Review Article

Smoothened Inhibitors to Block the Sonic Hedgehog Signaling for Cancer Treatment

Dwight Qing Teng[1] and Xixian Li[2,#]

[1]*Claremont High School, 1601 North Indian Hill Blvd, Claremont, CA 91711, USA;*
[2]*Oakcrest School, 1619 Crowell Rd, Vienna, VA 22182, USA*

ABSTRACT

The Sonic Hedgehog (sHH) pathway is vital for embryonic development and adult tissue maintenance. Aberrant sHH pathway activation is implicated in tumors like basal cell carcinoma (BCC), medulloblastoma, and pancreatic cancer, prompting significant efforts to develop pathway inhibitors. Smoothened (SMO), a pivotal protein in the sHH signaling pathway, is a key drug target for the treatment of tumors. To date, many chemical compounds have been developed to target SMO, including vismodegib and sonidegib which have been approved by FDA to treat advanced BCC with aberrant sHH pathway activation. In this article, we review recent advances in drug development by targeting SMO to inhibit the sHH signaling pathway for tumor treatment.

Keywords: Sonic Hedgehog signaling, tumor, SMO inhibitors, drugs, basal cell carcinoma, vismodegib and sonidegib.

INTRODUCTION

The sHH signaling pathway plays essential roles in embryonic development and adult organ homeostasis [1, 2]. For instance, the sHH signaling initiated by sHH ligand is implicated in correct formation of the limbs, somites and neural tube in early mammal embryogenesis [2, 3]. In the absence of sHH ligand, its receptor Patched (PTCH), a 12-span transmembrane protein, functionally inhibits SMO and prevents SMO from accumulating to primary cilia, leading to phosphorylation of the Full-length GLI proteins [4] (Figure 1). The phosphorylated GLI is then subjected to proteolytic process, resulting in repressor GLI (GLI^R) generation which suppresses the sHH target gene transcription [4]. In contrast, in the presence of sHH, the ligand binds to its receptor PTCH and relieves the PTCH inhibition to SMO. The activated SMO then translocates into primary cilia and dissociates GLI from a suppressive Suppressor of Fused (SUFU), leading to the activation of downstream GLI [5] (Figure 1). The activated GLI subsequently translocates into the nucleus to regulate the expression of target genes involved in cell proliferation, survival, and differentiation [1].

Aberrant activation of the sHH pathway has been implicated in various types of cancer including BCC, medulloblastoma, and pancreatic cancer. Therefore, significant efforts have been made to develop the sHH

Corresponding author: Xixian Li, E-mail: cece2022lee@gmail.com.

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[#]These two authors contributed equally to this work.
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signaling pathway inhibitors for cancer treatment. Among all the targets in the sHH pathway for drug development, SMO has attracted significant attention as mutations or overexpression of SMO promote tumor growth, invasion, and metastasis [1]. To date, FDA has approved two SMO inhibition-based drugs, vismodegib and sonidegib, to treat BCC, a type of skin cancer where the sHH signaling pathway is aberrantly activated. In this article, we will review recent advances in drug development by targeting SMO to inhibit the sHH signaling pathway for cancer treatment.

PTCH/SMO AND CANCER

PTCH/SMO in BCC

BCC, accounting for 90% of all skin cancer, is primarily resulted from exposure to ultraviolet (UV) of sunlight or ionizing radiation which leads to DNA damages [6]. About 85- 90% of BCCs show a loss of Patched1 (PTCH1) function by inactivating PTCH1 mutations or constitutive activation of SMO through SMO mutations in the sHH signaling [7]. PTCH1, one of the two isoforms of the PTCH gene, has been well studied as a key component in the sHH signaling pathway. while PTCH2, the other PTCH isoform, is less well understood and is believed to partially overlap with PTCH1 [8]. PTCH2 may compensate for the loss of PTCH1 in certain contexts and may have distinct or redundant roles depending on the tissue type [8].

