Basal cell carcinoma pathogenesis and therapy involving hedgehog signaling and beyond

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Abstract

Basal cell carcinoma (BCC) of the skin is driven by aberrant hedgehog signaling. Thus blocking this signaling pathway by small molecules such as vismodegib inhibits tumor growth. Primary cilium in the epidermal cells plays an integral role in the processing of hedgehog signaling-related proteins. Recent genomic studies point to the involvement of additional genetic mutations that might be associated with the development of BCCs, suggesting significance of other signaling pathways, such as WNT, NOTCH, mTOR, and Hippo, aside from hedgehog in the pathogenesis of this human neoplasm. Some of these pathways could be regulated by noncoding microRNA. Altered microRNA expression profile is recognized with the progression of these lesions. Stopping treatment with Smoothened (SMO) inhibitors often leads to tumor reoccurrence in the patients with basal cell nevus syndrome, who develop 10–100 of BCCs. In addition, the initial effectiveness of these SMO inhibitors is impaired due to the onset of mutations in the drug-binding domain of SMO. These data point to a need to develop strategies to overcome tumor recurrence and resistance and to enhance efficacy by developing novel single agent-based or multiple agents-based combinatorial approaches. Immunotherapy and photodynamic therapy could be additional successful approaches particularly if developed in combination with chemotherapy for inoperable and metastatic BCCs.

Keywords

BCC; hedgehog; photodynamic immunotherapy; therapeutics

1 | INTRODUCTION

Skin cancer is the most common form of malignancy in the world. A total of 3.5 million cases of nonmelanoma skin cancer (NMSC) are diagnosed each year, of which 80% represent basal cell carcinoma (BCC). The average cost of treatment for NMSC amounted to
4.8 billion dollars between 2007 and 2011. Additionally, there has been a 3–8% yearly increase in the incidence of NMSC worldwide since 1960. The incidence of BCC alone continues to increase by 10% per year. Forty to fifty percent of patients with a primary malignancy are likely to develop one or more BCC within 5 years. A recent retrospective cohort study found that locally advanced BCC could be identified in 0.8% of BCC cases and metastatic BCC occurred in 0.4% of the BCC cohort. BCC prevalence tends to be much higher in fair-skinned individuals with high cumulative ultraviolet (UV) susceptibility factors, such as light eyes, hair color, and the inability to tan. In fact, the lifetime risk for developing BCC ranges from 33 to 39% in Caucasian men and 23 to 28% in Caucasian women. The incidence of NMSCs directly increases depending on exposure to UV radiation. Intense solar UVB light exposure is the most important environmental risk factor for the development of NMSCs. UVB-induced DNA damage promotes the development of pyrimidine photoproducts that ultimately lead to mutations in key genes promoting the alterations of signal transduction pathways, cell cycle deregulation and immunosuppression. Our and other earlier reviews described in detail the molecular pathogenesis of NMSCs including BCCs. This review focuses on certain aspects of the molecular pathogenesis of BCCs, which have not been previously been discussed in greater detail while providing a cursory discussion of other aspects that have been reviewed earlier.

2 | CANONICAL HEDGEHOG SIGNALING

Mutations that lead to the up-regulation of hedgehog (HH) signaling are associated with the development of BCC. For instance, mutations in the tumor suppressor gene Patched (PTCH) are implicated in the growth of sporadic BCCs and those that develop due to Gorlin syndrome. Gorlin or Nevvoid basal cell carcinoma syndrome (NBCC) is a rare autosomal dominant disorder characterized by the development of multiple BCCs from an early age. Individuals are typically affected by numerous clinically advanced BCC lesions (ranging from dozens to thousands), dyskeratotic palmar and plantar pitting, rib and spine abnormalities, premature calcification of the falx cerebri, frontal bossing, hypertelorism, macrocephaly, and cleft lip or palate. Patients have an increased disposition for developing other malignancies, such as medulloblastomas, rhabdomyosarcomas, benign ovarian cysts, cardiac fibromas, and mesenteric cyst.

