Biochemical Pharmacology 80 (2010) 712–723

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Small molecule modulation of HH-GLI signaling: Current leads, trials and tribulations

Christophe Mas, Ariel Ruiz i Altaba *

Department of Genetic Medicine and Development, University of Geneva Medical School, 1 rue Michel Servet, CH-1211 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 12 February 2010
Accepted 13 April 2010

Keywords:
Hedgehog
GLI
Smoothened
Cancer
Small molecule

ABSTRACT

Many human sporadic cancers have been recently shown to require the activity of the Hedgehog-GLI pathway for sustained growth. The survival and expansion of cancer stem cells is also HH-GLI dependent. Here we review the advances on the modulation of HH-GLI signaling by small molecules. We focus on both natural compounds and synthetic molecules that target upstream pathway components, mostly SMOOTHENED, and those that target the last steps of the pathway, the GLI transcription factors. In this review we have sought to provide some bases for useful comparisons, listing original assays used and sources to facilitate comparisons of IC50 values. This area is a rapidly expanding field where biology, medicine and chemistry intersect, both in academia and industry. We also highlight current clinical trials, with positive results in early stages. While we have tried to be exhaustive regarding the molecules, not all data is in the public domain yet. Indeed, we have opted to avoid listing chemical structures but these can be easily found in the references given. Finally, we are hopeful that the best molecules will soon reach the patients but caution about the lack of investment on compounds that lack tight IP positions. While the market in developed nations is expected to compensate the investment and risk of making HH-GLI modulators, other sources or plans must be available for developing nations and poor patient populations. The promise of curing cancer recalls the once revered dream of El Dorado, which taught us that not everything that GLI-tters is gold.

Contents

1. Introduction

Control of tissue and organ size in normal development and homeostasis appears to depend on similar mechanisms, and cancer

* Corresponding author.
E-mail address: ariel.ruizaltaba@unige.ch (A. Ruiz i Altaba).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.04.016

and degenerative diseases may have a common link in mechanisms that regulate cell numbers. The last decades have seen the realization that embryonic patterning pathways utilized for cellular communication and involved in cell fate, survival and proliferation are deregulated in multiple human diseases that have abnormal pattern and too few or too many cells. Harnessing the power of these pathways promises to allow the understanding and manipulation of cell behavior and improved therapies for so far deadly human diseases that include many types of terminal and metastatic cancers, as well as degenerative diseases and the general wasting and lack of regenerative abilities associated with aging. Most interestingly, these embryonic pathways, notably including Hedgehog (HH)-Gli, Wnt-βCatenin/Tcf and Notch, also regulate stem cell self-renewal and survival.

In this review we focus on anti-cancer efforts but also mention areas of interest in regenerative medicine. Critically, HH-GLI signaling has been implicated in a large number of human cancers (reviewed in [1,2]): from familial basal-cell carcinomas [3,4] to sporadic basal-cell carcinomas [5], medulloblastomas [6,7], prostate [8,9,118], lung [10,11], pancreas [12,13], breast [14] and colon [15,16] cancers, as well as gliomas [6,17,18,119], leukemias [19,20], lymphomas [21] and melanomas [22]. HH-GLI not only controls the growth of the bulk of the tumor by promoting cell survival and proliferation, but it is also required for cancer stem cell self-renewal in gliomas [17,18], leukemias [19,20] and colon cancers [16]. For example, inhibition of HH-GLI activity in epithelial cells through RNA interference (RNAi) in human carcinomas in vitro and in mouse xenografts leads to tumor disappearance, inhibition of metastatic growth and tumor recurrence [16,17,22]. Targeting HH-GLI signaling is thus an anti-cancer priority. Developing a full understanding of Hedgehog-GLI function, however, will require the intersection and synergism of many disciplines, including importantly, chemical genetics with small molecules.

HH-GLI signaling is a complex pathway that involves a plethora of factors even though the core pathway has few components. Briefly (Fig. 1), secreted HH glycoproteins bind and inactivate the 12-transmembrane protein PATCHED1 (PTCH1), which itself normally inhibits the activity of the 7-transmembrane G-protein coupled receptor-like protein SMOOTHENED (SMOH). Thus, upon presence of the ligand, SMOH is freed from PTCH1 inhibition to act in primary cilia and send a signal that leads to the activation of the GLI transcription factors. Upon HH-SMOH signaling, the GLI proteins cease to act as repressors and turn into full-length activators of target genes. For a more detailed view of signal transduction, SMOH function and general signaling see recent reviews [2,23–26].

The reason(s) why HH-GLI is central to so many kinds of human cancers (Table 1), including those of brain, skin, prostate, colon, lung and pancreas is not trivial. One hypothesis we have put forward is that it is the central and possibly basic role of this pathway in the control of multipotency and stemness that represents the common denominator [1,2]. Moreover, we have also hypothesized that the GLI code integrates HH and non-HH signals [2,25,27]. Recent evidence supports these two ideas and provides a rational, wide-spectrum, basis for new anti-cancer and

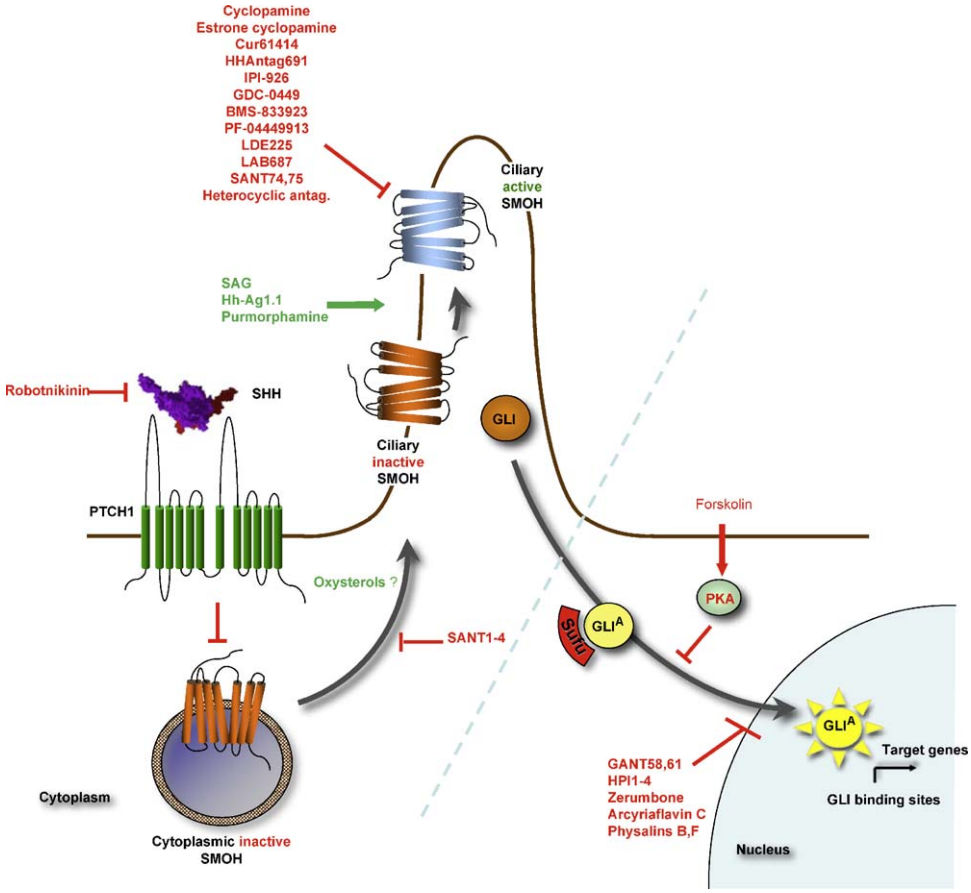

Fig. 1. Schematic diagram of HH signal reception and signal transduction at the primary cilium, focusing on the roles of PTCH1, SMOH and GLI proteins. Small molecules known to affect HH signaling are indicated. The binding of the ligand Sonic Hedgehog (SHH) to the transmembrane receptor PTCH1 results in the translocation of the protein Smoothened (SMOH) to the primary cilium, a subcellular compartment essential for signal transduction to the positive acting isoforms of the GLI zinc finger transcription factors. GLIs then accumulate in the nucleus and activate target gene transcription. The dashed line highlight an approximate general boundary of two arbitrary parts of the pathway (SMOH upstream versus GLI downstream) that are currently targeted for therapeutical applications. See Tables 2 and 3 for details on the different small molecules.

Table 1
Evidence for the involvement of HH-GLI signaling in multiple major sporadic human cancers.

Sporadic cancer type	First references
Skin	
Basal-cell carcinoma	[5]
Melanoma	[22]
Brain	
Medulloblastoma	[6]
Glioma	[6]
Lung	
SCLC	[10]
Non-SCLC	[11]
Pancreas	[12,13]
Prostate	[8]
Stomach	[13]
Colon	[15]
Ovarian	[115]
Breast	[14]
Liver	[116]
Blood	
Multiple myeloma	[19]
Leukemia (CML)	[20]

pro-regenerative approaches in biology and medicine. For instance, major human oncogenes and the pathways that regulate them (e.g. EGF, RAS, AKT) and major human tumor suppressors (e.g. PTEN, p53) regulate GLI1 activity [22,28]. A combinatorial approach with agents that alter pathway activity not only at different levels but also by affecting integrating inputs could thus prove useful.

These results and ideas have prompted a general interest in targeting HH-GLI signaling. Most large pharmas and several biotechs have invested in programs aimed to develop HH-GLI small molecule modulators. Clinical trials with novel anti-cancer compounds have already started (Tables 2 and 3). Yet, an assessment of the field indicates that there is an early massive interest in the blockade of SMOH (Tables 2 and 3), which for reasons that remain unclear, is highly druggable. The many SMOH blockers identified to date appear different and beg the question of mode of action and specificity. Moreover, since a number of cancer cells have been found that are insensitive to SMOH inhibition but require GLI1 activity [e.g. 8,17] an attractive addition or alternative is to develop anti-GLI small molecules. Recent data supports the idea that such molecules can be found [29] (Table 3). Indeed, even though GLI1 is a transcription factor and thus a priori a bad target, it is a rather unusual factor with multiple lives in different cellular compartments [29]. GLI1, and the other GLIs, are exquisitely regulated at different levels, including phosphorylation, acylation, sequestration and degradation [28,30–34]. Each of these steps as well as the partners that physically interact with the GLI proteins provides possible sites for small molecule action. GLI1 is not only a valid target but so far it is also the only reliable and general marker of a cell's response to HH signaling. Measuring *GLI1* levels in relevant human cells is thus a requisite [5,35].