PTCH functionally inhibits SMO activation without sHH ligand, and inactivation of PTCH would release SMO for the sHH signaling activation, and PTCH1 inactivating mutations have been identified in 70–90% of BCCs [9, 10]. Numerous studies have shown that sporadic BCC carries inactivating point mutations and loss of heterozygosity of PTCH1 allele. For instance, about half of PTCH1 somatic point mutations contain the "UV-signature" C-T and CC-TT changes [10, 11]. In addition, loss of heterozygosity of PTCH1 allele (located at chromosome 9q22.3) is often identified in BCC [12, 13]. Danaee et al analyzed loss of heterozygosity of the PTCH gene in 276 keratinocyte tumors and found a high prevalence (75.5%) of any 9q22.3 loss of heterozygosity of the PTCH in BCC with PTCH gene lost in 60% of BCC [13]. SMO is another important gene implicated in BCC, and approximately 10-20% of BCC harboring activation mutation in SMO gene. For instance, Bonilla conducted genomic analysis of 293 BCC biopsies from 263 sporadic BCC patients and 30 patients with Gorlin syndrome (a syndrome predisposing individuals to develop BCC) [9]. Their study identified 20% of BCC containing SMO

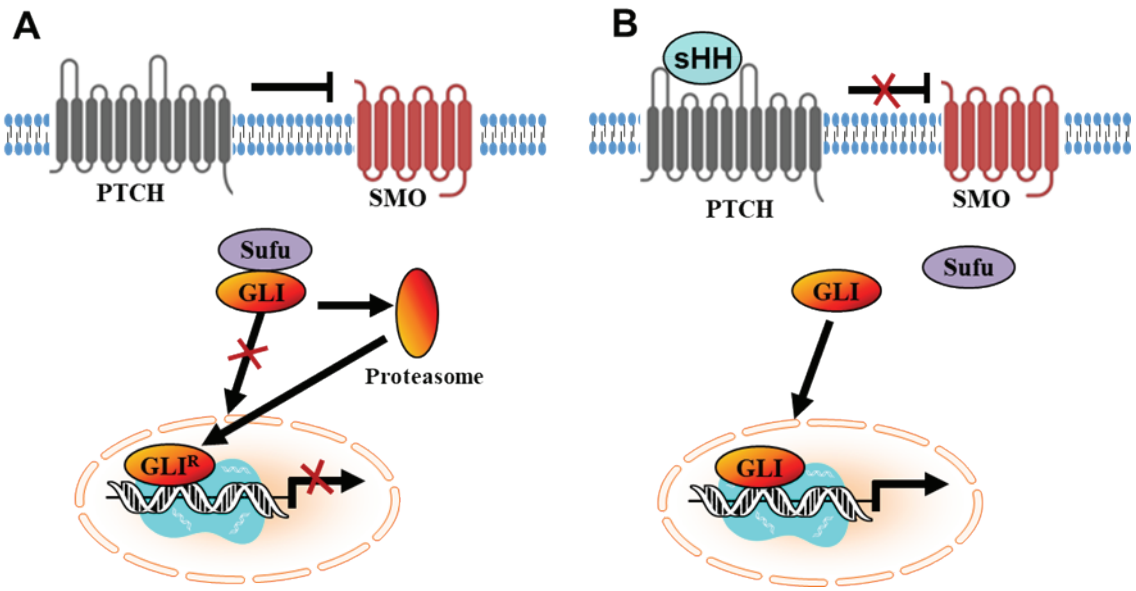

Figure 1. Sonic Hedgehog signaling (sHH) pathway in the absence (A) and presence (B) of Shh ligands. In A, PTCH inhibits SMO, leading to GLI1 sequestration in the cytoplasm by SuFu. In B, sHH ligand binds to PTCH, releasing PTCH suppression to SMO, resulting in the nuclear translocation of GLI which activate target genes the sHH signaling.

mutations. In another separate study, Reifenberger et al reported approximately 10% SMO mutations were identified from 42 BCC tumors [14]. In summary, both mutations and loss of heterozygosity of PTCH and SMO are implicated in BCC.

