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Review

Inhibition of the Hedgehog pathway in lung cancer

A. Dimou[a,*], A. Bamias[b], H. Gogas[c], K. Syrigos[d]

[a] University of Colorado, Division of Medical Oncology, 12801 E. 17th Avenue, Mail Stop 8117, Research 1 South, Aurora, CO, USA
[b] Alexandra Hospital, National and Kapodistrian University of Athens School of Medicine, Department of Clinical Therapeutics, Alexandra Hospital, 80 Vasilisis Sofias Avenue, Athens, Greece
[c] Laiko General Hospital, National and Kapodistrian University of Athens School of Medicine, 1st Department of Medicine, 17 Agiou Thoma St. Athens, Greece
[d] Sotiria Hospital, National and Kapodistrian University of Athens School of Medicine, 3rd Department of Medicine, 152 Masogeion Avenue, Athens, Greece

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ABSTRACT

Inhibitors of the hedgehog pathway are effective in patients with basal cell carcinoma and a subgroup of patients with medulloblastoma with active hedgehog signaling. Despite preclinical work suggesting otherwise, clinical trials in solid tumors of epithelial origin have not shown added benefit with these drugs. Here, we review the preclinical and clinical data of hedgehog pathway inhibition in the most common histologic types of lung cancer. We focus on highlighting areas of uncertainty, where further research might define a niche for hedgehog pathway inhibition in patients with lung cancer.

1. Introduction

Lung cancer is the leading cause of cancer related mortality globally and is classified as either Small Cell Lung Cancer (SCLC) or Non-Small Cell Lung Cancer (NSCLC) upon pathology review [1,2]. The main histologic types of NSCLC include squamous cell carcinoma and adenocarcinoma [2]. The majority of patients have non-curable disease stage at the time of diagnosis. Advances in systemic treatments including chemotherapy, targeted therapies and immune check point inhibitors have improved prognosis in recent years. Nevertheless, most patients with metastatic lung cancer die within a few years from diagnosis, stressing the need for more and novel therapeutic approaches. Targeted therapies benefit patients classified in well-defined molecular groups identified by the presence of activating mutations in key genes like *EGFR*, *ALK*, *ROS1* and *BRAF* that follow the oncogene addiction paradigm [3]. The list of driver oncogenes with actionable mutations is expanding over time. On the other hand, targeting molecular pathways in the absence of an addicting oncogene mutation is a more challenging goal.

The Hedgehog signaling pathway (HH pathway hereafter) is vital for development and tissue homeostasis [4]. In recent years, studying the role of the HH pathway in carcinogenesis and cancer stemness has highlighted the HH inhibitors as putative cancer therapeutics [5]. However, clinical application of HH inhibition in cancer improves outcomes only for patients with basal cell carcinoma [6] and a subgroup of patients with medulloblastoma with active HH signaling [7]. Both these tumor types harbor oncogenic mutations in key molecules of the HH pathway. In contrast, clinical trials have largely yielded negative results for unselected populations of patients with solid tumors of epithelial origin despite preclinical rationale [8,9]. Herein, we review the preclinical and clinical data on the use of HH inhibitors with respective to the major histologic types of lung cancer, including small-cell lung cancer (SCLC), lung adenocarcinoma and squamous cell lung carcinoma. The goal of this review is to highlight areas of uncertainty and guide future research for HH pathway inhibition in lung cancer.

2. Hedgehog pathway overview

Stem cells are capable for both self-renewal and evolution to more differentiated states, a feature known as asymmetric cell division [10]. The identification of stem cells originated from the observation that a limited number of bone marrow cells are able to reconstitute the entire

Abbreviations: HH, Hedgehog; SCLC, small-cell lung cancer; NSCs, neural stem cells; CICs, cancer initiating cells; SHH, sonic Hedgehog; IHH, Indian Hedgehog; DHH, desert Hedgehog; PTCH, patched; GPCR, G-protein coupled receptor; SMO, smoothened; SUFU, suppressor of fused; KIF7, kinesin family member 7; CK1α, casein kinase 1α; NRP1, neuropilin 1; NRP2, neuropilin 2; GSK3β, glycogen kinase 3β; HHIP, hedgehog inhibitory protein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ATO, arsenic trioxide; PFS, progression free survival; OS, overall survival; PKCι, protein kinase iota; EMT, epithelial to mesenchymal transition

* Corresponding author at: University of Colorado School of Medicine, Division of Medical Oncology, 12801 E. 17th Avenue Mail Stop 8117, Research 1 south, Anschutz Medical Campus, Aurora, CO, 80045, USA.