Here we review the very rapidly expanding field of small molecule HH-GLI regulators, attempting to provide a useful primer for ongoing and future studies and comparisons. Two major divisions in this review represent (1) the division of small molecules depending on their source: synthetic versus natural products, which traditionally has proven the most effective source; and (2) molecules that inhibit SMOH versus those that act downstream of SMOH including those that may block GLI function directly. Blocking antibodies, peptides and small RNA inhibitors are not included in this review.

2. HH-GLI antagonists

2.1. Natural compounds

2.1.1. Natural SMOH inhibitors

*2.1.1.1. **Jervine**. Source*: Jervine is an alkaloid isolated from the corn lily *Veratrum californicum*. As Cyclopamine (see below), it

Table 2
Small molecule HH pathway inhibitors and indicative targets in current clinical trials.

Compound	Organization	Target	Cancer type	Status
GDC0449	Roche/Genentech/Curis	SMOH	Medulloblastoma	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Recurrent glioblastoma multiforme	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Basal-cell nevoid syndrome	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Advanced basal-cell carcinoma	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Stomach or gastroesophageal junction	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Metastatic colorectal	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Small cell lung	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Ovarian	Phase II
GDC0449	Roche/Genentech/Curis	SMOH	Metastatic pancreatic	Phase II
+Gemcitabine	Roche/Genentech/Curis	+DNA replication	Metastatic pancreatic	Phase II
BMS-833923	BMS/Exelixis	SMOH	Basal-cell carcinoma	Phase I
BMS-833923	BMS/Exelixis	SMOH	Basal-cell nevoid syndrome	Phase I
BMS-833923	BMS/Exelixis	SMOH	Small cell lung	Phase I
+Carboplatin	BMS/Exelixis	+DNA alkylation	Small cell lung	Phase I
+Etoposide	BMS/Exelixis	+Topo II	Small cell lung	Phase I
BMS-833923	BMS/Exelixis	SMOH	Met. Gastric and esophageal	Phase I
+Cisplatine	BMS/Exelixis	+Topo II	Met. Gastric and esophageal	Phase I
+Capecitabine	BMS/Exelixis	+DNA replication	Met. Gastric and esophageal	Phase I
BMS-833923	BMS/Exelixis	SMOH	Multiple myeloma	Phase I
+Lenalidomide	BMS/Exelixis	+Not known	Multiple myeloma	Phase I
+Bortezomib	BMS/Exelixis	+Proteosome inh.	Multiple myeloma	Phase I
LDE225	Novartis	SMOH	Medulloblastoma	Phase I
LDE225	Novartis	SMOH	Skin basal-cell carcinoma	Phase II
PF0449913	Pfizer	?	Chronic myeloid leukemia	Phase II
+Dasatinib	Pfizer	+Src kinase inh.	Chronic myeloid leukemia	Phase II
IPI-926	Infinity	SMOH	Metastatic solid tumors	Phase I

Table 3
(a) HH-GLI antagonists and (b) HH-GLI agonists.

	Target	Origin	Original assay	Cells	IC50 (μM)[a]	First references
(a) HH-GLI antagonists						
Natural and derivatives						
Jervine	Smo	*Veratrum californicum*	HNF3β mRNA level	Chick neural plate	0.5	[42]
Cyclopamine	Smo	*Veratrum californicum*	Gli Luc reporter	NIH3T3	0.3	[42,45,46]
KAAD-Cyclopamine	Smo	Cyclopamine	Gli Luc reporter	NIH3T3	0.02	[47]
Cyclopamine tartrate	Smo	Cyclopamine	?	?	?	[55]
Cabohydrate–Cyclopamine (5f)	Smo	Cyclopamine	MTS assay	A549	33	[57]
Antigen peptide–Cyclopamine	Smo	Cyclopamine	MTS assay	DU145	?	[58]
IPI-269609	Smo	Cyclopamine	Gli Luc reporter	NIH3T3	?	[59]
IPI-926	Smo	Cyclopamine	Alkaline phophatase induction	C3H10T1/2	0.007	[60]
Vitamine D3	?	Activated 7-Dehydrocholesterol	?	?	?	[64]
Curcumin	?	*Curcuma longa*	*GLI1* and *PTCH1* mRNA levels	DAOY	?	[54]
Zerumbone	Gli	*Zingiber zerumbet*	Gli Luc reporter	HaCaT	7.1	[67]
Staurosporinone	Gli	*Nocardiopsis* sp.	Gli Luc reporter	HaCaT	1.8	[67]
Arcyriaflavin C	Gli	*Arcyria ferruginea*	Gli Luc reporter	HaCaT	11.3	[67]
Physalin B	Gli	*Physalis minima*	Gli Luc reporter	HaCaT	0.66	[67]
Physalin F	Gli	*Physalis minima*	Gli Luc reporter	HaCaT	0.62	[67]
Triazole itraconazole	?	(FDA approved)	?	?	?	[70]
Synthetic						
Robotnikinin	Shh	Macrocycle	Gli Luc reporter	NIH3T3	4	[72]
Estrone Cyclopamine	Smo	Steroidal precursors	Gli Luc reporter	HaCaT	?	[74]
SANT1	Smo	?	Gli Luc reporter	HaCaT	0.02	[91]
SANT2	Smo	Benzimidazole	Gli Luc reporter	HaCaT	0.03	[91]
SANT3	Smo	?	Gli Luc reporter	HaCaT	0.1	[91]
SANT4	Smo	?	Gli Luc reporter	HaCaT	0.2	[91]
TC132	Smo	SANT2 derivative	Gli Luc reporter	HaCaT	0.08	[92]
SANT74	Smo	Chlorobenzothiophene deriv.	Gli Luc reporter	HaCaT	0.07	[93]
SANT75	Smo	Chlorobenzothiophene deriv.	Gli Luc reporter	HaCaT	0.02	[93]
Cur-61414	Smo	Aminoproline	*GLI1* mRNA levels	HaCaT	0.2	[90]
HhAntag691	Smo	Benzimidazole deriv.	*GLI1* mRNA levels	HaCaT	0.04	[75]
GDC-0449	Smo	Benzimidazole deriv.	*GLI1* mRNA levels	C3H10T1/2	0.013	[79]
BMS-833923/XL139	Smo	?	?	?	0.006–0.035	[95]
LAB687	Smo	Ortho-biphenyl carboxamide	Gli Luc reporter	TM3	0.01	[84]
1-Amino-4-benzylphtalazine	Smo	Benzylphtalazine	Gli Luc reporter	TM3	0.003	[85]
LDE225	Smo	?	?	?	?	[87]
Heterocyclic antag.	Smo	Heterocycles	Alkaline phophatase induction	C3H10T1/2	0.000026–42	[98]
PF-04449913	?	?	?	?		[117]
JK184	Gli	Imidazopyridine deriv.	Gli Luc reporter	C3H10T1/2	0.03	[99]
GANT58	Gli	Thiophene with pyridine rings	Gli Luc reporter	NIH3T3	5	[29]
GANT61	Gli	Hexahydropyrimidine	Gli Luc reporter	NIH3T3	5	[29]
HIP1	Gli	?	Gli Luc reporter	NIH3T3	15	[101]
HIP2	Gli	?	Gli Luc reporter	NIH3T3	20	[101]
HIP3	Gli	?	Gli Luc reporter	NIH3T3	30	[101]
HIP4	Gli	?	Gli Luc reporter	NIH3T3	30	[101]
(b) HH-GLI agonists						
Natural and derivatives						
20α-Hydroxycholesterol	?	Cholesterol deriv.	*Ptc1-LacZ* expression	$PZp53^{MED}$	0.1	[103]
22(S)-hydroxycholesterol	?	Cholesterol deriv.	*Ptc1-LacZ* expression	$PZp53^{MED}$	0.2	[102,103]
24-Hydroxycholesterol	?	Cholesterol deriv.	*Ptc1-LacZ* expression	$PZp53^{MED}$	3	[103]
25-Hydroxycholesterol	?	Cholesterol deriv.	*Ptc1-LacZ* expression	$PZp53^{MED}$	1	[103]
Synthetic						
Hh-Ag1.1 and deriv.	Smo	Chlorobenzothiophene	Gli Luc reporter	C3H10T1/2	3	[88]
SAG	Smo	Chlorobenzothiophene	Gli Luc reporter	NIH3T3	0.003	[91]
Purmorphamine	Smo	Purine deriv.	Gli Luc reporter	NIH3T3	1	[106]

[a] IC50 values may not be comparable across different assays and cells.

induces cyclopia, limb and craniofacial malformations in several animal species [e.g. 36,37].

Mode of action (MoA): Jervine was described as a teratogen targeting specifically the cranial neuroepithelium [37]. It was also reported to decrease the proliferation of the cell line C3H10T1/2 [38] and to inhibit the early differentiation of mesenchyme into cartilage [39]. Jervine (10 μM) has also been shown to inhibit the growth of human colon carcinoma HT29 (18% growth inhibition) and liver HepG2 (15% growth inhibition) cell lines [40]. Structurally, Jervine resembles cholesterol and has the ability to inhibit cholesterol synthesis [41]. Jervine, like Cyclopamine, inhibits the response of chick neural plate cells to Sonic Hedgehog (Shh), as indicated by the induction of the dorsal marker Pax7 and the repression of the Isl1 and HNF3β ventral markers [42]. Mechanistically, Jervine binds to Smo and induces its accumulation to the primary cilium in an inactive conformation and prevents its conversion to an active state as Cyclopamine does [43].

In vivo evaluation: Teratogenic effects of Jervine have been reported in sheep, rabbits, hamsters, and chicks [37] but its HH signaling inhibitory activity has not been investigated *in vivo*.

*2.1.1.2. **Cyclopamine**. Source*: Cyclopamine (11-deoxojervine) is another alkaloid isolated from the corn lily *V. californicum*. It was identified as responsible for cyclopia, anophtalmia and abnormalities of midline development observed in the livestock that ate this plant [44,45]. It is active in diverse range and farm animals given

via multiple routes, including intraperitoneally, orally and intramuscular [120].