PTCH/SMO in Medulloblastomas

Medulloblastoma is a type of brain tumor accounting for approximately 20% of childhood brain cancers and 10% of all childhood cancer deaths [15, 16]. One subtype of medulloblastoma, known as Sonic Hedgehog-activated medulloblastoma (sHH-Activated MB), comprises approximately 25% to 30% of medulloblastoma [17]. Dysregulation of PTCH and SMO plays important roles in the pathogenesis of the sHH-Activated MB. Yang et al reported that granule neuron precursors and stem cells are the cell types for the origin of medulloblastoma, and deletion of PTCH to activate sHH signaling in stem cells leads to medulloblastoma in mice by 3 months of age [18]. Raffel et al examined 24 sporadic medulloblastomas and their data showed loss of heterozygosity of PTCH in 5 of 24 tumors. Among these cases, a mutation of the remaining allele was identified in three cases in PTCH gene. suggesting that inactivation of PTCH function is implicated in a SHH-Activated Medulloblastomas [19]. In addition, Xie reported that 2 of 14 sporadic medulloblastomas bear somatic nonsense mutations (Stop codon introduction) in one copy of the PTCH gene and also deletion of the other alle of PTCH gene [20].

Additional to PTCH gene mutations or lose of its heterogeneity in MB, activating mutations in SMO are collectively found in approximately 10-15% of sporadic medulloblastomas [21]. The importance of SMO mutations in medulloblastomas development has been evidenced by medulloblastomas formation in 94% of the transgenic mouse model in which homozygous transgenic mice contain activating mutations in both alleles of SMO [22].

PTCH/SMO in other types of cancer

Addition to BCC and the sHH-Activated MB, PTCH/SMO mutations and loss of heterozygosity also have significant effects on the development of other types of cancer i.e. liver cancer, pancreatic cancer and breast cancer. Sicklick et al demonstrated that SMO overexpression is correlated with liver tumor size, and identified a novel SMO^{K575M} mutation which may play a critical role in liver cancer development [23]. The knockdown of SMO could inhibit self-renewal, epithelial-mesenchymal transition, pulmonary metastasis, tumorigenesis of pancreas cancer stem cells, suggesting that inhibition of SMO could be a therapeutic strategy to treat pancreatic cancer [24]. Moreover, abnormal expression of sHH ligand without genomic mutations was believed to sufficiently trigger the initiation of pancreatic cancer [25].

Furthermore, mutations in PTCH and SMO have been reported in breast cancer as well. Wang and college showed that PTCH1 mutations, especially mutations in exons 22 and 23, are associated with early recurrence of breast cancer patients and could serve as a powerful predictor for recurrence of breast cancer [26]. Moraes et al demonstrated that expression of activated human SMO mutation in transgenic mice leads to dysplasia of the mammary ducts [27].

In summary, aberrant activation of the sHH signaling pathway, particularly by PTCH / SMO mutations or loss of heterozygosity of PTCH1 gene allele plays key roles in pathogenesis of multiple types of cancer. Therefore, significant efforts have been made to develop drugs to inhibit sHH signaling, especially SMO inhibitors, for the treatment of cancers.

TARGETING SMO FOR CANCER TREATMENT

Dysregulation of the sHH pathway, often due to overexpression of sHH ligand, PTCH and SMO mutations, is implicated in various cancers. As it positions at the downstream of sHH ligand and PTCH for the sHH signaling regulation, SMO is an attractive target for drug development. Thus, significant endeavors have been made in the past to develop SMO inhibitors to interfere the aberrant activation of the sHH pathway in malignancies including two of SMO inhibitors, Vismodegib (Erivedge) and Sonidegib (Odomzo) approved by FDA to treat advanced basal cell carcinoma (Figure 2).

Cyclopamine

Cyclopamine is the first reported SMO inhibitor, which can be dated back to the 1950s, when researchers investigated the causes of lamb cyclopia, a rare birth defect characterized by the development of a single eye in the center of the lamb face. They found that lambs born to ewes that had grazed on the corn lily plant (Veratrum californicum) during pregnancy exhibited this abnormality. Further investigation led to the identification of chemical compound cyclopamine responsible for the lamb cyclopia induction [28]. In 1998, Beachy group and Incardona et al. discovered that cyclopamine effectively inhibits the Shh signaling pathway [29, 30]. Subsequently, Beachy research group reported that cyclopamine specifically targets SMO protein for the sHH signaling

inhibition [31, 32]. Since its discovery, cyclopamine has become a valuable tool in scientific research, and a potential therapeutic agent for various cancers and diseases linked to abnormal Shh signaling. Intensive preclinical studies have also demonstrated that cyclopamine effectively inhibits growth of tumors, including human glioma, melanoma, colon, pancreatic, prostate cancers, small cell lung cancer, and medulloblastoma [33-37].