PTCH is a 12-pass transmembrane receptor protein that suppresses the HH signaling cascade. HH ligands, including Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog (DHH), repress the functions of tumor suppressor, PTCH, upon binding. This binding interaction allows the release of the seven-pass transmembrane protein Smoothened (SMO), which migrates to the primary cilium and regulates the transcription of GLI transcription factors (GLI1, GLI2, and GLI3). In the absence of the HH ligand, PTCH blocks this migration of SMO into the primary cilium. The majority of sporadic BCCs have loss-of-function mutations in at least one allele of PTCH1, preventing repression of the HH cascade, and others have gain-of-function mutations in SMO, leading to over-activation of the pathway. A recently published study found that intrafollicular epidermal stem cells, rather than committed progenitor cells, are able to develop into BCC upon HH signaling due to their enhanced self-renewing ability. This leads to rapid clonal expansion and resistance to p53-mediated apoptosis. HH signaling also has some role in the development of squamous
cell carcinoma since cells carrying mutant *PTCH* within the interfollicular epidermis, hair follicular bulge, and hair germ have the potential for developing into either of the two NMSCs. However, HH signaling is not considered to be a driver pathway for this neoplasm.

In the absence of ligand, GLI’s are phosphorylated, ubiquitinated and partly cleaved to generate repressor forms that prevent downstream HH signaling. Upon translocation of SMO into the primary cilium, such proteolytic processing is prevented and the lengthy active form of GLI allows for the transcription of target genes. The translocation of GLI 1/2 also involves the disassociation of the complex from its inhibitor suppressor of fused (SUFU) (Fig. 1). A loss-of-function mutation in *SUFU*, which has been found in sporadic BCCs, leads to increased GLI transcription. For an elaborate description of these events, please refer to our and other recent reviews.

### 3 | NONCANONICAL HEDGEHOG SIGNALING

GLI transcription also occurs via alternate pathways that comprise noncanonical HH signaling, as shown in Fig. 1. In this way, the characteristic ligand binding of *PTCH1* and activation of SMO is bypassed to induce GLI expression. Epidermal growth factor receptor (EGFR) signaling through RAS/RAF/MEK/ERK synergistically modulates expression of downstream GLI target by activating JUN/AP-1, which cooperates with GLI to induce target gene expression. In addition, EGFR-induced activation of ERK1/2 also prevents proteasome mediated GLI2 degradation in keratinocytes. Transforming growth factor (TGFβ) signaling up-regulates GLI2 transcription by promoting SMAD and β-Catenin interaction at the GLI promoter site. Employing mouse BCC cell lines, activation of aPKC, an atypical protein kinase C, was shown to function downstream of SMO to phosphorylate and activate GLI1. WNT/β-Catenin regulates HH transcription by targeting Coding Region Determinant Binding Protein (CRD-BP), which binds with GLI1 mRNA and induces BCC development. SUFU binds to β-catenin and blocks its nuclear translocation. Kinases such as Cdc211, unc-51-like-kinase 3 (Ulk3), mitogen-activated protein kinase 10 (MAPK 10) and dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 (DYRK2) are considered important in the regulation of HH signaling. Stimulation of PI3K/AKT by IGF-1 induces GLI even in the presence of low SHH. AKT also regulates SHH signaling through PKA-mediated GLI inactivation. SHH signaling regulates metastasis through the activation of PI3K/AKT, which promotes epithelial-mesenchymal transition (EMT) and matrix metalloproteinase 9 (MMP-9). Snail, a direct transcriptional repressor of E-cadherin, drives skin cancer progression and metastasis. We also showed that there is an enhancement of Snail in BCCs. NF-κB is a transcription factor that is associated with cutaneous inflammation and carcinogenesis triggered by chemical or UVB. NF-κB allows for SMO independent GLI activation by binding to the GLI promoter in EMT and claudin-low cell lines. This complex network of mechanisms may underlie the pathogenesis of both slow growing as well as locally invasive BCCs into a rare metastatic disease.