MoA: Cyclopamine disrupts HH signaling by inhibition of SMOH [42,46]. Using a derivative of Cyclopamine that could be crosslinked after light activation, it was shown that Cyclopamine binds directly to SMOH in green monkey COS1 cells [39]. Using BODIPY-Cyclopamine, a fluorescent derivative, Cyclopamine binding was localized to the SMOH heptahelical transmembrane bundle. These data suggest that Cyclopamine inhibits SMOH via a protein conformational shift. Indeed, SMOH is thought to exist in 2 states, active and inactive [47,48]. PTCH1 itself is suspected to regulate the switch between active and inactive SMOH trough a small molecule [49]. Moreover, all the activating SMOH mutants identified, with mutations along transmembrane domains TM6 or TM7, show resistance to Cyclopamine [47]. Surprisingly, recent evidence suggest that Cyclopamine disturbs SMOH trafficking and promotes its accumulation in the primary cilium similar to that reported for HH ligand and several HH agonists [50,51]. These data support a model in which SMOH is activated through a multi-step process, with Cyclopamine preventing the conversion between a ciliary inactive into a ciliary active SMOH [51]. Cyclopamine synergizes with the classical chemotherapeutic temolozomide, a DNA alkylating agent [17], with Gemcitibine, a pyrimidine analogue [53], and with Curcumin [54].

In vivo evaluation: Cyclopamine inhibits tumor growth in a $Ptc1^{(+/-)};p53^{(-/-)}$ mouse medulloblastoma model [52], in human orthotopic glioma [17], melanoma [22], colon cancer [16], pancreas [53] and prostate [9] xenograft models.

2.1.2. Cyclopamine derivatives

2.1.2.1. ***Cyclopamine tartrate salt.*** *Source*: A tartaric acid salt of purified Cyclopamine has increased solubility as compared to Cyclopamine [55].

MoA: Same as Cyclopamine.

In vivo evaluation: This Cyclopamine derivative has been used in a recent toxicological study attempting to define the window of susceptibility to synophtalmia in sheep. Results reveal that ingestion of *V. californicum* must occur during gestational day 13 or 14 to induce developmental defects in offspring. Later ingestion does not disturb development. Toxicokinetic analyses conducted on ewes injected intravenously with Cyclopamine tartrate indicated that the compound was completely eliminated from serum after 8 h [56].

2.1.2.2. ***KAAD-Cyclopamine.*** *Source*: KAAD-Cyclopamine is a N-aminoethyl aminocaproyl dihydrocinamoyl Cyclopamine derivative [47].

MoA: Same as Cyclopamine. KAAD-Cyclopamine was reported to exhibit 10–20 fold higher potency than Cyclopamine as demonstrated by activity reduction in primary fibroblasts derived from $Patch1^{-/-}$ mice [47].

In vivo evaluation: Not available (NA).

2.1.2.3. ***Carbohydrate–Cyclopamine conjugate.*** *Source*: A library of carbohydrate–Cyclopamine conjugates was developed to increase structural diversity in an attempt to improve its bioavailability, notably the low solubility in aqueous or polar solvents [57].

MoA: Same as Cyclopamine. The activity of these compounds was tested in human A549 lung cancer cells using a colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)) assay to analyze cell viability and cytotoxicity. Most of the compounds were less efficient than Cyclopamine, except one conjugated to L-rhamnose, compound 5f, which displayed higher potency and increased solubility [57].

In vivo evaluation: NA.

2.1.2.4. ***Antigen peptide–Cyclopamine conjugates.*** *Source*: To target prostate cancer cells, two novel peptide–Cyclopamine conjugates with prostate-specific antigen (PSA)-activated prodrugs were synthetized [58].

MoA: Same as Cyclopamine. Cyclopamine coupled at the second amine to a specific peptide (HSSKLQ or SSKYQ) is inactive (prodrug) until the peptide is recognized as substrate and cleaved by the prostate tissue-specific serine protease PSA, then rendering the Cyclopamine active. The ability of these prodrugs to be cleaved in human and mouse blood serum was investigated, Mu-SSKYQ-Cyclopamine prodrug being the most efficiently hydrolyzed. The efficiency on growth inhibition of the two prodrugs was assayed in PSA-non-expressing human DU145 prostate cancer cell line, exposed or not to PSA. Both drugs showed potency to impair cancer cell growth after activation by exogenous PSA [58].

In vivo evaluation: NA.

2.1.2.5. ***IPI-269609 and IPI-926.*** *Source*: Chemical tinkering with Cyclopamine yielded IPI-269609, which was selected due to its ability to decrease GLI-mediated luciferase reporter activity in mouse NIH3T3 stably transfected cells [59]. IPI-926 resulted from the medicinal chemistry modification of IPI-269609 [60].

MoA: Same as Cyclopamine. IPI-269609 affects cell motility and in vitro colony formation of human pancreatic cancer cell lines. IPI-269609 is water-soluble and shows no signs of toxicity or side effects [59]. However, its development as a drug candidate was discontinued because of limited potency and metabolic stability [60].

IPI-926 has improved potency, pharmacokinetics and metabolic stability over IPI-269609 as demonstrated by efficient inhibition of HH signaling in mouse C3H10T1/2 cells, longer elimination half-life in mouse, dog and monkey plasma and greater stability in human liver microsomes. Competition binding experiments using BODIPY-Cyclopamine in C3H10T1/2 show that IPI-926 is a strong SMOH inhibitor [60].

In vivo evaluation: IPI-269609 did not significantly inhibit tumor growth in mice, but inhibited distant metastases from orthotopic xenografts of human pancreatic cancer cells in mice. In pancreatic xenografts, IPI-269609 reduced intra-tumoral *Ptch1* mRNA levels and reduced a subpopulation of aldehyde dehydrogenase-bright cancer cells which have been identified as clonogenic, tumor-initiating cells [59].

IPI-926 anti-tumor activity was assayed in a mouse genetic model of medulloblastoma ($Ptch1^{+/-};Hic1^{+/-}$). Treatment led to tumor regression after daily oral administration (40 mg/kg), with no recurrence after 30 days of total treatment [60]. This compound also inhibited lung and pancreatic xenograft growth [61,62]. Strangely, IPI-926 did not affect epithelial tumor cells. However, co-administration of IPI-926 (40 mg/kg) and gemcitabine (50 mg/kg) to a mouse genetic model of pancreatic ductal carcinoma led to the depletion of desmoplastic stroma, stimulation of angiogenesis and improvement of gemcitabine delivery to the epithelial tumor [63]. IPI-926 clinical trial Phase I is ongoing.

2.1.2.6. ***(Pro-)Vitamin D3.*** *Source*: 7-Dehydrocholesterol is a cholesterol precursor in mammals that also yields vitamin D3 by UV light conversion.

MoA: The presence of 3β-hydroxysteroid in culture medium has been suggested to be directly proportional to the expression levels of *Ptch1*, indicating that (pro-)vitamin D3 could be the endogenous compound that naturally inhibits Smo [64]. (Pro-)vitamin D3 showed potency to inhibit Gli-mediated

transcription as reported by luciferase assay in various cell lines including mouse C3H10T1/2 cells and in *Ptch1*$^{-/-}$ MEFs. Radioactive competition experiments indicated specific binding of (pro-)vitamin D3 to SMOH at the same site as Cyclopamine. All together, these data suggest that Vitamin D3 or one of its close precursors could be the naturally occurring inhibitor of the Hh pathway by targeting smoothened [64] and could be therefore useful as a tumor suppressor [65]. However, few articles have appeared since these initial findings.

In vivo evaluation: Treatment of zebrafish embryos with (pro-) vitamin D3 recapitulated the phenotype observed in *Smo*$^{-/-}$ mice [64].

2.1.2.7. ***Curcumin.*** *Source*: Curcumin (diferuloylmethane) is a natural product extracted from the rhizome of *Curcuma longa* (turmeric), a traditional Indian spice [66]. Interestingly, turmeric is a member of ginger family (*Zingiberaceae*), from which another natural Hh inhibitor has been identified (see below Zerumbone [67]).

MoA: Not known. Curcumin is a polyphenolic pharmacologically active substance that has been used in traditional medicine mainly because of its antiseptic activity. Recently, anti-cancer properties of Curcumin, including inhibition of cell proliferation and induction of cell death have been reported in several cancer animal models as well as in human clinical trials [68]. The action of Curcumin appears to be pleiotropic and affect several pathways, among them Wnt signaling (Curcumin decreased βCatenin levels in human medulloblastoma cells) and HH signaling [54]. Treatment of primary human medulloblastoma cells and the DAOY medulloblastoma cell line with 40 μM Curcumin resulted in the downregulation of SHH as well as *PTCH1* and *GLI1* and the downstream proteins N-MYC, C-MYC, CYCLIN D1 and BCL2. Moreover Curcumin and Cyclopamine produce synergistic effects against medulloblastoma primary cells [54]. The exact MoA of Curcumin on the HH pathway is not known.

In vivo evaluation: A number of clinical trials have investigated the anti-cancer potency of Curcumin in colon, pancreas and breast cancers with a low response rate ($\sim$10%) revealing the poor bioavailability of native Curcumin but validating its anti-proliferative effect [68,66].

2.1.3. Natural GLI antagonists

2.1.3.1. ***Triazole itraconazole.*** *Source*: Marketed as Sporanox this compound is a triazole prescribed to patients with fungal infections [69].

MoA: Triazole itraconazole acts to inhibit pathway activity downstream of Ptch1 but apparently by a mechanism distinct from that of Cyclopamine and other Smo antagonists that is not detailed [70].

In vivo evaluation: Systemically administered Triazole itraconazole suppresses the growth of medulloblastoma in a mouse allograft model [70].

2.1.3.2. ***Zerumbone, Staurosporinone, Arcyriaflavin C, Physalin B, and Physalin F.*** *Source*: Ninety-four natural products and 192 substances from an extract library of tropical plants from southeastern Thailand were tested to identify GLI-mediated transcription repressors, using a human keratinocyte (HaCaT) cell-based reporter assays in which tetracycline inducible GLI1 or GLI2 induce the activity of a luciferase reporter [67].