Nevertheless, the therapeutic potential of cyclopamine as an sHH signaling inhibitor for human cancers was limited by its side effects, low solubility in normal saline, and other physiological solutions as well as instability under acidic conditions [38, 39]. To overcome these issues, several cyclopamine derivatives that display more-drug like properties have been developed, including KAAD-cyclopamine and IPI-926 [32, 40] , 11].

KAAD-Cyclopamine

Among the cyclopamine derivatives, KAAD-Cyclopamine displays several advantages over cyclopamine. Chen et al demonstrated that KAAD-Cyclopamine exhibits greater potency in inhibiting the sHH signaling pathway compared to natural cyclopamine. This enhanced potency can potentially lead to improved therapeutic outcomes with lower doses to minimizeits potential side effects [32]. In addition, Chen et al also showed that KAAD-Cyclopamine offers greater selectivity in targeting SMO in comparison to cyclopamine leading to reduced off-target effects and improved safety profiles [32]. These advantages collectively position KAAD-cyclopamine as a promising candidate for further development as a therapeutic agent for diseases associated with dysregulated sHH pathway.

IPI-926

IPI-926, also known as IPI-269609, is another synthetic derivative of cyclopamine that has been developed as a potential anticancer agent targeting SMO. In comparison to cyclopamine, it exhibits improved pharmacokinetic properties and enhanced potency. For

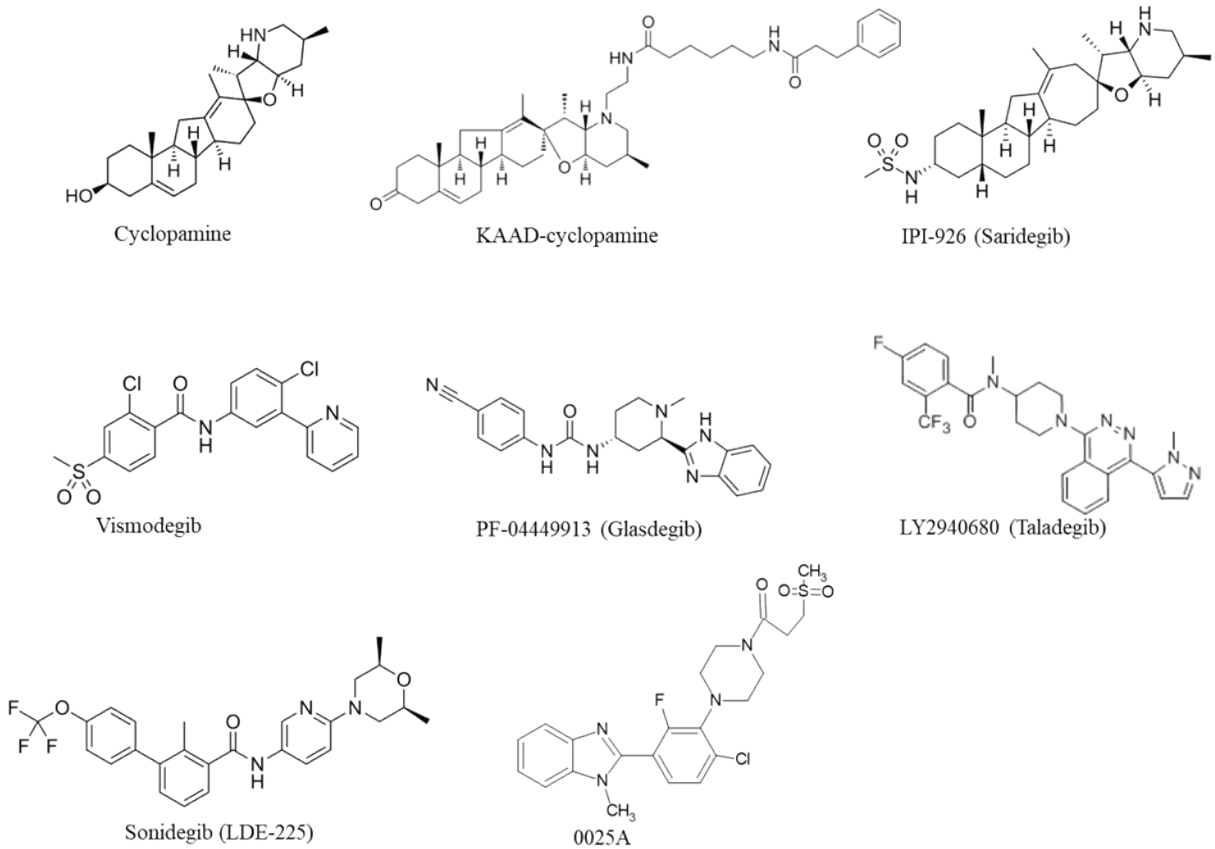

Figure 2. Chemical Structures of SMO inhibitors.

