has proven to be well tolerated in adults, it remains to be seen whether the normal roles of Hh signaling in early childhood development will present challenges for translating this mode of therapy to a younger patient population (Box 1).

■ Ligand-dependent & -independent Hh signaling

The rationale for use of a Hh-targeted inhibitor as a therapeutic intervention for different cancers varies depending on the contributions of Hh signaling to the etiology of specific disease states. Aberrant activation of the Hh pathway in cancer has been shown to occur primarily through two mechanisms: ligand-independent signaling within tumors as a result of mutational activation and ligand-dependent signaling occurring through interactions mediated by Hh secretion.

Ligand-independent signaling in cancer

Constitutive activation of the Hh pathway occurring through ligand-independent mechanisms has been identified in BCC and medulloblastoma. Patients with the rare condition of basal cell nevus syndrome (BCNS) provided the first link between Hh-pathway activation and cancer. Also known as Gorlin syndrome, BCNS is associated with sporadic formation of BCCs throughout life and is caused by germline loss-of-function mutation in PTCH [53]. In these cancers, cell proliferation and growth of the bulk tumor is driven by constitutive, ligand-independent pathway activation, resulting from inactivating mutations in PTCH leading to deregulation of SMO. In addition to the majority of BCC cases demonstrating activating mutations in PTCH, activating mutations in SMO are associated with an additional 10% of BCCs [54,55]. Similarly, individuals with Gorlin syndrome are also predisposed to developing medulloblastoma [56–59]. In addition to mutations in PTCH and SMO, inactivating mutations in SUFU have also been described as a marker of increased risk for development of ligand-independent medulloblastoma [60].

The rationale for delivering Hh-pathway-targeted therapies with respect to ligand-independent disease is relatively straightforward. In this setting, Hh-pathway inhibitors demonstrate robust single-agent efficacies and are capable of producing complete cytoreductive responses in the preclinical stage [26,27]. The dramatic responses captured in preclinical systems are the result of direct targeting of the bulk tumor-cell population, where proliferation and survival is dependent and largely 'addicted' to Hh-pathway activation. Rhabdomyosarcoma (RMS) has proven to be an exception to this understanding. Sporadic or BCNS-mediated Hh-pathway activation is also associated with the formation of RMS [61,62]. Mouse models of BCNS reiterate this predisposition [63,64], but unlike medulloblastoma and BCC, Hh-pathway-targeted therapies do not significantly impact RMS tumor growth in the preclinical setting, indicating loss of dependency on SMO-mediated Hh-pathway activation [65,66].

Clinical data from studies of Hh antagonists in ligand-independent disease

The large majority of clinical experience regarding Hh inhibitors in ligand-independent disease comes from patients with locally advanced or metastatic BCC. Most BCCs can be well managed through surgical resection of small, early-detected lesions. In the rare cases when advanced, unresectable or metastatic disease arises, no other effective standard therapies are available. In this setting, Hh-pathway inhibitors are appealing as they target the primary force driving tumor growth and represent the only available pharmacological treatment option. A cohort analysis from a Phase I study of vismodegib in solid tumors provided the first glimpse of clinical responses for a SMO inhibitor in locally advanced and metastatic

BCC [67]. In this cohort, 33 individuals with locally advanced or metastatic BCC were enrolled, with 18 of these patients presenting distant metastases. Of these 33 patients, 18 had objective responses consisting of two complete responses and 16 partial responses, resulting in an overall objective response rate of 58% and 12.8 month median duration of response. Stable disease was demonstrated in an additional third of patients. A 12% minority had progressive disease as a best response in this study. A comparably robust profile for response was seen in a second, investigator-initiated trial of vismodegib in a similar BCC population [68]. In this study, seven of 11 evaluable patients showed histological clearance 3 months after initiation of therapy.

Other preliminary Phase I evaluations of SMO antagonists also indicate some level of efficacy within this disease setting. Preliminary analysis of a 24 patient locally advanced or metastatic BCC cohort from a Phase I study of IPI-926 in advanced or metastatic solid tumors demonstrated six partial responses (25%) and two instances of disease progression (8%) at time of data presentation [49]. Within the cohort defined by locally advanced and metastatic BCC, ten patients remained on the drug for greater than 10 months. Of these patients, five (50%) demonstrated partial responses. Additional evidence from a Phase I study of LDE225 produced one complete response and four partial responses in locally advanced and metastatic BCC [51]. While clearly dramatic responses are seen in these Phase I trials, we are limited only to information gleaned from preliminary analyses of trials designed to measure efficacy, as currently no reports of mature data from completed Phase II trials are available. Preliminary results from the pivotal, single-arm Phase II ERIVANCE study evaluating efficacy of vismodegib in patients with locally advanced and metastatic BCC have been presented by Seculik *et al.* [69]. The results of this larger Phase II study confirms the activity of vismodegib implied by the Phase I cohort analysis and contribute to the basis for the recent approval for the use of vismodegib

Box 1. Therapeutic challenges and opportunities in pediatric cancers.