E-mail addresses: anastasios.dimou@ucdenver.edu (A. Dimou), abamias@med.uoa.gr (A. Bamias), hgogas@med.uoa.gr (H. Gogas), ksyrigos@med.uoa.gr (K. Syrigos).

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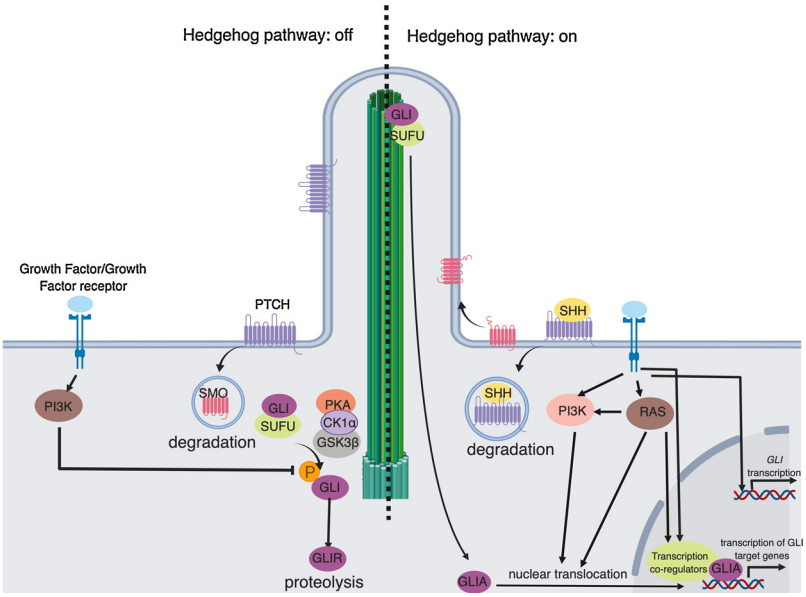

Fig. 1. Canonical and non-canonical activation of Hedgehog pathway. The canonical Hedgehog pathway in vertebrates requires the primary cilium, a solitary plasma membrane structure with controlled entrance for plasma bound molecules. In the absence of Hedgehog ligands, the pathway is inactive (left). The patched (PTCH) receptors inhibit smoothened (SMO) which subsequently undergoes endocytosis and degradadation. In this state, GLI transcription factors are sequestered by Suppressor of Fused (SUFU), phosphorylated successively by protein kinase A (PKA), glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α) and either degraded (GLI2), or truncated to a HH pathway repressor form, GLIR (GLI3). In the active state of the pathway (right), sonic hedgehog (SHH) and other hedgehog ligands bind to their receptor PTCH which then undergoes endocytosis and degradation. SMO then re-localizes into the primary cilium. Also, the SUFU/GLI complex localizes at the tip of the cilium, GLI dissociates from SUFU, exits the primary cilium and enters the nucleus in its active form, GLIA (mainly GLI2), where it activates the transcription of *GLI* responsive genes. GLI1 functions as an amplifier of the pathway. In the non-canonical activation of the pathway, PI3K, RAS and growth factors (like EGF and TGFβ) might either inhibit the phosphorylation of GLI by PKA, induce the nuclear localization of GLI, activate transcription co-regulation factors *in cis* with GLI or induce the transcription of the *GLI* genes. Created with BioRender.