instance, IPI-926 displays ~30-fold greater potency than cyclopamine at inhibiting Gli-luciferase reporter activity in both murine and human cell lines [41]. Additionally, IPI-926 was shown to exhibit a significant increase in plasma half-life due to low clearance and high tissue distribution, and oral administration of IPI-926 results in downmodulation of the sHh pathway in primary chondrosarcoma xenografts [40, 42]. Pancreatic cancer is among the most lethal human cancers, in part because it is insensitive to many chemotherapeutic drugs. In studying a mouse model of pancreatic cancer, co-administration of IPI-926 significantly increases the delivery and efficacy of gemcitabine in the mice [43]. Due to its excellent preclinical properties, IPI-926 was examined in a clinical phase I trial to treat patients with Patients with Advanced and/or Metastatic Solid Tumor Malignancies (NCT01130142) [44].

Non-cyclopamine-derivatives-based SMO inhibitors

PF-04449913, LY2940680 (Taladegib) and 0025A, three SMO inhibitors structurally completely different from cyclopamine and its derivatives, have been reported as well. In one study, Munchhof et al. showed remarkable potency and beneficial pharmacological characteristics of PF-04449913[45]. Further study demonstrated that PF-04449913 effectively attenuates the leukemia-initiation potential, and enhances acute myeloid leukemia therapy by sensitizing dormant leukemia stem cells to chemotherapy and overcoming resistance in the bone marrow microenvironment [46]. Early Phase I trials of PF-04449913 have confirmed its favorable safety, tolerability, and potential efficacy in various hematologic malignancies, including acute myeloid leukemia, myelodysplastic syndrome, myelofibrosis, chronic myelomonocytic leukemia, and advanced solid tumors (NCT [47-49]. Current Phase II trials of PF-04449913 are underway to assess its effectiveness in acute myeloid leukemia, high-risk myelodysplastic syndrome (NCT01546038), myelofibrosis in patients previously treated with ruxolitinib (NCT02226172), and refractory/relapsed myelodysplastic syndrome or chronic myelomonocytic leukemia (NCT01842646).

LY2940680 (Taladegib), another non-cyclopamine related SMO inhibitor was shown to target the extracellular end of SMO's transmembrane-helix bundle for Hedgehog signaling inhibition [50, 51]. Phase I and Phase II trials of LY2940680 have been conducted for advanced solid tumors and esophageal cancers. In a phase I clinical trial, LY2940680 exhibited favorable pharmacokinetic profiles, and it can inhibit both the wild-type SMO and the mutant SMO^{D473H} (NCT02530437) [52] [53]. In an ongoing phase II clinical trial, LY2940680 efficacy and safety are being evaluated specifically in patients with solid tumors characterized by PTCH1 loss-of-function mutations (NCT05199584). However, no results from this trial have been reported.

Recently, Fan et al reported a novel potent SMO antagonist 0025A, which may represent a new therapy for refractory cancers [54]. 0025A can bind to both wild-type SMO or mutant SMO^{D473H}, and reduce the accumulation of SMO on primary cilia and the expression of Gli upon the sHH ligand stimulation. In addition, the Shh signaling is closely related to regulation of hair follicle morphogenesis, and the inhibitory effect of 0025A on hair follicle morphogenesis and hair growth were examined [55, 56]. . The studies showed that 0025A suppressed Shh signaling-mediated-hair growth in C57BL/6 mice, which warrants further investigation of 0025A compound in the treatment of human cancer.