MoA: Five compounds were identified that present the ability to inhibit both GLI1 and GLI2 mediated transcription with similar efficacy. Zerumbone, a sesquiterpene isolated from *Zingiber zerumbet*, Staurosporinone and Arcyriaflavin C, two bisindoles alkaloids, and Physalin B and F, isolated from the tropical plant *Physalis minima*, decreased the expression of GLI1, PTCH1 and BCL2 in HaCaT cells [67]. Because bisindoles alkaloids have been reported as kinase inhibitors, Staurosporinone and Arcyriaflavin C could inhibit PKC-δ, a protein kinase required for adequate GLI1 transcription [33,121]. Physalins are also known modulators of NF-κB cascade [71]. Zerumbone, Staurosporinone and Physalin B and F decreased the proliferation of the human pancreatic cancer cell line PANC1 [67].

In vivo evaluation: NA.

2.2. Synthetic compounds

2.2.1. Synthetic HH inhibitors

2.2.1.1. ***Robotnikinin.*** *Source*: Identified by binding screening of ShhN against a library of 10,000 small molecules covalently linked to a glass surface (Small Molecule Microarray) [72,73].

MoA: This methodology led to the identification of a first small molecule (hit 1) able to bind Shh. It showed moderate inhibition of HH signaling as indicated by GLI-Luciferase reporter activity in mouse NIH3T3 cells. Chemical tinkering led to the synthesis of Robotnikinin [73], which inhibits SHH mediated GLI transcription with a similar potency than Cyclopamine. The inhibition of the Hh pathway was not observed in mouse *Ptch1*$^{-/-}$ cells, indicating that Robotnikinin acts upstream of Ptch1. As expected, the inhibitory effect of Robotnikinin is rescued by treatment with Smo agonists like SAG and purmorphamine (see below). These results were recapitulated in mouse C3H10T1/2 cells where Robotnikinin antagonized the induction of SHH-dependent alkaline phosphatase and in human primary keratinocytes where it decreased endogenous *GLI1* and *GLI2* levels. In a synthetic human derived skin model, Robotnikinin was also able to reduce *GLI1* and *GLI2* mRNAs levels [72].

In vivo evaluation: NA.

2.2.2. Synthetic SMOH inhibitors

2.2.2.1. ***Estrone-derived Cyclopamine analogue.*** *Source*: Structurally simplified, Cyclopamine-like compound made fully synthetically from rather inexpensive commercially available steroidal precursors [74].

MoA: Estrone-derived Cyclopamine analogue demonstrates efficient SHH signaling inhibition at micromolar concentration (10 μM) in different biological models: It decreases SHH-induced proliferation of mouse granule neuron precursors by 10-fold. It is equipotent with Cyclopamine in blocking SHH activity as measured by a GLI-Luciferase reporter assay in mouse Light2 cells [74].

In vivo evaluation: NA.

2.2.2.2. ***HhAntag691.*** *Source*: High-throughput screening of a library of small molecules using the GLI-luciferase reporter assay in mouse C3H10T1/2 cells first identified HhAntag691 [75]. HhAntag691 belong to the class of benzimidazole from which SANT2, another SMOH antagonist, originates.

MoA: HhAntag691 works by suppressing Smo activity and decreases *Gli1* and *Gli2* levels [75]. HhAntag691 is able to cross the blood brain barrier after oral delivery. Oral gavage reduced the tumoral expression of *Gli1* (but not *Ptch1*), and blocked tumor growth by increasing apoptosis in a mouse Ptch1$^{+/-}$p53$^{+/-}$ model of medulloblastoma [75]. HhAntag691 also blocks Shh-induced proliferation of cerebellar granule neural precursor isolated from postnatal day 6 (P6) mice [75]. HhAntag691 also inhibits ATP-binding cassette (ABC) transporters ABC2 and Pgp [76]. HhAntag691 sensitized tumoral cells like NCI-H460 (human non-small

cell lung carcinoma cells) to cytotoxic agents that are usually exported out of the cells by ABC transporters [76,77].

In vivo evaluation: In addition to impairing medulloblastoma growth in Ptch1$^{+/-}$p53$^{+/-}$ mice, oral administration of HhAntag691 reduced the growth of pancreatic and colon adenocarcinoma primary cells xenografted in nude mice by affecting the stroma only [78]. Strangely, no effects were reported in the epithelial tumor cells themselves. HhAntag691 inhibited also the growth of xenografted HT29 and HT55 colon cancer cell lines but had no effect on tumors derived from xenografted colon cancer DLD1 cells in which SHH production was not detectable [78]. HhAntag691 downregulated Hh targets in the mouse stromal microenvironement, but not within the human tumor epithelium as suggested by PCR analyses [78].

*2.2.2.3. **GDC-0449**. Source*: Derived from the optimization of HhAntag691 by medicinal chemistry, GDC-0449 has improved solubility and absorption properties [79].

MoA: GDC-0449 pharmacokinetic and drug metabolism properties have been evaluated in rat, mouse, dog and *Cynomolgus* monkey (GDC-0449 hydrochloride salt in this case) pre-clinically [80]. Globally, results indicated a low to moderate (monkey) plasma clearance, a high metabolic stability based on hepatocyte concentration and secondary metabolite production, and good oral bioavailability. GDC-0449 does not inhibit P450 isoforms or P-glycoprotein. GDC-0449 half-life ranges from 5 to 20 days in ptients [80].

In vivo evaluation: GDC-0449 induced tumor regression of a mouse medulloblastoma allograft derived from *Ptch1*$^{+/-}$ mice [79]. To date, GDC-0449 has been evaluated in two Phase I clinical trials. The first one concerned 68 subjects with advanced basal-cell carcinoma [81] with duration of 9.8 months. More than half the patients treated responded. A second clinical trial was performed on a patient with metastatic medulloblastoma [82,83], whom treatment resulted in tumor regression and improvement of general symptoms. However, the initial response to the treatment ceased after 3 months. A mutation in SMOH was detected in tumor tissue that may explain resistance [83]. GDC-0449 is now in Phase II focusing on BCCs, medulloblastoma, advanced stomach cancer, gastroesophageal junction cancer, small cell lung cancer, metastatic colorectal cancer, basal-cell nevus syndrome, metastatic solid tumors, recurrent glioblastoma, pancreatic and ovarian cancer (Table 2).

*2.2.2.4. **LAB687**. Source*: Isolated from the screening of a library of synthetic small molecules on a GLI-luciferase reporter assay in mouse TM3 Leydig cells [84].

MoA: LAB687 was first known as an antagonist of microsomal triglyceride transfer protein (MTP) that inhibits the secretion of ApoB, the primary apolipoprotein of LDL. It was then identified as a potent inhibitor of GLI-mediated transcription as reported by a GLI-luciferase reporter assay, and as an anti-proliferative agent using the MTS assay [84]. LAB687 derivatives with improved HH antagonist potency and decreased MTP activity were produced. Structure–activity relationship (SAR) studies established that benzylamines are better than carboxamides or sulfonamides to antagonize the HH pathway. Competition SMOH binding assays using a tritiated form of the Hh pathway agonist Hh-Ag1.1 revealed that these compounds (8b, 21b and 22b) target both mouse and human SMOH proteins [84].

In vivo evaluation: NA.

2.2.2.5. 1-Amino-4-benzylphtalazines. Source: Antagonists were identified by cell-based screening from the Novartis compound collection and analogues synthesized, leading to the selection of a molecule that is orally bioavailable and has anti-tumor activity [85].

MoA: Compound 24, a 1-amino-4-benzylphtalazines analogue, was able to inhibit the HH pathway with an IC_{50} of ~3 nM, as reported by a Gli-luciferase assay in stably transfected mouse TM3 cells stimulated with the HH agonist Ag1.5 [85]. Radiolabeled competition assays demonstrated that compound 24 interacts both with the mouse and human SMOH proteins.

In vivo evaluation: Pharmacokinetic properties (plasma clearance, body distribution volume, half-life, oral bioavailability) of compound 24 were evaluated in nude mice. Compound 24 (20 and 40 mg/kg) efficiently decreased tumor volume in a *Ptch*$^{+/-}$;*p53*$^{-/-}$ mouse medulloblastoma allograft model and reduced intratumoral levels of *Gli1* mRNAs by 80% 4 h after initiation of treatment [85].

*2.2.2.6. **LDE225**. Source*: LDE225 is an orally bioavailable small molecule from the Novartis collection that selectively binds to SMOH [86].

MoA: LDE225 potency was evaluated in vitro in CD34^{+} chronic myeloid leukemia (CML) primary cells. LDE225 did not reduce proliferation and did not increased apoptosis in short-term cultures [87]. LDE225 may target CML stem cells as it reduced secondary colony formation in long-term cultures [87].

In vivo evaluation: NA.

*2.2.2.7. **CUR61414**. Source*: Isolated from the screening of 100,000 synthetic organic molecules on a GLI-luciferase reporter assay in mouse C3H10T1/2 [88].

MoA: CUR61414 is more potent than Jervine in reducing Gli-mediated-luciferase activity in C3H10T1/2 cells [88]. The specificity of this activity was confirmed in the chick neural plate assay where the expression of Hh activity markers (Pax7 and Nkx2.2) was regulated by CUR61414, while markers for BMP or Wnt pathways were not affected. Moreover, CUR61414 is also able to block Hh signaling in Ptch1$^{-/-}$ mouse fibroblasts indicating that its target is downstream of Ptch1 [88].

Competition and immunobinding experiments in human HEK 293T cells with Cyclopamine and an agonist of the Hh pathway, Hh-Ag, demonstrated that CUR61414 is a direct ligand of SMOH and prevents its activation [88]. Moreover, using a constitutively active mutant of SMOH (W539L) [89] to perform competition binding analysis, CUR61414 showed reduced affinity for the mutated form of SMOH (by 7-fold), indicating that as Cyclopamine and Jervine, CUR61414 binds to a site on SMOH that is affected by this mutation [88].

In vivo evaluation: Positive activity in *ex vivo* chick neural plate assays and in a *Ptch1*$^{+/-}$ mouse embryonic skin basal-cell carcinoma model [90]. CUR61414 was able to block the formation and to induce the regression of basaloid lesions appearing in this model or induced by UV exposure, whereas the proliferation of normal basal keratinocytes was unaffected. It increased the rate of tumor cell apoptosis [90]. A phase I clinical trial on basal-cell carcinomas was stopped after negative results, likely due to the failure of the drug and/or formulation to penetrate human skin [122].

*2.2.2.8. **SANT-1, SANT-2, SANT-3, SANT-4, and TC132** (SANT2 derivative). Source*: Isolated from the screening of a 10,000 compounds library acquired from Chembridge using the Gli-luciferase assay in mouse NIH3T3 cells [91].