- Evaluation of Hedgehog (Hh) inhibitors in pediatric indications is sparked by the function of aberrant Hh-pathway activation in the tumorigenesis of medulloblastoma and other malignant diseases, where median age of incidence ranges from early childhood to adolescence. Although the clinical safety profiles for these agents have been well characterized in adult patients, the roles of Hh signaling in early- through to late-developmental stages complicates the translation of these inhibitors in younger patient populations.
- In addition to the critical functions of Hh signaling during postnatal cerebellar development [34,112], multiple immediate and latent defects related to bone growth and development have been identified as potential hazards in this young population. Contrary to the tolerability of pathway inhibition in adult mice, young mice (P10–P14) exposed to SMO antagonists demonstrate loss in bodyweight and permanently stunted growth [113]. The diminutive size of exposed mice is also associated with premature fusion of growth plates and defective epiphyseal architecture, as well as shortening of long bones, resulting from decreased proliferation and increased differentiation of chondrocytes. In addition to these effects on bone growth and development, effects on growing teeth is also of possible concern, as treatment of mice with SMO antagonists has been shown to inhibit Hh-dependent differentiation of amelioblast progenitors, resulting in the stunted growth of mouse incisors [113,114].
- Results from a Phase I study demonstrated that vismodegib is well tolerated in young patients (median age: 11.6 years; range: 4.4–20.9 years) with recurrent or resistant medulloblastoma [115]. In addition to reporting promising tolerability, this Phase I also captured the first evidence of efficacy in this young population in one case of PTCH mutated medulloblastoma.
- Current therapies for pediatric medulloblastoma include surgical resection, radiation therapy and intensive treatment with combination high-dose chemotherapies. These therapeutic options are associated with their own set of long-term health risks, including neurological defects affecting speech, compromised locomotor function, cognitive deficits, growth defects due to hormonal imbalance, hearing loss and increased risk of developing secondary malignancies later in life.
- Similar to the risks associated with current standard-of-care, noted skeletal defects documented in postnatal mice are more likely to be evident in the youngest of treated patients. Like all therapies with potentially hazardous effects, the risk/benefit must be weighed with the application of Hh-pathway inhibitors in pediatric indications. In the case of medulloblastoma, selection of patients demonstrating mutational activated Hh-signaling is paramount to the rational application of Hh-targeted therapies in young patients, as these patients are less likely to respond to current therapies and more likely to benefit from Hh-pathway inhibition. This strategy is currently being employed in a Phase II trial of vismodegib in pediatric recurrent or refractory medulloblastoma (NCT01239316) [205].

in this patient population. Though fully-mature analyses from this study are pending, preliminary results indicate over three-quarters of patients derived clinical benefit from receiving vismodegib. Objective response rates of 43 and 30% were demonstrated in the locally advanced and metastatic BCC cohorts, respectively. Over 80% of patients in both cohorts demonstrated stable disease or better as their best response. The results of this study are impressive and have catalyzed an extraordinarily rare drug approval based on the responses seen in a Phase II study.

Opportunities for evaluating clinical efficacy in adults with advanced metastatic medulloblastoma are limited by the epidemiology of the disease. Medulloblastoma primarily remains a pediatric disease with a median age of 5 years at time of diagnosis. As clinical evaluation of this class of agents is proceeding cautiously in pediatric populations, currently available data on clinical responses is limited to a handful of rare adult cases. Additionally, the proportion of medulloblastomas representing the Hh molecular subgroup most likely to benefit from SMO-targeted agents is less prevalent compared with that found in BCC (Table 2) [70]. The first clinical report of a Hh inhibitor used in a patient with medulloblastoma was in the initial Phase I trial of vismodegib; he was a 26-year-old male with medulloblastoma and widespread skeletal metastases [71]. Within 1 month of starting treatment with vismodegib, resolution of palpable nodules and of bone pain was evident. By the second month of treatment the patient's response was maintained and imaging by fluorodeoxyglucose-positron emission tomography demonstrated dramatic improvement in previously measurable disease. However, within a month following this response, the patient's disease aggressively returned and was no longer responsive to therapy. Sequencing of genomic DNA from recurrent tumors identified a missense mutation resulting in the substitution of histadine for aspartate at the D473 position in SMO, significantly reducing the binding potential for vismodegib [72]. Additional clinical data in medulloblastoma is limited to preliminary evidence of efficacy derived from similarly rare patients currently enrolled in Phase I trials of XL-139, where a single patient remained on the drug for over 450 days at time of data presentation [48], and with LDE225, where a single patient achieved 40% reduction in tumor volume by RECIST as their best response at time of presentation [50].