hematopoietic system when transplanted in syngeneic mice [11]. Since then, a number of stem cell populations have been characterized both in normal adult and embryonic tissues including the hematopoietic stem cells [12], the neural stem cells (NSCs) [13], the embryonic stem cells [14] and others [15,16]. Likewise to stem cells in normal tissues, the stem cell theory of cancer introduces the concept of the cancer initiating cell (CIC) sitting at the top of cancer cell hierarchy [17]. According to this theory, a limited number of CICs can form a tumor xenograft when implanted in mice [18]; CICs are responsible for resistance to drug therapy, disease relapse and metastasis [17]. Importantly, both normal stem cells and CICs activate common molecular pathways including morphogens WNT, NOTCH and HH [19]. Especially the HH pathway activation follows a concentration HH ligand gradient during the development of the normal lung and is also important for the maintenance of adult stem cells in many tissues [20,21].

The HH pathway (illustrated in Fig. 1) was initially described in *Drosophila melanogaster* where it was found to be required for segment polarity and ventral-dorsal differentiation [22]. In vertebrates, the SHH, IHH and DHH (Sonic, Indian and Desert Hedgehog respectively) ligands bind to the 12-pass transmembrane receptors Patched1 and Patched2 (PTCH1 and PTCH2 respectively) [23]. In the absence of ligands, the PTCH receptors suppress the 7-pass transmembrane G-protein coupled receptor (GPCR)-like protein Smoothened (SMO) [24]. The binding of lipid modified SHH, IHH and DHH ligands to the PTCH receptors [25,26], activates SMO in the primary cilium, a solitary organelle with sensory function in most mammalian cells [27–29]. The exact interaction of PTCH with SMO is not fully elucidated but involves control of cholesterol availability [30,31]. This process inhibits the processing of the zinc finger transcription factors GLI. Following SMO activation, full length GLI enters the cilia in the form of a complex with the HH negative regulator Suppressor of Fused (SUFU) [32]. The complex accumulates at the cilia tip where GLI disassociates [32] and exits the cilia in a Kinesin Family Member 7 (KIF7) dependent process to function as a transcriptional activator for HH pathway target genes [4]. There are currently three identified GLI transcription factors in vertebrates, GLI1, GLI2 and GLI3. GLI3 functions mainly as a pathway repressor in its truncated form. GLI1 lacks the N-terminal repressor sequence and has activating effect only, whereas GLI2 is the main pathway activator [33–35].

Several layers of regulation for this canonical activation of the HH pathway exist. First, the protein kinase A (PKA), the casein kinase 1α (CK1α) and GSK3β can phosphorylate and mark for proteosomal degradation the GLI transcription factors [36]. Second, Suppressor of Fused (SUFU) binds to GLI and sequesters GLI in the cytoplasm in the absence of hedgehog ligands [37]. Third, genes encoding for negative regulators of the HH pathway, such as *PTCH* 1 and 2 and hedgehog inhibitory protein (*HHIP*) are GLI target genes, therefore they engage in a negative feedback loop with GLI [38,39]. Also, neuropilin 1 and 2 (NRP1 and NRP2), activate a positive feedback loop with GLI as described recently [40,41]. Neuropilins enhance HH signaling in both a PKA dependent [41] and independent [42] way. In the mouse, non-canonical Hedgehog pathway also exists, where astrocyte derived Shh activates nestin in a medulloblastoma model. Importantly, this paracrine loop is Ptc1 and Gli independent but Smo dependent [43]. Last but not least, pathways other than the HH pathway, for example RAS, TGFβ and PI3K, can induce *GLI* expression in cancer in a number of ways [44,45]. First, RAS, as well as PI3K induce the nuclear localization and activate GLI1 in melanoma models [46]. Further, PI3K/AKT signaling inhibits GLI phosphorylation by PKA and prevents its degradation [47]. Third, signaling through EGFR activates c-JUN via MAPK which functions as a transcription co-regulator factor with GLI for certain GLI targets [48]. Fourth, SMAD transcription factors are regulated by TGFβ and synergize with GLI1 to induce TGFβ and HH dependent *CCND1* expression [49]. Finally, TGFβ signaling increases transcription of the *GLI* genes [50]. Fig. 1 illustrates the canonical and non-canonical HH pathway and its regulation.