MoA: SANT compounds bind directly to SMOH as demonstrated by their ability to inhibit the association of BODIPY-Cyclopamine with human HEK SMOH expressing cells in a competition assay. SANT may target the same site than Cyclopamine [51,91]. However, unlike Cyclopamine and its derivatives, the SANT compounds inhibit Gli reporter expression driven by the SMOH

mutant SMOA1 (W539L), indicating that they inhibit Smo activity by different mechanisms [92].

It has also been shown that SANT1 and SANT2 prevent the Shh-induced enrichment of SMOH in the primary cilia of mouse NIH3T3 cells [51]. SANT1 and SANT2 thus seem to inhibit the Hh pathway by locking SMOH in an inactive cytoplasmic state [51] in contrast to Cyclopamine which prevents SMOH activation in cilia. A series of compounds have been derived from SANT2 by synthetic chemistry and tested for their potency to inhibit Gli-luciferase reporters, and for their teratogenic effects in Medaka fish [92]. The benzimidazole ring is essential for inhibitory properties. A SANT2 derivative (TC132) presenting slightly more potency has been reported [92].

In vivo evaluation: Neither SANT2 nor TC132 were able to induce cyclopia in Medaka embryos, due to reduced bioavailability as compare to Cyclopamine [92].

*2.2.2.9. **SANT74 and SANT75**. Source*: A small molecule library of 61 SAG analogues, a SMOH agonist, was screened on a Gli-GFP transgenic zebrafish model to identify in vivo potent and specific Hh signaling inhibitors [93].

MoA: Two compounds, SANT74 and SANT75, blocked Gli-GFP expression and produced the expected developmental defects consecutive to Hh pathway inhibition in zebrafish. No change in *Axin2* expression, a Wnt pathway target gene, was detected, suggesting specificity. Endogenous *Gli1* and *Ptc1* mRNAs levels were decreased in a dose dependent manner by both compounds. In mouse NIH3T3, SANT74 and SANT75 decreased Gli-luciferase activity with an IC_{50} of 70 and 20 nM, respectively. Competition experiments in human HEK 293T cells with BODIPY-Cyclopamine demonstrated that SANT75 is a SMOH antagonist. FRET analyses indicated that SANT74 and SANT75 prevent the conformational change of SMOH from an inactive to active state [93].

In vivo evaluation: SANT74 and SANT75 inhibit Hh signaling in zebrafish embryos [93].

*2.2.2.10. **BMS-833923/XL139**. Source*: NA [94].

MoA: BMS-833923, previously XL139, is an oral SMOH antagonist. It competes BODIPY-Cyclopamine in SMOH binding [95]. In vitro it decreases *GLI1* and *PTCH1* mRNA expression and it also inhibits the proliferation of various human hematologic cancer cell lines [95].

In vivo evaluation: *In vivo*, BMS-833923 reduced medulloblastoma and pancreatic carcinoma xenograft growth in mice [95] and also reduced Gli1 protein and mRNA levels in regenerating mouse skin during wound healing [96]. An ongoing Phase I study of BMS-833923 in subjects with advanced or metastatic solid tumors, indicates that treatment is well tolerated by patients and that BMS-833923 has pharmacological activity in human tissues [97].

*2.2.2.11. **Heterocyclic antagonist**. Source*: Synthetic heterocyclic compound [98].

MoA: SMOH antagonist found to compete BODIPY-Cyclopamine in human HEK293 cells. IC_{50} ranging from 26 pM to 42 μM in mouse C3H10T1/2 cell alkaline phospatase induction assay.

In vivo evaluation: NA.

2.2.3. Synthetic GLI antagonists

*2.2.3.1. **JK184**. Source*: A small molecule library of about 20,000 heterocycles was screened on mouse C3H10T1/2 cells stably transfected with Gli-luciferase reporters and induced by recombinant SHH-N [99].

MoA: JK184 is a derivative of an imidazopyridine able to inhibit GLI-dependent transcriptional activity with an IC_{50} value of 30 nM. In mouse C3H10T1/2 cells, *Gli1* and *Ptch1* mRNA levels are also decreased after JK184 treatment. JK184 is efficient to inhibit *in vitro* proliferation of various human cancer cell lines (e.g. pancreas, medulloblastoma) as well as 50% inhibition of pancreatic cancer xenograft growth after oral gavage for 7 days (0.2 mg/day) [99]. The imidazopyridine core is required for this property. JK184 may not act through direct Smo binding as it does not compete with BODIPY-Cyclopamine. JK184 binds Adh7 protein (a class IV alcohol dehydrogenase) as determined by affinity chromatography. It is able to block the in vitro enzymatic oxidation of retinol that is a substrate of Adh7. Therefore, JK184 seems to antagonize the Hh pathway via Adh7. Knockdown of *Adh7* with siRNAs resulted in 60% decreased of *Gli1* and *Ptch1* in C3H10T1/2 cells activated with 100 nM Hh–Ag. Cimetidine and ranitidine, two known inhibitors of Adh7, reproduced this result [99]. However, a second study showed that JK184 is a microtubule-destabilizing agent that caused depolymerization by acting directly on tubulins subunits [100], and that its effect on Adh7 is indirect. Moreover, microtubule disassembly is a complex mechanism that apparently regulates both positively and negatively Gli function, making JK184 not a highly selective Hh pathway inhibitor [100].

In vivo evaluation: NA.

*2.2.3.2. **GANT58 and GANT61**. Source*: A library of 1990 compounds from the USA National Cancer Institute was screened using the GLI-luciferase reporter assay in human HEK293 cells induced by GLI1 overexpression [29].

MoA: Two compounds GANT58 (thiophene core) and GANT61 (hexahydropyrimidine derivative) were selected for their efficiency to reduce GLI1- and GLI2-dependent reporter transcription [29]. GANT compounds also inhibit: (i) GLI-mediated transcription in mouse NIH3T3 cells stably transfected with the GLI-Luciferase reporter construct and stimulated by SAG agonist, (ii) *Gli1* mRNA levels in *Ptch1*$^{-/-}$ mouse embryonic fibroblasts and (iii) alkaline phosphatase induction in mouse C3H10T1/2 cells. Because GANT compounds were able to reduce *Gli1* and *Hip1* levels in Sufu$^{-/-}$ MEFs whereas Cyclopamine had no effect, GANT compounds antagonize the Hh pathway by acting on targets downstream of Smo and Sufu. Microarray data suggest the specificity of GANT61. GANTs inhibit human tumor cell line proliferation (PANC1 pancreas, 22Rv1 prostate) and growth of their xenografts in mice. Importantly, Cyclopamine and GANT58 prevented additional growth of the tumors while GANT61 induced their regression, without any obvious side effects. Absence of Creb phosphorylation after GANTs treatment suggests that these compounds do not inhibit GLI through PKA like forskolin. EMSA assays revealed that GANT61 reduce the DNA binding ability of Gli1 [29]. However, GANT61 did not have any effects in an independent study [101].

In vivo evaluation: These two compounds blocked human prostate cancer cell growth in an *in vivo* xenograft model in mice by increasing apoptosis [29].

*2.2.3.3. **HPI1, HPI2, HPI3, and HPI4**. Source*: 122,755 compounds from the Stanford University high-throughput bioscience center collection were screened in a Gli-luciferase reporter assay in mouse NIH3T3 stably transfected cells stimulated with 500 nM SAG, a Smo agonist, in order to preferentially identify antagonists acting downstream of Smo [101].

MoA: Four Hh pathway inhibitors were identified, that do not compete with the binding of BODIPY-Cyclopamine to Smo, but antagonize the Shh-induced differentiation of mouse C3H10T1/2 cells, and inhibit *Ptch1* endogenous expression in mouse NIH3T3 cells [101]. Absence of activity of these compounds on Wnt signaling suggests specificity. The four HPI compounds inhibit Hh activity in *Sufu*$^{-/-}$ fibroblasts suggesting that they act downstream Sufu. Indeed, HPI1 was able to antagonize Gli-luciferase reporter activity of exogenous GLI1 and GLI2 in mouse NIH3T3 cells. HPI2

selectively acted on GLI2 while HPI3 and HPI4 were not able to inhibit the effect of GLI overexpression. Additional assays showed that the HPIs do not modulate PKA, PI3K/AKT or MAPK signaling. Instead, HPI1 increased GLI1 stability. HPI2 and HPI3 seem to target GLI2 activator formation, but through different mechanisms. HPI4 may inhibit signaling by disturbing ciliogenesis [101].

In vivo evaluation: Only HPI1 and HPI4 were able to inhibit the proliferation of mouse perinatal cerebellar granule neuron precursors harboring a mutant Smo (Smo2 mouse), the growth of which is Cyclopamine resistant [101].

3. HH-GLI agonists

3.1. Natural compounds

Oxysterols. *Source*: Oxidized cholesterol derivatives.

MoA: Treatment of the multipotent mouse marrow stromal cell line M2 with 20(S) and 22(S) hydroxycholesterols [102] led to the up-regulation of *Gli1* and *Ptch1*. Human disorders resulting from disruption of the sterol synthesis mimic embryonic Shh signaling inhibition, resulting in holoprosencephaly [103]. Like Shh, oxysterols possess osteoinductive properties [102], suggesting that cholesterol derivatives, like oxysterols, are possible Shh pathway agonists. Inhibition of sterol synthesis blocked the growth of medulloblastoma from $ptc1^{+/-}$;$p53^{-/-}$ mice and decreased *Gli1* and *Ptch1* mRNA levels. These effects were rescued by addition of 20α, 22(S)-, 24- and 25-hydroxycholesterol, but not by 7β-hydroxycholesterol, suggesting specificity [103]. Overexpression of Gli1 renders the medulloblastoma cells resistant to cholesterol synthesis inhibitors, suggesting Smo or other component above Gli as target of 25-hydroxycholesterol. Competition experiments with Cyclopamine suggest that 25-hydroxycholesterol could act at the level of Smo [103]. Gli-luciferase reporter activity was also induced by oxysterols in mouse M2 cells and in mouse C3H10T1/2 cells [102]. $Smo^{-/-}$ mouse embryonic fibroblasts did not respond to oxysterol stimulation suggesting that 20(S) and 22(S) hydroxycholesterols also target Smo. However, binding competition experiments using BODIPY-Cyclopamine in human HEK293 cells do not support oxysterol mode of action through direct binding to Smo. Pharmacologic inhibition of PKC by Rottlerin suggests that PKC signaling is required for oxysterol HH signaling induction [102].