Ligand-dependent Hh signaling in cancer

While ligand-independent mechanisms of pathway activation are highly prevalent in the case of BCC, mutational activation of the Hh-signaling pathway is rare across the spectrum of other malignancies (Table 2). Increased expression of Hh ligand, leading to ligand-dependent signaling, is more commonly represented in cancer and has been purported to contribute to multiple functional hallmarks of malignant disease. Ligand-dependent signaling has been shown to mediate aspects of tumor growth, metastasis, angiogenesis and interactions within the tumor microenvironment supporting tumor homeostasis. Overexpression of Hh-family ligands has been implicated in cancers of the pancreas [73–75], prostate [76,77], liver [78–80], breast [81], melanoma [82] and lung [83–86] (Table 2).

Four putative models describing the potential mechanisms for Hh-pathway activation contributing to cancer have been proposed [87], and include ligand-mediated signaling through autocrine, paracrine and reverse-paracrine tumor signaling (Figure 2), in addition to a fourth model describing Hh-mediated cancer stem cell renewal that can occur through either of these signaling modes. In the paracrine signaling model, tumors expressing Hh ligand signal to surrounding stroma. In response to ligand stimulation, cells of the surrounding stroma promote tumor growth and homeostasis through putative signaling-mediated expression of tumor growth factors. In reverse paracrine signaling, Hh ligand expressed in the stromal

Table 2. Hedgehog-pathway status in several cancers.

State	Hedgehog expression	PTCH expression	SMO expression	Frequency (%)	Ref.
Normal	*Off*	*On*	*Off*	-	-
Basal-cell carcinoma	*Off*	Mutant-off *On*	On Mutant-on	95	[53,55,54]
Medulloblastoma	*Off*	Mutant-off	On	30–40	[26,57,64]
Pancreatic cancer	On	Off	On	100	[74,75]
Prostate cancer	On	Off	On	100	[76,77]
Small-cell lung cancer	On	Off	On	50	[83]
Hepatocellular cancer	On	Off	On	N/A	[78]
Breast cancer	On	Off	On	100	[81]
Ovarian cancer	On	Off	On	58	[111]

Pathway activating events are non-italic and pathway inhibitory events are italicized.
N/A: Not available.