3. HH pathway inhibitors

There are currently several strategies to inhibit the hedgehog pathway in the clinic. Interfering directly with SMO activity is a well-studied means to inhibit the HH pathway. Cyclopamine belongs to *Veratrum* alkaloids, plant derived compounds known to cause teratogenesis including cyclopia [51,52]. Cyclopamine binds to the extracellular loops of the transmembrane domain of SMO and is a SMO inhibitor [51,53]. Vismodegib, saridegib and sonidegib are more potent inhibitors of SMO which also bind to the transmembrane domain [54]. On the contrary, oxysterol and other oxidized derivatives of cholesterol activate SMO by binding to the cysteine rich domain located in the extracellular domain of SMO [55–57]. Indeed, statins which are known to block cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) inhibit medulloblastoma growth *in vivo* [58]. Additionally, cholesterol is necessary and sufficient to activate SMO and might represent the missing link between PTCH1 and SMO [30,31].

In this model, PTCH1 negatively regulates plasma membrane cholesterol in the vicinity of SMO further supporting the rationale for cholesterol synthesis inhibition as a HH targeting strategy.

Itraconazole, a triazol antifungal agent was identified as an inhibitor of HH signaling in a library screen of 2400 drugs with FDA approval or in post phase I drug development process [59]. Despite the well-established target of itraconazole, 14-α-lanosterole demethylase which is necessary for ergosterol synthesis in fungi and cholesterol in mammals with higher potency for the fungal enzyme, the inhibitory effects of itraconazole on the HH pathway result from direct binding and inhibition of SMO at a site different from the binding site for oxysterols or cyclopamine [59]. Itraconazole effectively prevents the accumulation of SMO in the primary cilium and inhibits the growth of Hh dependent medulloblastoma *in vivo* [59]. The itraconazole doses necessary to inhibit SMO are higher compared to doses used to inhibit ergosterol synthesis in fungi but still clinically feasible.

Another approach to inhibit the HH pathway is to target the transcription factors GLI. The active form of GLI2 is the most significant mediator of HH activity in mammals, GLI3 is mostly a suppressor and GLI1 serves as a pathway output amplifier [34,35,60]. Arsenic trioxide (ATO) is used clinically for the treatment of acute promyelocytic leukemia because it degrades PML-RARA [61]. ATO inhibits GLI1 in a primary cilium independent manner, blocks Gli2 accumulation in the primary cilium and exerts anti-tumorigenic effects in a wide array of cancer cell lines and HH dependent *in vivo* mouse models [62,63]. GANT-58 and GANT-61 were identified in a drug screen as inhibitors of GLI1 as they prevent binding of GLI1 transcription factor to DNA [64]. SSTC3 is an agonist for CK1α, which along with GSK3β phosphorylates the GLI transcription factors marking them for degradation [65]. SSTC3 inhibits *TRP53* mutated, *MYCN* amplified medulloblastoma mouse models by promoting GLI degradation [65].

3.1. Hedgehog pathway inhibition in small cell lung cancer

The HH pathway is active in the developing airway as well as in regeneration of neuroendocrine cells following airway injury [66]. In the latter case, diffuse intraepithelial expression of Gli1 and Shh occurrs right before the appearance of neuroendocrine cells in the lungs of mice during regeneration following injury with naphthalene [66]. Additionally, the same group reported juxtacrine activity of SHH in human SCLC cell lines, expression of multiple members of the HH pathway in SCLC cell lines and tumors and anti-proliferative and anti-tumorigenic activity of cyclopamine in these models [66]. GLI1 is frequently expressed in SCLC tumors but not in cell lines [67]. In transgenic mouse SCLC models with conditional knockout of *Rb1* and *Trp53* cell autonomous Hedgehog signaling is activated and promotes the development and maintenance of the SCLC tumors [67,68]. Activation of HH pathway has been demonstrated to be involved in development of resistance to chemotherapy in human SCLC models, *in vitro* and *in vivo* [67]. Interestingly, the proportion of cells having primary cilia increases in cell culture and mouse xenograft models upon development of resistance [67].