Genomic analysis has identified novel mutations both downstream of GLI and independent of the HH pathway that may be considered important in the development and/or progression of BCC. Yes-associated protein (YAP) is a co-transcriptional activator with oncogenic
potential that also has a role in maintaining basal epidermal progenitors, regulating hair follicles, and promoting proliferation in normal skin.\textsuperscript{27} In vitro studies demonstrated that YAP activation is associated with accelerated proliferation, diminishing apoptosis, and the suppression of differentiation of primary mouse keratinocytes.\textsuperscript{27} Upon Hippo signaling activation, YAP is phosphorylated and transported to the cytoplasm where it is sequestered by 14-3-3\textsigma and can no longer induce target gene transcription.\textsuperscript{28, 29} Bonilla et al\textsuperscript{30} analyzed 293 BCCs lesions and found that YAP target genes are significantly upregulated suggesting their role in tumorigenesis. PTPN14 and LATS1 loss-of-function mutations promote BCC through the nuclear localization and consequent transcriptional activation of YAP.\textsuperscript{30} Two novel genes related to BCCs, serine/threonine protein phosphatase genes PPP6C and serine/threonine protein kinase gene STK19 have also been identified.\textsuperscript{30} PPP6C manifests an inhibitory effect on cyclin D1 and is involved in promoting phosphorylation-dependent activation of LATS1.\textsuperscript{31} Missense mutations in N-MYC, an oncogene associated with neuroblastoma and medulloblastoma, were found in 30\% of BCCs analyzed.\textsuperscript{30} MYC is a key regulatory factor in embryonic development and plays a dominant role in oncogenesis, which occurs more frequently in highly recurrent BCCs.\textsuperscript{30, 32} MYC genes are involved in regulating multiple cellular mechanisms such as DNA repair, proliferation, metabolism, cellular differentiation, regulation of noncoding RNA, and protein synthesis.\textsuperscript{33} The majority of mutations located in the MYC box 1 region (MB1) compromised the interaction of N-MYC with FBXW7, the substrate-binding component of an ubiquitin ligase complex that leads to proteasome-mediated degradation of MYC.\textsuperscript{30} Mutations in FBXW7 further augment the oncogenic impact of enhanced N-MYC stability in BCCs.\textsuperscript{30} Mutations in the conserved binding sites for the transcription factor GATA3 induce BCC genesis by disrupting epidermal differentiation.\textsuperscript{34} GATA3 knockout mice show increased proliferation in basal epidermal cells, decreased apoptosis, aberrant hair follicle, and epidermal differentiation along with an upregulation of NOTCH 1 and WNT signaling.\textsuperscript{35} Mutations in Kinastin (kinetochore-localized ASTRIN/SPAG5 binding protein (KNSTRN)), which encodes a kineto-chore-associated protein in the mitotic spindle that is vital to chromosomal segregation, are also implicated in BCC.\textsuperscript{30, 36} Mutant KNSTRN in a murine BCC cell line disrupted sister chromatid cohesion during mitosis further emphasizing its role in promoting chromosomal instability.\textsuperscript{36} KNSTRN mutations seem to be acquired in the later stages in BCC development implicating their potential as biomarkers of aggressive disease, although this remains to be established.\textsuperscript{36} Oncogenic mutations noted in the genes of the MAPK pathway included ErbB2, Ras, PIK3CA, and RAC1. NOTCH1 and NOTCH2 mutations were observed in 26\% and 29\% of BCCs, respectively.\textsuperscript{30} Furthermore, single nucleotide polymorphisms associated with caspase 8 splice variants potentially inhibiting apoptosis are associated with BCCs.\textsuperscript{30, 34} Inactivation of caspase-8 in basal epidermal keratinocytes triggers chronic skin inflammation and hyperproliferation in mouse skin.\textsuperscript{37}
HH signaling is mediated in the primary cilium, a microtubule-based, membrane-enclosed structure. Primary cilia are nonmotile and sensory structures involved in trafficking receptors molecules to cilial membrane. Motile cilia participate in both generation and detection of mechanical signal during development. Both motile and nonmotile types of cilia have some common structural proteins such as core components of tubulin and intraflagellar transport (IFT) proteins. Some proteins such as inner and outer dynein arms (IDAs) and nexin-dynein regulatory complex (NDRC) and radial spokes are only present in motile cilia to regulate waveform and beat frequency. Ciliary and basal body proteins act as signaling hubs for various developmental as pathophysiological pathways. Morphogenesis and homeostasis of epidermis and hair follicles require involvement of multiple SHH, WNT, NOTCH, Hippo signaling pathways which have been closely linked with nonmotile ciliary proteins. Besides these pathways, ciliary proteins also participate in vesicle transport, cytoskeleton, signaling, ubiquitination. Lehman et al have demonstrated that ciliary function disruption of dermal cells of the skin results in loss of SHH or GLI2 function, and arrest of follicle development. Primary cilia’s temporally and spatially distinct functions participate in crosslink of signaling, proliferation and differentiation in epidermis, hair follicle, and in the bulge stem cells whose normal morphogenesis is relies on SHH and NOTCH signaling. Loss of epidermal cilia leads to hyperplasia, expanded clone size and modulate keratinocyte differentiation. In a recent affinity proteomic analysis, 217 tagged ciliary proteins were analyzed to identify the new disease-relevance. Lewis et al showed that growth arrest specific 8 (Gas8) is needed for cilial motile functions and its two missense variants (A391V and E199K) were detected in human primary ciliary dyskinesia (PCD) patients. The involvement of primary cilia and interactions of various ciliary proteins through which epidermis-mesenchymal cells receive signals remain elusive but it remains very interesting to understand the pathophysiology of PCD.