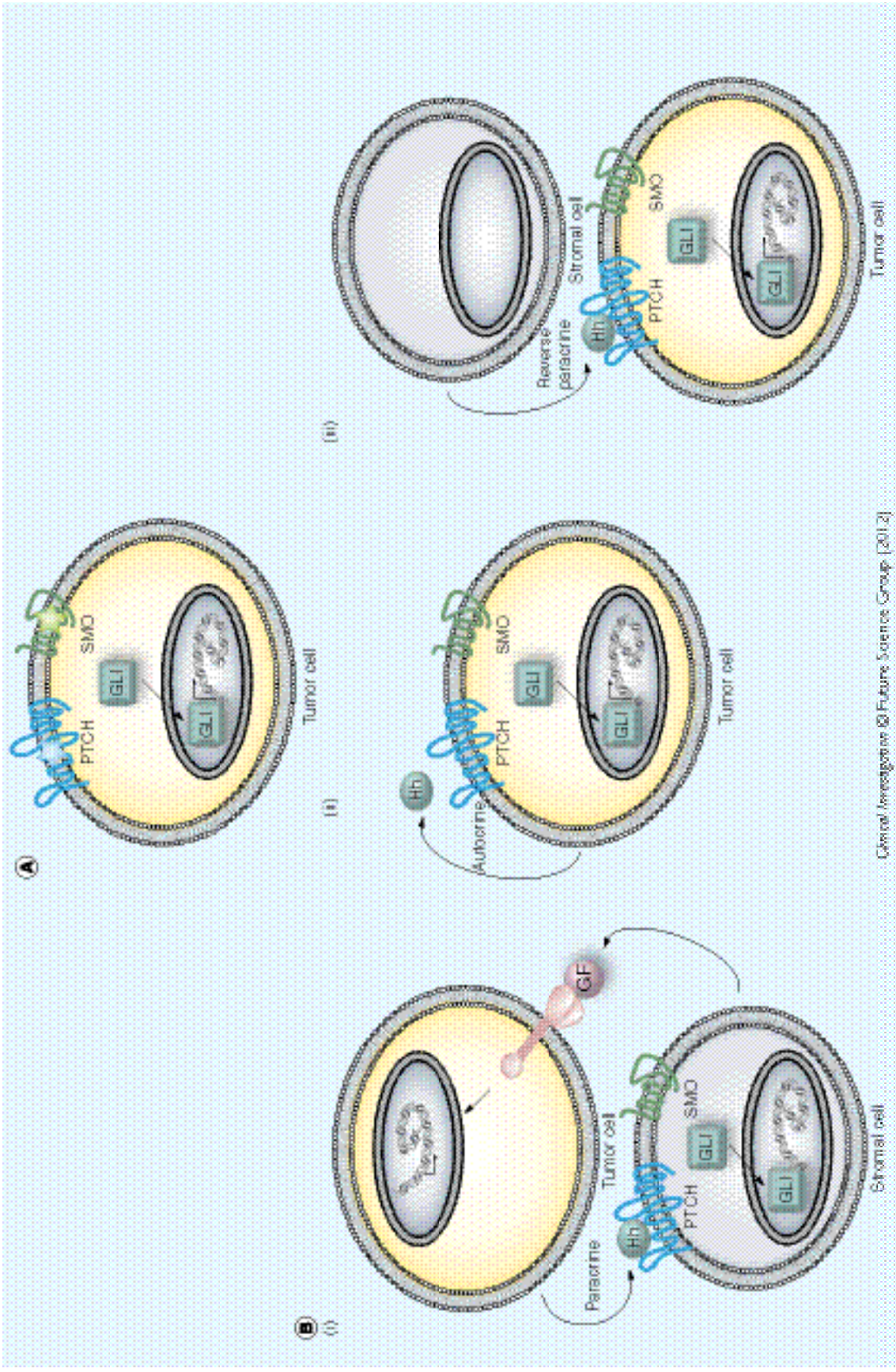

Figure 2. Modes of Hedgehog signaling in cancer. (A) Ligand-independent signaling can occur through pathway activating mutations in PTCH or SMO. Loss-of-function mutations in PTCH (blue star) or activating mutations in SMO (green star) result in constitutive pathway activation, as is commonly seen in basal-cell carcinoma. **(B)** Ligand-dependent signaling can occur by three proposed modes: (i) paracrine signaling, which is stimulated by Hh expression from tumors signaling to stromal cells; (ii) autocrine signaling, which is stimulated by tumor cells secreting Hh to auto-activate the Hh-signaling pathway; and (iii) reverse paracrine signalling, which is stimulated through stromal cell activation of the Hh-pathway back to the tumor (secretion of GFs is depicted).
GF: Growth factor; Hh: Hedgehog.

compartment signals to tumors that express machinery necessary for Hh signaling, stimulating GLI-activated transcriptional programs affecting, for example, proliferative and metastatic potential. The contribution of paracrine Hh signaling to tumor growth has been demonstrated through preclinical modeling, and the notion of Hh-mediated tumor–stroma interactions is believed to be a contributing factor to tumor growth and support in pancreatic, lung, breast and colon cancers [77,81,88,89].

Autocrine or juxtacrine signaling has also been implicated in the instance of small-cell lung cancer, where Hh signaling is thought to promote the survival of a highly clonogenic, chemoresistant subpopulation of cells capable of tumor repopulation following cytoreductive therapies [83,86]. Additionally, the ability of Hh signaling to control differentiation and self-renewal of cancer stem cells is evident in chronic myeloid leukemia, multiple myeloma, breast cancer, glioblastoma and pancreatic adenocarcinoma [89–95].