In vivo evaluation: NA.

3.2. Synthetic compounds

Purmorphamine. *Source*: Purmorphamine, a 2,6,9-trisubstituted purine derivative compound, was identified through high-throughput screening of a combinatorial library consisting of 50,000 heterocycle compounds on a mouse cell-based model of osteogenesis [104].

MoA: Purmorphamine was selected as a HH signaling agonist due to its potency to differentiate mouse mesenchymal progenitor (C3H10T1/2) cells [104]. Microarray experiments performed in C3H10T1/2 cells suggest specificity [105]. Purmorphamine activity was confirmed with a Gli-luciferase reporter assay in mouse NIH3T3. Purmorphamine restores HH signaling in $Ptch1^{-/-}$ mouse embryonic fibroblasts treated with the antagonist BODIPY-Cyclopamine, showing that this compound targets the pathway downstream of Ptch1 [106]. In $Smo^{-/-}$ mouse fibroblasts, Purmorphamine does not induce HH signaling, indicating that it requires Smo for its activity [106]. Competition experiments in human HEK 293T cells with BODIPY-Cyclopamine suggest that Purmorphamine modulates Smo function through direct interaction [106].

In vivo evaluation: NA.

Hh-Ag1.1. *Source*: Agonist isolated from the screening of 140,000 synthetic compounds coming from diverse libraries and using a Gli-luciferase reporter assay in mouse C3H10T1/2 cells [88].

MoA: Hh-Ag1.1 was reported to increase Gli-luciferase reporter activity in mouse C3H10T1/2 stably transfected cells and to increase *Gli1* and *Ptch1* levels [88]. A series of 300 derivatives was synthesized. Some displayed greater potency (1000-fold for Hh-Ag1.5), others were more stable or less toxic (Hh-Ag1.2, Hh-Ag1.3). Hh-Ag1.1 is able to increase the proliferation of rat primary cortex neuronal precursors and to modulate the expression of transcription factors normally regulated by Hh signaling during neurogenesis of the chick neural plate (reduction of *Pax7* in dorsal spinal cord, induction of *Mnr2* in motor neuron progenitors and of *Nkx2.2* in ventral interneuron progenitors). Hh-Ag1.1 competed with Cyclopamine, Jervine, Cyclopamine-KAAD and Cur61414 in increasing Gli-luciferase activity in mouse C3H10T1/2 cells, indicating that this agonist targets SMOH. Binding experiments with tritiated Hh-Ag1.1 demonstrated that it is a ligand of SMOH [88]. Importantly, Hh-Ag1.1 binding to SMOH is not affected by the presence of gain of function SMOH mutations (W539L) [89], contrary to Cyclopamine, Jervine and other SMOH inhibitors that are not able to bind this SMOH mutant.

In vivo evaluation: Oral administration of Hh-Ag1.1 induced Hh pathway activity in $Ptch1^{lacZ/+}$ mice as reported by extended X-gal staining of the dorsal neural tube and by the apparition of the characteristic open rostral neural tube phenotype usually observed in $Ptch1^{-/-}$ embryos. Hh-Ag1.1 also partially rescued midline defects of $Shh^{-/-}$ but not of $Smo^{-/-}$ embryos [88].

SAG. *Source*: Isolated from the screening of 140,000 synthetic compounds using a Gli-luciferase reporter assay in mouse C3H10T1/2 cells [88,91].

MoA: SAG was reported to activate the HH pathway in mouse NIH3T3 cells to a similar extent as ShhN, at low concentrations ($<$1 μM) [91]. At higher concentrations, SAG was found to inhibit Gli-luciferase reporter activity and this inhibition was not due to cytotoxicity.

The cellular target of SAG seems to be downstream of Ptch1 since SAG was able to restore Hh pathway activity inhibited by KAAD-Cyclopamine in mouse $P2^{Ptch1-/-}$ cells. SAG appears to bind to the heptahelical bundle of Smo as demonstrated by its ability to inhibit the association of BODIPY-Cyclopamine [91].

In vivo evaluation: NA.

4. Future perspectives

Multiple efforts described in this review highlight the generalized interest in HH-GLI signaling in human disease and its modulation by small molecules. Cancer is the second killer in developed nations and the cancer market alone may reach 60 billion $US worldwide this year.

It may seem, at first glance, that the field is crowded. A more careful look, however, reveals the fact that it is wide open. While finding SMOH antagonists has been easy – most companies have quite a few – sorting out their specificity, action and effectiveness has proven very difficult. For example the first candidate from Curis/Genentech, Cur-61414, a synthetic SMOH inhibitor that was efficient in reducing basal-cell carcinoma growth in $Ptch1^{+/-}$ mice ex vivo [90], failed in humans, possibly due to inadequate formulation resulting in poor delivery [122]. For other classes of potential drugs, less than 1 out of 20 very promising candidates make it to patients. It thus remains to be seen how many of these early hits will actually deliver therapeutic action. Indeed, the progress of this early great leap forward, that is the fast finding of many SMOH antagonists in cell line-based assays, could be delayed

by the lack of precise understanding of biological function. Time spent deciphering the intricacies of HH-GLI signaling in relevant human cells may allow late players to move ahead as the choice of small molecule – and the costs involved – can make or break HH-GLI targeting programs. It will also be interesting to find out if natural products can provide new entities that may have the best activities as compared with synthetic compounds. Natural products hold a comfortable lead over synthetics (with hit rates of ~0.3% and <0.001%, respectively) in delivering drugs to patients [107].

In this sense, the natural alkaloid Cyclopamine could prove useful to treat cancer patients. It has been shown to be specific at effective doses in vitro and in mice, it can be delivered orally, and it has been shown to have specific effects in range and farm animals at minute concentrations without important side effects [e.g. 9,16,17,22,44,45,120]. Since Cyclopamine was discovered by Richard Keeler and coworkers over 30 years ago [e.g. 44,45], IP issues are largely mute although there is a use patent [108]. Perhaps this is a reason why pharmas are not interested in embarking on clinical tests with Cyclopamine itself, although several derivatives have been made and at least one (apparently fully patented [109]) is in clinical trials. At present, the lack of interest in the use of Cyclopamine itself, which is available today albeit in limited quantities, for terminally ill cancer patients poses ethical questions from a health policy point of view. Developing nations with large patent populations may well choose to test this or other "free" compounds at a fraction of what fully IP protected SMOH inhibitors will likely cost. Alternatively, they may choose a road less traveled, but not virgin, and produce generics for their citizens, as colon cancers and others are reaching epidemic proportions.

Finding the best SMOH antagonists, that is, those with the highest level of specific inhibition and lowest level of non-specific actions, will be important nonetheless, as these kinds of HH-GLI antagonists may be the first to reach the market. Phase I results for Roche's GDC-0449 on metastatic basal-cell carcinoma, which normally harbor HH-GLI activating mutations, suggest excellent early tumor-reduction in responding patients, but not all patients responded [81]. The increased number of patients in subsequent trials should resolve this issue. However, classifying patients and finding the potential high responders who can benefit the most from anti-SMOH and other HH-GLI blockers, through testing for *GLI1* levels and other biomarkers, remains a priority.

Another pressing issue is whether anti-SMOH compounds will act on the epithelial tumor cells themselves in cases of colon, prostate, melanoma, glioma and other cancers that do not frequently harbor pathway-activating mutations. Such tumor epithelial cells have been found to have active signaling and require SMOH and GLI function as determined by cell-autonomous RNAi (e.g. [8,16,17,22]), but inhibitors seem to affect only the stroma in xenograft models [78,63], raising questions about the action of the inhibitors and possible differences between humans and mice (see [110]).

An important goal is the use of HH-GLI inhibitors in combinatorial therapies in which potential resistance by mutations in SMOH [82,83] could be bypassed by the simultaneous use of both anti-SMOH and anti-GLI compounds, for instance. Moreover, these inhibitors could be likely combined in turn with anti-oncogenic RAS, AKT or MEK molecules, for example, plus classical chemotherapeutics [17,53] (Table 2). Anti-GLI agents will be essential in those cases in which activation occurs through oncogenic or loss of tumor suppressive inputs [25].

These are exciting times. We shall soon know if the excellent results in different species can be translated to humans to offer new possibilities for a cancer treatment and an improvement on current outcomes.

Similarly, the promise of enhancing and prolonging regenerative mechanisms by transiently up-regulating HH-GLI activity [111–114] (thus bypassing the tumorigenic effect derived from its sustained activation) may translate into a great improvement of wound healing, for instance, specially in cases of impaired or diminished repair such as in chronic ulcers and in aged patients.

The race to find the best HH-GLI inhibitors, and to the market, is on but not all that GLI-tters is gold...

Acknowledgments

We thank all Ruiz i Altaba lab members for comments and discussion. We apologize for any authors whose work could not be included due to space limitations. This work was supported by grants from the NCCR Frontiers in Genetics, Swissbridge, FNS and the EU-FP7 HEALING.