GORAB, a golgin that localizes at the golgi apparatus, induces formation of the primary cilium. For this reason, GORAB deficient dermal mesenchymal cells that exhibit defects in primary cilia development are unable to fully respond to HH signaling in vitro. SMO initially originates from the cell surface and translocates to the ciliary membrane. Various proteins participate in the extensive mechanisms involved in the process of ciliary translocation. The IFT machinery mediates the movement of SMO from the ciliary base to the tip. For instance, mutant IFT27 and IFT25 mice experienced impaired hair follicular morphogenesis in association with disruptions in the trafficking of SMO and impaired transcription of GLI. Following the translocation of SMO to the primary cilia, protein kinase A (PKA) induced repression of GLI transcription is suppressed and GLI proteins are released from their inhibitor SUFU. The ciliary accumulation of SMO following HH signaling activation forms the Evc-SMO complex at a distinct ciliary compartment known as the EvC zone. This critical association is required for the SMO mediated suppression of PKA and SUFU, the subsequent GLI3 repressor inhibition and GLI2/3 activator formation. EF-hand calcium binding domain 7 (EFCAB7) and IQ domain-containing protein E (IQCE) are two ciliary proteins that
positively regulate HH signaling by anchoring the EVC-EVC2 complex in a signaling microdomain at the base of the cilia.\textsuperscript{55} Moreover, a heteromeric transient receptor potential channel, polycystic kidney disease like 1 (PKD1L1)-(PKD2L1) controls ciliary calcium concentration and regulates SMO mediated GLI activation.\textsuperscript{55} These data suggest the involvement of complex interactions of ciliary proteins and SHH signaling proteins. The physiological importance of many of these interactions is not yet clear.

The distribution of phosphatidylinositol 4-phosphate (PI(4)P) in the ciliary membrane and phosphatidylinositol 4,5 phosphate 2 (PI(4,5)P2) at the ciliary base is created by a ciliary phosphoinositide 5-phosphatase (INPP5E).\textsuperscript{56} This distribution is known to promote normal HH signaling by limiting the ciliary accumulation of G-protein coupled receptor 161 (GPR161), an inhibitor of HH signaling.\textsuperscript{56} Upon inactivation of INPP5E, PI(4,5)P2 accumulates at the cilia tip and leads to the recruitment of PI(4,5)P2 interacting protein and Gpr161, which then represses GLI transcription.\textsuperscript{57} Thus, in the absence of signaling, GPR161 localizes to the cilium and may lead to the activation of PKA and subsequent processing of GLI3 to its repressor form.\textsuperscript{51} In the presence of signaling, GPR161 binds to β-arrestin and subsequently, clathrin-mediated endocytosis promotes its removal.\textsuperscript{58}

Additionally, Jiang et al\textsuperscript{59} demonstrated that a phospholipid, (PI(4)P), shuttles between PTCH and SMO to mediate HH signaling. The binding of PI(4)P to the arginine motif in the SMO C-terminal tail promotes phosphorylation dependent activation of SMO and its ciliary localization. Studies also suggest that Pitchfork (PIFO) and the G protein-coupled receptor associated sorting protein 2 (GPRASP2) are integral components of the ciliary targeting complex that facilitates SMO translocation into the primary cilium.\textsuperscript{60} Kuzhandaivel et al\textsuperscript{61} identified that Costal (COS 2) and Fused (Fu) are required for SMO ciliary transport involved in \textit{Drosophila} olfactory sensory neurons. These core components are conserved from \textit{Drosophila} to vertebrates.