Unlike cancers that demonstrate ligand-independent Hh signaling, the wealth of evidence supporting the contribution of ligand-dependent signaling in cancer is not indicative of the Hh-pathway functioning as the predominant force driving tumor growth. Rather, ligand-dependent Hh signaling is hypothesized to function in the capacity of supporting tumor growth, metastatic potential or homeostasis, through promoting stromal interaction or maintenance of a cancer-progenitor cell niche. The anticipated efficacy derived from Hh inhibitors in the treatment of cancers demonstrating ligand-dependent signaling is also distinct from that observed in ligand-independent disease. Unlike the encouraging cytoreductive responses generated in preclinical models of BCC and seen in a large portion of locally advanced and metastatic BCC patients, the effects of single-agent administration of Hh-targeted agents have not been found to produce robust primary responses in ligand-dependent systems.

Clinical data from studies of Hh antagonists in ligand-dependent disease

Therapeutic studies of Hh-pathway antagonists are ongoing in a wide array of tumor types. Trial designs represent scenarios for positioning Hh inhibitors as a concomitant therapy with standard-of-care cytotoxic agents or in a maintenance setting following standard cytoreductive therapy. As such, indicators of clinical response with respect to Hh-targeted therapies must also be inclusive of the functional context of Hh signaling in disease-specific tumor biology.

In the instance of small-cell lung cancer, where evidence supports both paracrine and autocrine modes of signaling supporting a population of chemoresistant tumor cells, Hh-targeted therapies are being explored together with chemotherapy and in the maintenance setting following chemotherapy doublet treatment with platinum and etoposide (NCT00887159 and NCT00927875) [201,202]. In this setting, the primary end point for clinical response is a prolongation of progression-free survival (PFS). In a primary xenograft model of small-cell lung cancer, targeting Hh signaling at a state of minimal residual disease following treatment with a chemotherapy doublet prolonged duration of cytoreductive response [86]. A Phase II evaluation in patients with chemotherapy-responsive ovarian cancer under a similar therapeutic strategy has recently been completed (NCT00739661) [96]. This randomized, double-blinded, placebo-controlled, multicenter trial of vismodegib as a single-agent in 104 patients with ovarian cancer, was carried out under the rationale that prolongation of a minimal residual disease state could be achieved through targeting of a subpopulation of cells capable of recapitulating tumor growth [57,97]. However, this study failed to meet its primary objectives as measured by PFS. With a median follow-up time of 5.7 months for all patients, the PFS for placebo was 5.8 months versus 7.5 months in the vismodegib arm. In this study the hazard ratio (HR) for PFS was 0.79 (95% CI: 0.46–1.35; p = 0.39). Cohorts for patients in their second and third remissions demonstrated HRs of 0.66 (95% CI: 0.36–1.20) and 1.79 (95% CI: 0.50–6.48), respectively.

Scenarios also exist supporting concomitant application of Hh-targeted agents with chemotherapy. A total of 11 of the 52 currently registered clinical trials involving vismodegib, IPI-926, LDE225 or XL-139, target pancreatic adenocarcinoma. Evidence for the existence of a Hh-dependent subpopulation of pancreatic adenocarcinoma have been reported and support treatment in a maintenance setting [95]. Additionally, Hh-ligand expression in pancreatic epithelium may promote intraepithelial neoplasia through a process restricted to paracrine signaling to the stroma [74]. Paracrine-Hh signaling to the stroma also contributes to tumor growth and metastasis in models of pancreatic ductal adenocarcinoma that express high levels of Hh ligand [88,95,98]. In addition to stromal components of pancreatic adenocarcinoma contributing to tumor growth through Hh-mediated tumor–stroma interactions, deficiencies in stromal vascularity and the resulting sub-optimal tumor–drug exposure associated with this disease is believed to contribute to general resistance to systemic therapies. In a surprising result, treatment of preclinical mouse models of pancreatic ductal adenocarcinoma with

IPI-926 was able to stimulate angiogenesis of tumor-associated stroma and increase tumor vascularity, facilitating delivery of gemcitabine to tumor tissues and improving antitumor responses [99]. The specific combination of gemcitabine plus Hh-inhibitor therapy to treat pancreatic adenocarcinoma serves as the basis of six clinical investigations across three Hh-targeted agents. Preliminary data from the Phase Ib portion of a Phase Ib/II study evaluating IPI-926 in combination with gemcitabine in metastatic pancreatic cancer showed promising hints of activity, with five out of 16 (31%) of evaluable patients demonstrating partial responses [100]. However, the Phase II portion of this study failed to confirm patient benefit as measured by overall survival. In fact, the study was terminated early due to evidence of survival benefits in the gemcitabine-only arm compared with those receiving IPI-926 in combination [203].