Despite the preclinical data, incorporation of SMO inhibitors in the treatment of patients with SCLC does not yield significant benefit in unselected patients. Vismodegib failed to improve response rates, progression-free survival (PFS) and overall survival (OS) when added to cisplatin and etoposide compared with chemotherapy alone [8]. In a phase I trial, sonidegib in combination with cisplatin and etoposide induced a durable response for at least 27 months in a patient with SCLC and *SOX2* amplification [69].

There are several possibilities that might explain the discrepancy between clinical trial data and preclinical models. First, it is possible that cell lines and animal models might not fully recapitulate the complexity of human tumors. Second, although the HH pathway might contribute to pro-tumorigenic phenotype in SCLC, other molecular pathways might salvage cancer cells from HH pathway inhibition in an adaptive resistance model. Additionally, timing of HH pathway inhibition might be important for optimal activity. In this respect, the findings of Park et al [67] suggest that SMO inhibitors should follow chemotherapy as HH pathway is activated as part of a resistance phenotype. Last, targeting other molecules of the HH pathway, like SHH or the GLI transcription factors could be more effective than targeting SMO, as these might promote SCLC progression in a non-canonical fashion. In a recent report, Szczepny et al. showed a Smo independent role of Shh ligand in activating cyclin B1 and inducing chromosomal instability in SCLC conditional Tp53;Rb1 mutant mouse models, in addition to the effect in the canonical pathway [68].

3.2. HH inhibitors in squamous cell NSCLC

Squamous cell histology accounts for up to 30% of all NSCLC cases [70]. Despite advances in targeted treatment for patients with lung adenocarcinoma, there is little progress for patients with squamous cell lung cancer. An important step for better molecular understanding of this disease is the classification of squamous cell lung cancers into four categories, primitive, classical, basal and secretory, on the basis of gene expression profiles [71]. This classification schema is more informative compared to the existing morphological WHO classification as it correlates with outcomes and is also validated in the TCGA genomic classification for squamous cell lung carcinomas [72]. Importantly, GLI1 and GLI2 expression as well as the GLI target PTCH, are upregulated and the expression of negative regulators of the HH pathway, GLI3 and SUFU is downregulated in the classical but not the other molecular groups [73]. Interestingly, inhibition of the GLI transcription factors with GANT61 increases apoptosis, reduces cell proliferation in a GLI dependent fashion and decreases tumor growth *in vivo*. Conversely, inhibition of SMO has a modest effect on these processes [73].

Amplification of chromosome 3q is a hallmark of classical subtype of squamous cell lung cancer [74]. *SOX2* and *PRKCi* are localized in this area of the genome and they are frequently amplified in these tumors [75]. A recent study showed that *SOX2* and *PRKCi* amplifications are enriched in lung spheres representing lung squamous cancer initiating cells. Consistently with the CICs phenotype, they induce higher anchorage independent growth in soft agar assays and tumorigenic capacity compared to parental cells [76]. Interestingly, these spheres are also dependent on the canonical HH pathway. Mechanistically, protein kinase C iota (PKCi) encoded by *PRKCi*, phosphorylates SOX2 at threonine in position 118 (T118) and induces SOX2 occupancy of the *HHAT* (Hedgehog acyltransferase) promoter. Subsequent induction of HHAT, palmitoylation of SHH and activation of an autocrine canonical HH pathway is required for CICs maintenance [76]. GLI1 also enhances cancer initiating properties in lung squamous cell carcinomas with *FGFR1* amplification [77]. This alteration is present in up to 16.6% in this population and often associated with *SOX2* amplification [77].

Clinical data are sparse and limited to case reports. A clinical response was documented in a patient with squamous cell lung cancer and a germline *SMO* mutation treated with vismodegib [78]. Whether there might be a role for HH pathway inhibition in the absence of mutations is unknown.