References

[1] Ruiz i Altaba A, editor. Hedgehog-Gli signaling in human disease. Austin, TX: Landes Bioscience/Springer; 2006.
[2] Ruiz i Altaba A, Mas C, Stecca B. The Gli code: an information nexus regulating cell fate, stemness and cancer. Trends Cell Biol 2007;17(9):438–47.
[3] Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, et al. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 1996;272:1668–71.
[4] Hahn H, Christiansen J, Wicking C, Zaphiropoulos PG, Chidambaram A, Gerrard B, et al. A mammalian patched homolog is expressed in target tissues of sonic hedgehog and maps to a region associated with developmental abnormalities. J Biol Chem 1996;271:12125–8.
[5] Dahmane N, Lee J, Robins P, Heller P, Ruiz i Altaba A. Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. Nature 1997;389:876–81.
[6] Dahmane N, Sanchez P, Gitton Y, Palma V, Sun T, Beyna M, et al. The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. Development 2001;128:5201–12.
[7] Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, et al. Medulloblastoma growth inhibition by hedgehog pathway blockade. Science 2002;297:1559–61.
[8] Sanchez P, Hernandez AM, Stecca B, Kahler AJ, DeGueme AM, Barrett A, et al. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. Proc Natl Acad Sci USA 2004;101:12561–6.
[9] Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, et al. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 2004;431:707–12.
[10] Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003;422:313–7.
[11] Yuan Z, Goetz JA, Singh S, Ogden SK, Petty WJ, Black CC, et al. Frequent requirement of hedgehog signaling in non-small cell lung carcinoma. Oncogene 2007;26:1046–55.
[12] Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003;425:851–6.
[13] Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, et al. Widespread requirement for HEDGEHOG ligand stimulation in growth of digestive tract tumours. Nature 2003;425:846–51.
[14] Kubo M, Nakamura M, Tasaki A, Yamanaka N, Nakashima H, Nomura M, et al. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer. Cancer Res 2004;64:6071–4.
[15] Qualtrough D, Buda A, Gaffield W, Williams AC, Paraskeva C. Hedgehog signalling in colorectal tumour cells: induction of apoptosis with cyclopamine treatment. Int J Cancer 2004;110:831–7.
[16] Varnat F, Duquet A, Malerba M, Zbinden M, Mas C, Gervaz P, et al. Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. EMBO Mol Med 2009;1:338–51.
[17] Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 2007;17:165–72.
[18] Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. Stem Cells 2007;25:2524–33.
[19] Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci USA 2007;104:4048–53.
[20] Dierks C, Beigi R, Guo GR, Zirlik K, Stegert MR, Manley P, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell 2008;14:238–49.

[21] Dierks C, Grbic J, Zirlik K, Beigi R, Englund NP, Guo GR, et al. Essential role of stromally induced hedgehog signaling in B-cell malignancies. Nat Med 2009;13:944–51.
[22] Stecca B, Mas C, Clement V, Zbinden M, Correa R, Piguet V, et al. Melanomas require HEDGEHOG-GLI signaling regulated by interactions between GLI1 and the RAS-MEK/AKT pathways. Proc Natl Acad Sci USA 2007;104:5895–900.
[23] Philipp M, Caron MG. Hedgehog signaling: is Smo a G protein-coupled receptor? Curr Biol 2009;19:R125–7.
[24] Jiang J, Hui CC. Hedgehog signaling in development and cancer. Dev Cell 2008;15:801–12.
[25] Stecca B, Ruiz i Altaba A. Context-dependent regulation of the GLI code in cancer by HEDGEHOG and non-HEDGEHOG signals. J Mol Cell Biol 2010;2:84–95.
[26] Goetz SC, Ocbina PJ, Anderson KV. The primary cilium as a Hedgehog signal transduction machine. Methods Cell Biol 2009;94:199–222.
[27] Ruiz i Altaba A, Stecca B, Sanchez P. Hedgehog-Gli signaling in brain tumors: stem cells and paradevelopmental programs in cancer. Cancer Lett 2004;204:145–57.
[28] Stecca B, Ruiz i Altaba A. A GLI1-p53 inhibitory loop controls neural stem cell and tumour cell numbers. EMBO J 2009;28:663–76.
[29] Lauth M, Bergstrom A, Shimokawa T, Toftgard R. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc Natl Acad Sci USA 2007;104:8455–60.
[30] Canettieri G, Di Marcotullio L, Greco A, Coni S, Antonucci L, Infante P, et al. Histone deacetylase and Cullin3-REN(KCTD11) ubiquitin ligase interplay regulates Hedgehog signalling through Gli acetylation. Nat Cell Biol 12:132–42.
[31] Di Marcotullio L, Ferretti E, Greco A, De Smaele E, Po A, Sico MA, et al. Numb is a suppressor of Hedgehog signalling and targets Gli1 for Itch-dependent ubiquitination. Nat Cell Biol 2006;8:1415–23.
[32] Riobó NA, Lu K, Ai X, Haines GM, Emerson Jr CP. Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. Proc Natl Acad Sci USA 2006;103:4505–10.
[33] Riobó NA, Haines GM, Emerson Jr CP. Protein kinase C-delta and mitogen-activated protein/extracellular signal-regulated kinase-1 control GLI activation in hedgehog signaling. Cancer Res 2006;66:839–45.
[34] Chamoun Z, Mann RK, Nellen D, von Kessler DP, Bellotto M, Beachy PA, et al. Skinny hedgehog, an acyltransferase required for palmitoylation and activity of the hedgehog signal. Science 2001;293:2080–4.
[35] Methods and materials for the diagnosis and treatment of basal cell carcinoma. Patent US20030100032.
[36] Sim FR, Matsumoto N, Goulding EH, Denny KH, Lamb J, Keeler RF, et al. Specific craniofacial defects induced by jervine in the cultured rat embryo. Teratog Carcinog Mutagen 1983;3:111–21.
[37] Omnell ML, Sim FR, Keeler RF, Harne LC, Brown KS. Expression of *Veratrum* alkaloid teratogenicity in the mouse. Teratology 1990;42:105–19.
[38] Campbell M, Horton W, Keeler R. Comparative effects of retinoic acid and jervine on chondrocyte differentiation. Teratology 1987;36:235–43.
[39] Chen JK, Taipale J, Cooper MK, Beachy PA. Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. Genes Dev 2002;16:2743–8.
[40] Lee KR, Kozukue N, Han JS, Park JH, Chang EY, Baek EJ, et al. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J Agric Food Chem 2004;52:2832–9.
[41] Beachy PA, Cooper MK, Young KE, von Kessler DP, Park WJ, Hall TM, et al. Multiple roles of cholesterol in hedgehog protein biogenesis and signaling. Cold Spring Harb Symp Quant Biol 1997;62:191–204.
[42] Cooper MK, Porter JA, Young KE, Beachy PA. Teratogen-mediated inhibition of target tissue response to Shh signaling. Science 1998;280:1603–7.
[43] Wilson CW, Chen MH, Chuang PT. Smoothened adopts multiple active and inactive conformations capable of trafficking to the primary cilium. PLoS One 2009;4:e5182.
[44] Binns W, James LF, Keeler RF, Balls LD. Effects of teratogenic agents in range plants. Cancer Res 1968;28:2323–6.
[45] Keeler RF, Binns W. Teratogenic compounds of *Veratrum californicum* (Durand). V. Comparison of cyclopian effects of steroidal alkaloids from the plant and structurally related compounds from other sources. Teratology 1968;1:5–10.
[46] Incardona JP, Gaffield W, Kapur RP, Roelink H. The teratogenic *Veratrum* alkaloid cyclopamine inhibits sonic hedgehog signal transduction. Development 1998;125:3553–62.
[47] Taipale J, Chen JK, Cooper MK, Wang B, Mann RK, Milenkovic L, et al. Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature 2000;406:1005–9.
[48] Zhao Y, Tong C, Jiang J. Hedgehog regulates smoothened activity by inducing a conformational switch. Nature 2007;450:252–8.
[49] Taipale J, Cooper MK, Maiti T, Beachy PA. Patched acts catalytically to suppress the activity of Smoothened. Nature 2002;418:892–7.
[50] Wang Y, Zhou Z, Walsh CT, McMahon AP. Selective translocation of intracellular Smoothened to the primary cilium in response to Hedgehog pathway modulation. Proc Natl Acad Sci USA 2009;106(8):2623–8.
[51] Rohatgi R, Milenkovic L, Corcoran RB, Scott MP. Hedgehog signal transduction by Smoothened: pharmacologic evidence for a 2-step activation process. Proc Natl Acad Sci USA 2009;106:3196–201.
[52] Sanchez P, Ruiz i Altaba A. In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice. Mech Dev 2005;122:223–30.
[53] Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, et al. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. Cancer Res 2007;67:2187–96.
[54] Elamin MH, Shinwari Z, Hendrayani SF, Al-Hindi H, Al-Shail E, Khafaga Y, et al. Curcumin inhibits the Sonic Hedgehog signaling pathway and triggers apoptosis in medulloblastoma cells. Mol Carcinog 2009.
[55] Logan Natural Products. Patent WO/2009/099625. www.logannaturalproducts.com.
[56] Welch KD, Panter KE, Lee ST, Gardner DR, Stegelmeier BL, Cook D. Cyclopamine-induced synophthalmia in sheep: defining a critical window and toxicokinetic evaluation. J Appl Toxicol 2009;29:414–21.
[57] Zhang J, Garrossian M, Gardner D, Garrossian A, Chang YT, Kim YK, et al. Anticancer activity studies of cyclopamine derivatives. Bioorg Med Chem Lett 2008;18:1359–63.
[58] Kumar SK, Roy I, Anchoori RK, Fazli S, Maitra A, Beachy PA, et al. Targeted inhibition of hedgehog signaling by cyclopamine prodrugs for advanced prostate cancer. Bioorg Med Chem 2008;16:2764–8.
[59] Feldmann G, Fendrich V, McGovern K, Bedja D, Bisht S, Alvarez H, et al. An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. Mol Cancer Ther 2008;7:2725–35.
[60] Tremblay MR, Lescarbeau A, Grogan MJ, Tan E, Lin G, Austad BC, et al. Discovery of a potent and orally active hedgehog pathway antagonist (IPI-926). J Med Chem 2009;52:4400–18.
[61] Travaglione V, Watkins DN. A novel HH pathway inhibitor, IPI-926, delays recurrence post-chemotherapy in a primary human SCLC xenograft model. Presented at the ASMS Conference, Denver, CO; June 1–5, 2008.
[62] Travaglione V, Watkins DN. Activity of IPI-926, a novel inhibitor of the HH pathway, in subcutaneous and orthotopically implanted xenografts tumors that express SHH ligand. Presented at the EORTC-NCI-AARC symposium on "Molecular targets and cancer therapeutics", Geneva, Switzerland; 2008.
[63] Dierks C, Beigi R, Guo GR, Zirlik K, Stegert MR, Manley P, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 2009;324:1457–61.
[64] Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F, Peppelenbosch MP. Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. PLoS Biol 2006;4:e232.
[65] Bijlsma MF, Peppelenbosch MP, Spek CA. (Pro-)vitamin D as treatment option for hedgehog-related malignancies. Med Hypotheses 2008;70:202–3.
[66] Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 2008;65:1631–52.
[67] Hosoya T, Arai MA, Koyano T, Kowithayakorn T, Ishibashi M. Naturally occurring small-molecule inhibitors of hedgehog/GLI-mediated transcription. Chembiochem 2008;9:1082–92.
[68] Kunnumakkara AB, Anand P, Aggarwal BB. Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Lett 2008;269:199–225.
[69] Chen SC, Sorrell TC. Antifungal agents. Med J Aust 2007;187:404–9.
[70] Kim J, Gong R, Lee J, Chong C, Clemons K, Liu J, et al. Itraconazole as a novel Hedgehog pathway antagonist in cancer therapy. In: Abstract in Proceedings of the 100th annual meeting of the American association for cancer research; 2009.
[71] Damu AG, Kuo PC, Su CR, Kuo TH, Chen TH, Bastow KF, et al. Isolation, structures, and structure–cytotoxic activity relationships of withanolides and physalins from *Physalis angulata*. J Nat Prod 2007;70:1146–52.
[72] Stanton BZ, Peng LF, Maloof N, Nakai K, Wang X, Duffner JL, et al. A small molecule that binds Hedgehog and blocks its signaling in human cells. Nat Chem Biol 2009;5:154–6.
[73] Peng LF, Stanton BZ, Maloof N, Wang X, Schreiber SL. Syntheses of aminoalcohol-derived macrocycles leading to a small-molecule binder to and inhibitor of Sonic Hedgehog. Bioorg Med Chem Lett 2009;19:6319–25.
[74] Winkler JD, Isaacs A, Holderbaum L, Tatard V, Dahmane N. Design and synthesis of inhibitors of Hedgehog signaling based on the alkaloid cyclopamine. Org Lett 2009;11:2824–7.
[75] Romer JT, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, et al. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/−)p53(−/−) mice. Cancer Cell 2004;6:229–40.
[76] Zhang Y, Laterra J, Pomper MG. Hedgehog pathway inhibitor HhAntag691 is a potent inhibitor of ABCG2/BCRP and ABCB1/Pgp. Neoplasia 2009;11:96–101.
[77] Zhang Y, Bressler JP, Neal J, Lal B, Bhang HE, Laterra J, et al. ABCG2/BCRP expression modulates D-Luciferin based bioluminescence imaging. Cancer Res 2007;67:9389–97.
[78] Yauch RL, Gould SE, Scales SJ, Tang T, Tian H, Ahn CP, et al. A paracrine requirement for hedgehog signalling in cancer. Nature 2008;455:406–10.
[79] Robarge KD, Brunton SA, Castanedo GM, Cui Y, Dina MS, Goldsmith R, et al. GDC-0449-a potent inhibitor of the hedgehog pathway. Bioorg Med Chem Lett 2009;19:5576–81.
[80] Wong H, Chen JZ, Chou B, Halladay JS, Kenny JR, La H, et al. Preclinical assessment of the absorption, distribution, metabolism and excretion of GDC-0449 (2-chloro-N-(4-chloro-3-(pyridin-2-yl)phenyl)-4-(methylsulfonyl)benzamide), an orally bioavailable systemic Hedgehog signalling pathway inhibitor. Xenobiotica 2009;39:850–61.
[81] Von Hoff DD, LoRusso PM, Rudin CM, Reddy JC, Yauch RL, Tibes R, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med 2009;361:1164–72.