Results from clinical application of Hh antagonists in combination with chemotherapy in ligand-dependent cancers are limited to a single Phase II study of vismodegib in combination with either bevacizumab (anti-VEGF antibody) plus leucovorin, 5-fluorouracil, oxaliplatin (FOLFOX) or bevacizumab plus leucovorin, 5-fluorouracil, irintoecan (FOLFIRI) in metastatic colorectal cancer in the first-line setting (NCT00636610) [204]. This multicenter, randomized, double-blinded, placebo-controlled trial also failed to achieve its primary end point of significantly extending PFS with the addition of vismodegib to the standard-of-care regimen [101]. The HR between vismodegib and placebo receiving arms was 1.24 (95% CI: 0.83–1.87; p = 0.30). In this specific study, four deaths (two from sudden death and two from pneumonia) were reported in the experimental arm (98 patients) and no deaths were reported in the placebo-receiving arm (101 patients). Full efficacy and response data from this study are not yet available.

Although mature clinical data in ligand-dependent cancers are limited to two studies in metastatic colon cancer and chemoresponsive ovarian cancer, which failed to meet primary end points, it remains too soon to discredit the therapeutic potential for Hh inhibitors. Unlike the exceedingly prevalent nature of Hh-pathway activation serving a primary role driving BCC, demonstration of clinical benefit in ligand-dependent diseases may require enrichment based on demonstration of elevated tumor Hh-ligand expression or other predictive biomarkers. An additional challenge to the application of Hh antagonists in the maintenance setting comes from discontinuation of use due to a lower tolerance for adverse effects of these agents in patients with minimal or no residual disease [96]. The lack of statistically significant clinical benefit in the initial colon, ovarian and pancreatic cancer studies, should not be assumed to be predictive of outcome in other cancers linked to Hh signaling, especially in cases where preclinical and translational evidence support a link between Hh-dependent signaling and tumor response. Preliminary evidence of disease control has also been demonstrated in multiple myeloma, for which there is strong evidence supporting both paracrine and autocrine Hh signaling [91,102], with 60% of patients receiving XL-139 in a Phase I trial demonstrating stable disease [103].

The robust responses demonstrated in BCC has resulted in the first approval of a Hh-pathway inhibitor. However, the true potential for Hh-inhibitor therapies in Hh ligand-dependent disease remains uncertain. Results from multiple Phase II trials based in a list of ligand-dependent cancers, including small-cell lung cancer and myelofibrosis, are expected in the second half of 2012. The rolling release of results from ongoing studies will help to clarify the utility of these agents across a diverse set of malignancies.

Future perspective

■ Clinical resistance & therapeutic options for treating resistance to first generation pathway inhibitors

The importance of Hh-pathway activation in the survival of Gorlin syndrome-related cancers is exemplified by major primary clinical responses demonstrated in BCC following treatment with SMO antagonists. However, the dramatic responses yielded from Hh-targeted therapies can be undercut through multiple mechanisms of acquired drug resistance (Figure 3). In addition to the initial identification of the D473H SMO mutation described by the transient clinical response of a patient with metastatic medulloblastoma [71,72], several additional SMO mutations have been found that result in resistance to first-generation SMO antagonists in experimental models of disease. The nature of these mechanisms for resistance fall into four categories: mutations in SMO affecting drug binding [33,104]; pathway-activating mutations downstream of SMO that overcome drug sensitivity; overexpression of activating GLI-transcription factors, and activation of alternative signaling pathways implicated in cancer [104]. Although the incidence and nature of similar mechanisms of acquired resistance in BCC have yet to be defined, there are clear benefits to maintaining robust on-target responses in the face of these resistant phenotypes, especially if development of Hh inhibitors is successful in producing safe and meaningful responses in pediatric malignancies.