3.3. HH inhibitors in lung adenocarcinoma

HH target genes are expressed in lung adenocarcinoma cell lines and clinical specimens [79–81]. The majority of lung adenocarcinoma cases (76%) have some expression of GLI1 by immunohistochemistry according to a study, however almost half of the GLI1 positive cases do not express any SMO [82]. The same report showed frequent methylation of the *SMO* promoter in lung adenocarcinoma cell lines to explain low SMO levels. In contrast, GLI1 expression in SMO-low cell lines is induced by the MAPK pathway in a non-canonical fashion. Inhibition and knock down of GLI1 with GANT61 and small interfering RNA (siGLI) respectively inhibits proliferation and decreases the expression

Table 1
Overview of Hedgehog inhibitors.

Mechanism of Hedgehog inhibition	Example drugs	Clinical trials in lung cancer (ongoing)
Inhibition of oxysterol synthesis: Oxysterols are derivatives of cholesterol, they bind to and activate SMO.	• Statins	• NCT01441349: Irinotecan/Cisplatin plus simvastatin for patients with chemotherapy naïve extensive SCLC.
Inhibition of SMO	• Vismodegib, sonidegib • Triazole antifungals (e.g. itrakonazole)	• MATCH trial (NCT02465060): Vismodegib arm for patients with SMO or PTCH mutations • NCT02357836: Neoadjuvant itrakonazole in NSCLC • NCT03664115: Itrakonazole plus chemotherapy vs. chemotherapy alone for patients with treatment naïve metastatic NSCLC
Inhibition of GLI	• ATO • GANT61	• NCT02066870: Icotinib and ATO in Patients NSCLC and EGFR mutations, with Resistance to EGFR-TKI
Activation of CK1α (Hedgehog pathway regulation)	• SSTC3	none

SMO: Smoothened, CK1α: Casein Kinase 1α, ATO: arsenic trioxide, NSCLC: Non-Small Cell Lung Cancer, SCLC: Small Cell Lung Cancer, TKI: tyrosine kinase inhibitor.

of proteins associated with a cancer initiating phenotype in these cell lines. Other preclinical data also demonstrate the anti-tumor efficacy of SMO inhibitors or shRNA for SMO or GLI *in vitro* [79,81].

In a phase 2 study, addition of the SMO inhibitor itraconazole to pemetrexed in second line treatment of patients with lung non-squamous cell carcinoma prolonged median PFS (5.5 months) compared to pemetrexed alone (2.8 months). However, this difference was not statistically significant as the study was stopped prematurely and included only 23 patients [83]. Noteworthy, a low dose of itraconazole (200 mg) was used in this study.

In the mouse, Cyclin E overexpression induces HH pathway activation in both cancer and dysplasia lesions indicating a role for HH in lung carcinogenesis [84]. Additionally, in a transgenic $KRAS^{G12D}$ mouse model, loss of *EphA2* accelerates the tumorigenic process mediated by conditional activation of KRAS. Interestingly, these tumors have ERK dependent activation of the canonical HH pathway and cells from these tumors respond to SMO inhibitors sonigedib and vismodegib in cell viability assays [85].

A number of groups have reported activation of the HH pathway in *EGFR* mutation positive lung adenocarcinoma models developing resistance to EGFR inhibitors via epithelial to mesenchymal transition (EMT) [86–88]. EMT is a cellular process that allows epithelial cells to detach from a basic membrane and migrate by adopting a mesenchymal morphology [89]. It involves the activation of a network of transcription factors and plays crucial roles during the implantation of the embryo and organ development. In cancer biology, EMT promotes cancer cell migration, invasion, metastasis and drug resistance. Especially for lung cancers with activating EGFR mutations, EMT drives secondary resistance independently from other known mechanisms of resistance to EGFR tyrosine kinase inhibitors (TKIs) [90]. GLI promotes EMT in lung adenocarcinoma [91]. The role of HH pathway in resistance to EGFR inhibition has also been demonstrated in head and neck cancer and glioblastoma [92,93].