Vismodegib (GDC-0449) and Sonidegib (LDE-225)

Vismodegib (GDC-0449) is the first SMO inhibitor drug approved by the FDA for the treatment of adults with BCC. Developed by Genentech, Inc., this drug emerged from extensive clinical trials, including a phase I trial involving 68 patients. Throughout this trial, varying dosages of Vismodegib were administered, with promising outcomes across different patient groups. In subsequent phase II and III clinical trials, Vismodegib exhibited notable efficacy in patients with advanced BCC. Key assessments encompassed adverse effects, tumor responses, pharmacokinetics, and the down-regulation of GLI expression, and the results underscored the potential of Vismodegib as a viable anti-tumor therapy, particularly in advanced BCC and medulloblastoma cases. Eventually Vismodegib gained approval from FDA in January 2012 and from the European Commission in July 2013 to specifically treat adult patients with symptomatic metastatic BCC or locally advanced BCC.

Sonidegib (LDE-225) is the second SMO antagonist drug approved by the FDA. Sonidegib was discovered by Pan and colleagues in an *in-vitro* high-throughput screen, and it interacts with SMO in the drug-binding pocket to prevent downstream activation of sHh signaling pathway [57, 58]. In the clinical Phase I trial, Sonidegib was assessed for its safety, pharmacokinetics, and preliminary efficacy in patients with advanced BCC. Results confirmed Sonidegib's favorable safety profile, good pharmacokinetics and promising preliminary efficacy [59]. Subsequent clinical trials confirmed

significant tumor response rates in patients with locally advanced or metastatic BCC with manageable side effects [60-62]. In 2015, Sonidegib received approval from the FDA for the treatment of locally advanced BCC that has recurred following surgery or radiation therapy, or for patients who are not candidates for surgery or radiation therapy.

SMO DRUG RESISTANCE AND OVERCOMING STRATEGIES

The development of small molecule inhibitors targeting the sHH signaling pathway, specifically SMO inhibitors, has shown great efficacy in the treatment of cancers and other diseases. However, the emergence of drug resistance poses a significant challenge to their efficacy. One of the primary mechanisms of drug resistance is SMO mutations that can occur in the drug-binding pocket of SMO, rendering it insensitive to inhibition. For instance, most BCC patients experience significant clinical benefit on vismodegib at the early treatment, but some of them develop resistance eventually. Genomic analysis of tumor biopsies of those patients revealed that vismodegib resistance is predominantly associated with SMO mutations, which either directly impaired vismodegib drug binding or activated SMO to varying levels [63]. Additionally, Yauch et al identified a SMO^{D473H} mutation in Medulloblastoma patient, which confers resistance to vismodegib [64].

In addition, cancer cells can activate alternative signaling pathways that bypass the sHH pathway, allowing them to proliferate despite SMO inhibition. For example, Buonamici et al demonstrated that Sonidegib induces tumor regression in animal models of medulloblastoma effectively. However, resistance emerged during the treatment due to SMO mutations, which reactivates sHH signaling and tumor growth [65]. Unexpectedly, up-regulation of phosphatidylinositol 3-kinase (PI3K) signaling was identified as another potential resistance mechanism. Combination therapy of PI3K inhibitors BKM120 or BEZ235 with Sonidegib significantly delayed resistance development [65].

Overcoming resistance to SMO inhibitors requires innovative therapeutic strategies, such as development of drugs that target the downstream key components of sHH signaling (such as GLI) and combination therapies that combine SMO inhibitors with drugs targeting alternative signaling pathways, such as PI3K inhibitors or MEK inhibitors. These strategies may help overcome resistance associated with SMO inhibitors.

FUTURE PERSPECTIVES AND CONCLUSION

SMO inhibitor drugs hold promise in expanding treatment options for various cancers including BCC. Continued research efforts aim to elucidate the potential of SMO inhibitors in targeting other malignancies, including SHH-Activated Medulloblastomas and other solid tumors with aberrant sHH signaling. As our understanding of the intricate interplay between the sHh signaling and tumorigenesis deepens, novel strategies for refining SMO inhibitor therapies are likely to emerge, potentially leading to improved patient outcomes and survival rates in a broader spectrum of cancer types.

In summary, SMO has emerged as a compelling target for drug development due to its central role in the sHH signaling pathway. Continued advancements in SMO-targeted therapies hold promise for improving treatment outcomes for patients with SMO-dependent malignancies and related disorders. While challenges such as drug resistance and off-target effects persist, advancements in drug development, patient stratification, and combination therapy strategies offer hope for further optimizing SMO inhibitor treatments and extending their benefits to a wider patient population.

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