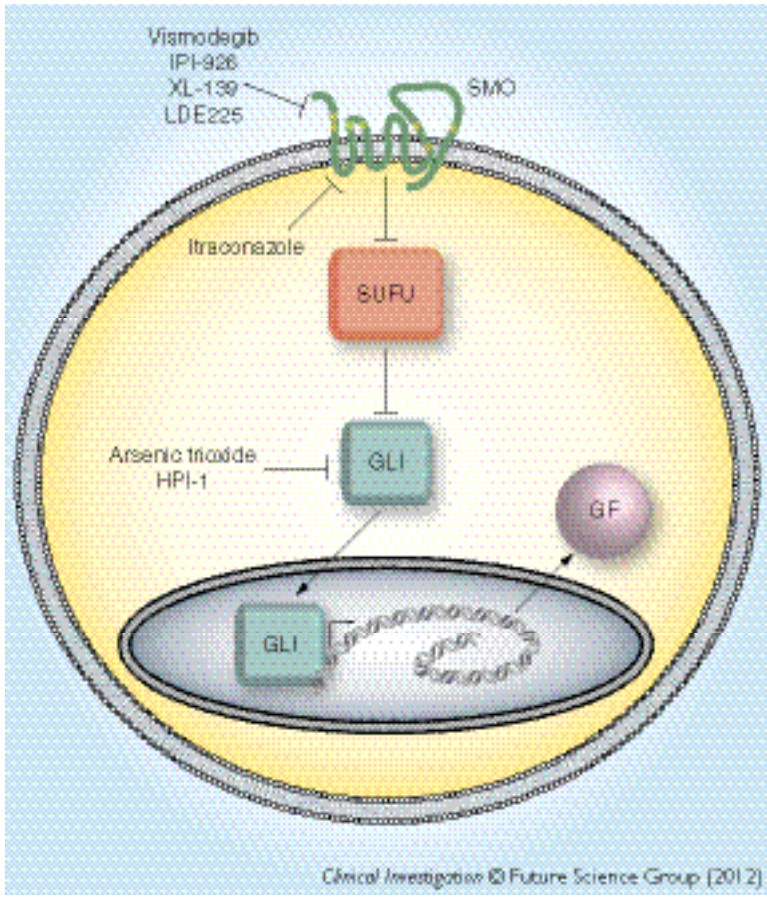

Figure 3. Targeting resistance to SMO inhibitors. Resistance to first-generation SMO antagonists can arise through four primary mechanisms: mutations in SMO that compromise binding of drug; loss-of-function mutations in SUFU; overexpression of activating GLI transcription factors; activation of alternative signaling pathways (putative expression of GFs is depicted). Itraconazole inhibits SMO-mediated pathway activation through a mechanism that is distinct from first-generation inhibitors and may retain potency despite mutations in SMO. Arsenic trioxide and HPI-1 inhibit pathway activation at the level of GLI. Inhibition of activated escape pathways (e.g., IGF1-R, PI3K and mTOR) also represents an option to impact drug-resistant disease (not depicted).
GF: Growth factor.

To address this problem, additional modes for targeting the pathway are necessary. Therapeutic potential in a population resistant to the initial generation of SMO inhibitors may be derived from agents active at multiple levels of the Hh pathway. Mutations in SMO that compromise the binding of first-generation SMO inhibitors emphasize the limitations of the group of compounds currently in clinical development. As all current SMO antagonists under clinical development are defined by the ability to compete for the cyclopamine-binding pocket, mutations that compromise the binding of one agent have the potential to significantly reduce potency across the class. Novel small molecules capable of targeting SMO-mediated signaling in the presence of binding mutations have been identified [32,33,105]. These agents retain potency against SMO mutations that block the binding of vismodegib, either through novel modes of binding within the cyclopamine-binding pocket or through other less well-characterized mechanisms. Not surprisingly, loss-of-function mutations in SUFU, similar to those reported in rare cases of ligand-independent medulloblastoma, also have potential for conferring resistance to SMO inhibitors. Overexpression of GLI-transcription factors also represents a viable route for pathway activation irrespective of the signaling capacity of SMO. In these instances, agents must function at or below the level of GLI in order to impact the fidelity of pathway activation. A few inhibitors have been described to fall into this category [32,33]. For example, the small molecule HPI-1 has been proposed to directly inhibit GLI-mediated transcription [105]. Although HPI-1 has less than optimal drug properties, *in vivo* experiments in models of vismodegib-resistant medulloblastoma using a novel formulation of HPI-1 to achieve adequate drug delivery demonstrate the potential for this proposed class of pathway inhibitors that act downstream of SMO [106].