Della Corte et al showed that HH pathway activation by *SMO* amplification is a mechanism of secondary resistance to gefitinib *in vitro* [94]. They also showed that SMO forms a complex with MET, creating a rationale for combined SMO and MET inhibition [94]. In a follow up study, they developed a series of EGFR TKIs resistance xenograft models from EGFR mutation positive HCC827 parental cells [87]. Interestingly, many of these *in vivo* models demonstrate up regulation of SMO and GLI1 protein levels along with an EMT profile. Additionally, combination of SMO and EGFR inhibition effectively decreases proliferation, invasion and anchorage independent growth of resistant cases [95]. In another study, Bora-Shingal et al showed the HH pathway and EGFR cooperate to activate the stem cell inducing transcription factor SOX2 in cell lines with EGFR activating mutations [88]. Also, in this study, GLI1 was enriched in the side population, thought to represent the CICs of these cells in culture.

4. Mechanism of HH pathway activation in NSCLC

Despite the role of the HH pathway in basal cell carcinoma and medulloblastoma, it has been postulated that epithelial tumors do not demonstrate cell autonomous HH ligand activity. The main evidence comes from the study by Yauch et al showing no correlation between *GLI1* expression levels and sensitivity of a large range of colorectal, pancreatic and lung cancer cell lines to SMO inhibitors [96]. Additionally, the concentrations of HH inhibitors required to inhibit proliferation in pancreatic and lung cancer cell lines are in the micromolar range whereas the concentrations needed to inhibit GLI1 luciferase activity as well as proliferation in known hedgehog responsive mesenchymal cell lines are 10 and 20 times lower respectively. Instead, the HH ligands secreted by the epithelial neoplastic cells may have a paracrine effect in mesenchymal cells in the tumor microenvironment [96]. This paracrine loop in HH ligand expressing tumor models is protumorigenic supporting a therapeutic role for SMO inhibitors. Nevertheless, a growing body of evidence, including experiments with specific knock downs of *SMO*, supports the autocrine activity of HH ligands in lung cancers in addition to the paracrine model [79–81]. The autocrine model is particularly active in the subpopulation of cancer initiating cells rather than cancer cells from the bulk of the tumor [76].

5. Ongoing clinical trials with HH inhibitors in lung cancer

HH pathway inhibition has been explored in clinical trials in unselected populations over the past decade. Despite approval in patients with non-resectable basal cell carcinoma and activity in patients with SHH type of medulloblastoma, the trials in epithelial solid tumors yielded negative results. Currently, few clinical trials are ongoing as summarized in Table 1. In one of them (NCT02357836), itraconazole is administered at a dose of 600 mg twice daily for 10–14 days as neoadjuvant treatment prior to surgery for patients with resectable NSCLC. This dose of itraconazole was selected to reflect the higher dose required to inhibit the HH pathway compared to the typical itraconazole dose when the drug is used for its antifungal properties. The study, which has a strong translational rationale will evaluate the resection specimen for changes in the HH pathway and tumor angiogenesis.

6. Areas of uncertainty and concluding remarks

Clinical trials did not show any benefit from HH inhibition in SCLC or other solid tumors of epithelial origin. Given the role of the HH pathway in lung carcinogenesis and lung cancer biology, we believe that further study of the mechanisms of lack of response to HH inhibitors might explain the failure to translate preclinical work. To this end, we identify three areas of uncertainty, where further research might propose novel directions for this domain. First, formation of complexes of SMO with other transmembrane receptors like MET [94] or generation of oxysterols that function as SMO activators [57] might limit the activity of SMO inhibitors and could support combination

strategies with MET inhibitors and statins respectively. Second, activity of HH inhibition in selected squamous cell lung cancer population on the basis of *SOX2* and *PRKCi* amplification is currently unknown and could be the focus of future studies. Last but not least, GLI inhibition could be a novel strategy in the treatment of lung cancer and the clinical value of GLI inhibitors should further be explored.

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Author declaration

I wish to confirm the following disclosures:

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I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm and approve the order of authors listed in the manuscript.

I confirm that I have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing I confirm that we have followed the regulations of our institutions concerning intellectual property.

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