[82] Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. N Engl J Med 2009;361:1173–8.
[83] Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, et al. Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. Science 2009;326:572–4.
[84] Peukert S, Jain RK, Geisser A, Sun Y, Zhang R, Bourret A, et al. Identification and structure–activity relationships of ortho-biphenyl carboxamides as potent Smoothened antagonists inhibiting the Hedgehog signaling pathway. Bioorg Med Chem Lett 2009;19:328–31.
[85] Miller-Moslin K, Peukert S, Jain RK, McEwan MA, Karki R, Llamas 3rd L, et al. 1-Amino-4-benzylphthalazines as orally bioavailable smoothened antagonists with antitumor activity. J Med Chem 2009;52:3954–68.
[86] Novartis: www.novartis.com.
[87] Da I, Copland M. Combination of the Hedgehog pathway inhibitor LDE225 and Nilotinib eliminates chronic myeloid leukemia stem and progenitor cells. In: Abstract from the 51st ASH annual meeting and exposition; 2009 [A1428].
[88] Frank-Kamenetsky M, Zhang XM, Bottega S, Guicherit O, Wichterle H, Dudek H, et al. Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. J Biol 2002;1:10.
[89] Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, et al. Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature 1998;391:90–2.
[90] Williams JA, Guicherit OM, Zaharian BI, Xu Y, Chai L, Wichterle H, et al. Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions. Proc Natl Acad Sci USA 2003;100:4616–21.
[91] Chen JK, Taipale J, Young KE, Maiti T, Beachy PA. Small molecule modulation of Smoothened activity. Proc Natl Acad Sci USA 2002;99:14071–6.
[92] Buttner A, Seifert K, Cottin T, Sarli V, Tzagkaroulaki L, Scholz S, et al. Synthesis and biological evaluation of SANT-2 and analogues as inhibitors of the hedgehog signaling pathway. Bioorg Med Chem 2009;17:4943–54.
[93] Yang H, Xiang J, Wang N, Zhao Y, Hyman J, Li S, et al. Converse conformational control of smoothened activity by structurally related small molecules. J Biol Chem 2009;284:20876–84.
[94] Exelixis, Inc. Inhibitors of the hedgehog pathway. Patent WO2008112913. www.exelixis.com; 2008.
[95] Gendreau SB, Fargnoli J. Preclinical characterization of BMS-833923 (XL139), a hedgehog (HH) pathway inhibitor in early clinical development. Mol Cancer Ther 2009;8(12 Suppl.):B192.
[96] Wang X, Bennett K. Assessment of Gli1 expression during skin regeneration in mouse models and normal healthy volunteers. Mol Cancer Ther 2009;8.
[97] Siu L, Tornout JMV. A first-in-human, phase I study of an oral hedgehog pathway antagonist, BMS-833923 (XL139), in subjects with advanced or metastatic solid tumors. Mol Cancer Ther 2009;8.
[98] Siena Biotech. Patent WO/2009/074300. www.sienabiotech.com; 2009.
[99] Lee J, Wu X, Pasca di Magliano M, Peters EC, Wang Y, Hong J, et al. A small-molecule antagonist of the hedgehog signaling pathway. Chembiochem 2007;8:1916–9.
[100] Cupido T, Rack PG, Firestone AJ, Hyman JM, Han K, Sinha S, et al. The imidazopyridine derivative JK184 reveals dual roles for microtubules in Hedgehog signaling. Angew Chem Int Ed Engl 2009;48:2321–4.
[101] Hyman JM, Firestone AJ, Heine VM, Zhao Y, Ocasio CA, Han K, et al. Small-molecule inhibitors reveal multiple strategies for Hedgehog pathway blockade. Proc Natl Acad Sci USA 2009;106:14132–7.
[102] Dwyer JR, Sever N, Carlson M, Nelson SF, Beachy PA, Parhami F. Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. J Biol Chem 2007;282:8959–68.
[103] Corcoran RB, Scott MP. Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. Proc Natl Acad Sci USA 2006;103:8408–13.
[104] Wu X, Ding S, Ding Q, Gray NS, Schultz PG. A small molecule with osteogenesis-inducing activity in multipotent mesenchymal progenitor cells. J Am Chem Soc 2002;124:14520–1.
[105] Wu X, Walker J, Zhang J, Ding S, Schultz PG. Purmorphamine induces osteogenesis by activation of the hedgehog signaling pathway. Chem Biol 2004;11:1229–38.
[106] Sinha S, Chen JK. Purmorphamine activates the Hedgehog pathway by targeting Smoothened. Nat Chem Biol 2006;2:29–30.
[107] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 2009;325:161–5.
[108] See cyclopamine in http://www.freepatentsonline.com/.
[109] Tremblay MR, Nesler M, Weatherhead R, Castro AC. Recent patents for Hedgehog pathway inhibitors for treatment of malignancy. Expert Opin Ther Patents 2009;19:1039–56.
[110] Ruiz i Altaba A. Therapeutic inhibition of Hedgehog-GLI signaling in cancer: epithelial, stromal, or stem cell targets? Cancer Cell 2008;14:281–3.
[111] Amankulor NM, Hambardzumyan D, Pyonteck SM, Becher OJ, Joyce JA, Holland EC. Sonic hedgehog pathway activation is induced by acute brain injury and regulated by injury-related inflammation. J Neurosci 2009;29:10299–308.
[112] Pola R, Ling LE, Silver M, Corbley MJ, Kearney M, Blake Pepinsky R, et al. The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors. Nat Med 2001;7:706–11.
[113] Mak KK, Bi Y, Wan C, Chuang PT, Clemens T, Young M, et al. Hedgehog signaling in mature osteoblasts regulates bone formation and resorption by controlling PTHrP and RANKL expression. Dev Cell 2008;14:674–88.
[114] Paladini RD, Saleh J, Qian C, Xu GX, Rubin LL. Modulation of hair growth with small molecule agonists of the hedgehog signaling pathway. J Invest Dermatol 2005;125:638–46.
[115] Chen X, Horiuchi A, Kikuchi N, Osada R, Yoshida J, Shiozawa T, et al. Hedgehog signal pathway is activated in ovarian carcinomas, correlating with cell proliferation: it's inhibition leads to growth suppression and apoptosis. Cancer Sci 2007;98:68–76.
[116] Sicklick JK, Li YX, Jayaraman A, Kannangai R, Qi Y, Vivekanandan P, et al. Dysregulation of the Hedgehog pathway in human hepatocarcinogenesis. Carcinogenesis 2006;27:748–57.
[117] Pfizer Products, Inc. Patent WO2008075196. www.pfizer.com; 2008.
[118] Sheng T, Li C, Zhang X, Chi S, He N, Chen K, et al. Activation of the hedgehog pathway in advanced prostate cancer. Mol Cancer 2004;3:29.
[119] Ehtesham M, Sarangi A, Valadez JG, Chanthaphaychith S, Becher MW, Abel TW, et al. Ligand-dependent activation of the hedgehog pathway in glioma progenitor cells. Oncogene 2007;26:5752–61.
[120] Keeler RF, Baker DC. Oral, osmotic minipump, and intramuscular administration to sheep of the Veratrum alkaloid cyclopamine. Proc Soc Exp Biol Med 1989;192:153–6.
[121] Lauth M, Bergström A, Toftgård R. Phorbol esters inhibit the Hedgehog signalling pathway downstream of Suppressor of Fused, but upstream of Gli. Oncogene 2007;26(35):5163–8.
[122] See http://www.curis.com; news 07/11/2006.