As novel classes of Hh-pathway-targeted agents are further developed, translation of these early-lead compounds to clinically relevant inhibitors is likely to become a primary focus of ongoing drug development. There is an urgent need for inhibitors that retain activity in the context of acquired resistance, as drug-resistant populations emerge alongside the recent approval of vismodegib as the only pharmacological agent for the treatment of unresectable, advanced BCC. Two clinically available therapeutics have been identified as possible strategies that could be subjected to relatively immediate testing. Itraconazole, an approved triazole antifungal drug, and arsenic trioxide, an anti-neoplastic agent approved for the treatment of acute promyelocytic leukemia, have both been shown to inhibit Hh-signaling and ligand-independent tumorigenesis through mechanisms that are distinct from that of cyclopamine. Itraconazole targets SMO activation through a cyclopamine-independent mechanism of action, whereas arsenic trioxide directly inhibits signal transduction through inhibition of GLI-mediated pathway activation [107,108]. Interestingly, biomarker analysis from patients with metastatic, castration-resistant prostate cancer from a Phase II trial demonstrated that clinically relevant GLI1 downregulation in non-involved skin was seen in 17 (68%) of the 25 patients receiving itraconazole

Executive summary

Introduction to the Hedgehog-signaling pathway

- Ordered Hedgehog (Hh) signaling is critical for normal embryogenesis and early development.
- Aberrant activation of the Hh-signaling pathway is implicated in many cancers and contributes to tumor growth, metastasis, homeostasis and clonogenicity.
- The Hh pathway is normally controlled by endogenous negative regulators of signaling.
- In cancer, overexpression of the Hh ligand or loss-of-function mutations in negative regulators are both common mechanisms for pathway activation.

Hh-pathway inhibitors

- The natural product, cyclopamine, inhibits SMO-mediated-pathway activation immediately downstream of Hh-ligand binding and is the archetypical inhibitor of Hh signaling.
- First-generation inhibitors are cyclopamine-competitive antagonists of SMO.
- Vismodegib (Genentech/Roche) is the first clinically approved Hh inhibitor; approval for use as a first-line agent in advanced basal cell carcinoma was granted in January 2012.
- Hh-pathway inhibitors are well tolerated in adults; tolerability in pediatric populations is amenable to therapeutic application in the Hh-molecular subgroup of medulloblastomas, but concerns exist for long-term effects.

Ligand-independent signaling in cancer

- Functional mutations in signaling proteins result in constitutive, ligand-independent activation of Hh signaling.
- Ligand-independent Hh-pathway activation is present in nearly all cases of basal-cell carcinoma and in a third of all medulloblastomas.
- Hh inhibitors have potent, single-agent cytoreductive efficacy in ligand-independent tumors.
- Clinical efficacy is impressive in advanced basal-cell carcinoma; over 80% of patients obtain meaningful clinical benefit.

Ligand-dependent signaling in cancer

- Overexpression of Hh ligand results in ligand-dependent activation of Hh signaling.
- Ligand-dependent Hh-pathway activation is present across a wide spectrum of cancers; roles of Hh activation in each cancer varies.
- Therapeutic strategies for Hh inhibitors in ligand-dependent cancer are context dependent and require combination with cytoreductive therapies.
- Therapeutic potential for Hh inhibitors in ligand-dependent cancers remains uncertain; disappointing results in ovarian, colon and pancreatic cancers may not be predictive for other cancers.

Future perspective: clinical resistance & therapeutic options for treating resistance to first-generation pathway inhibitors

- Multiple mechanisms of drug-resistance can undermine the robust efficacies demonstrated in ligand-independent cancers.
- Limited mechanistic diversity within current clinically developed inhibitors places substantial focus on developing new drug classes and novel therapeutic strategies for managing or delaying resistance.

[109]. These unexpected results serve as an interesting pharmacodynamic proof-of-principle, warranting further clinical evaluation in ligand-independent disease, especially in a population refractory to first-generation SMO inhibitors. Finally, activation of the PI3K/AKT/mTOR signaling axis has also been shown to facilitate resistance to clinical drug candidates targeting SMO [33]. Treatment of medulloblastoma allografts with concomitant LDE225 and a dual inhibitor targeting PI3K and mTOR resulted in potentiation of tumor response and a delay to onset of resistance. Translating this therapeutic approach to the clinic offers an enticing opportunity for targeting ligand-independent Hh activation, while simultaneously suppressing alternative pathways implicated in resistance. As the first generation of targeted SMO inhibitors begin to reach clinical application, a primary focus will shift to strategies for maintaining robust clinical responses in BCC and medulloblastoma patients.

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BT Aftab owns equitable shares of Exelixis, Inc., licensor of BMS-833923/XL-139. CM Rudin has previously consulted for Genentech, owner of vismodegib, and Novartis, owner of LDE-225. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

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