Other signaling pathways, such as WNT, NOTCH, mTOR, and Hippo that have been implicated in BCC promotion are also associated with the primary cilium. WNT signaling is required to modulate the cilia cytoskeleton suggesting that the cytoskeleton is vital to the onset of WNT signaling.\textsuperscript{62} The development of ciliopathies such as the Bardet-Biedl (associated with the knockdown of BBS1, BB4, and MKKS) have been linked to an overactive WNT response in cell cultures.\textsuperscript{63} Moreover, the presence of mutations in mice primary cilia has resulted in an increased canonical WNT signaling response.\textsuperscript{64} Canonical WNT signaling promotes stabilization of β-catenin in the cytoplasm, which then triggers the transcription of WNT target genes in the nucleus.\textsuperscript{62} Noncanonical WNT signaling, on the other hand, results in modification in cell shape and actin assembly.\textsuperscript{62} Cilia also function to regulate NOTCH signaling and, in doing so, preserve the balance between epidermal proliferation and differentiation.\textsuperscript{45} NOTCH receptors and NOTCH processing enzymes are localized within the cilia in epidermal cells and ciliary mutations lead to defects in NOTCH signaling.\textsuperscript{45} NOTCH regulates HH signaling by controlling the interaction of ciliary transport proteins with PTCH1 and SMO.\textsuperscript{65} miR-449 mediated inhibition of NOTCH is vital to the differentiation of ciliated cell progenitors.\textsuperscript{66} Moreover, cilia mediate cell size through downregulation of the mTOR pathway.\textsuperscript{66} The suppression of mTOR correlates with the activation of autophagy, which results in the inhibition of proteasome-mediated degradation.
of ciliary proteins. NPHP4, a cilia-associated protein, serves as a negative regulator of Hippo signaling by directly interacting with LATS1 and inhibiting LATS1-mediated phosphorylation of YAP. NPHP4 acts upstream of NPHP9, another protein that localizes to the primary cilia, in a common pathway that ultimately activates YAP and promotes proliferation.

5 | NONCODING RNA REGULATION

MicroRNAs (miRNA) are small regulatory RNA that have been implicated in the development of various forms of cancer. Mature miRNAs serve as post-transcriptional regulators of gene expression. DROSHA, an RNase III endonuclease involved with the initial stages of RNA processing in the nucleus, cleaves primary miRNA to precursor miRNA. DiGeorge syndrome critical region gene 8 (DGCR8), another essential component of the miRNA maturing microprocessor complex, stabilizes DROSHA and identifies the primary RNA substrate. Once the precursor miRNA is transported to the cytoplasm, DICER, an RNase III enzyme, further cleaves pre-miRNA to form mature strands that are incorporated into the RNA-induced silencing complexes (RISC). Incorporated miRNA guides RISC to its complementary mRNA so that translation can be interrupted. RISC is a multi-protein complex composed of several proteins; these include: the RISC core components argonaute-1 (AGO1) and argonaute-2 (AGO2), the RISC loading complex subunit TARBP, and the double-stranded RNA binding protein PACT. Altered expression of miRNA machinery components, such as the maturing microprocessor complex and RISC, may play a role in BCC development. mRNA expression levels of DROSHA, DGCR8, AGO1, AGO2, TARBP, and PACT were significantly upregulated in BCC tumor tissue as compared to the healthy skin controls. Conversely, DICER was found to be downregulated in BCC as compared to healthy skin. Phosphorylation of TRBP by MAPK leads to the stabilization of the miRNA complex, an upregulation of tumor promoting miRNAs and the downregulation of tumor suppressor miRNAs. Hence, modifications in this complex miRNA maturation process may lead to altered protein expression and resulting tumorigenesis.

Altered miRNA profiles in association with some of the key players in BCC pathogenesis provide a compelling story regarding the significance of noncoding RNA regulation in this context. Sand et al found 16 upregulated and 10 down regulated miRNA in BCC skin with connections to HH and MAPK signaling. miR-203 has been identified as a tumor suppressor that is suppressed by EGFR/MEK/ERK/c-JUN signaling. Heffelfinger et al found that distinctive miRNA expression correlates with nodular and infiltrative tumor BCC subtypes. Not only that but the expression level of miR-183, a miRNA that has been shown to inhibit metastasis in various other cancers, was consistently lower in the infiltrative than nodular BCCs. The upregulation of MiR-141, 200a, and 200c in nodular BCC may be linked to C-MYC and the WNT-β-catenin pathway.

Oncogene cluster, oncomiR-1 cluster (miR-17-92) has been implicated in SHH pathway regulated medulloblastoma in a PTC1 mouse model. miRNA belonging to this particular cluster were overexpressed in BCCs suggesting their role in BCC pathogenesis. Recently Sand et al identified differentially expressed cirRNAs in BCCs that regulate miRNA
activity by sequestering target sequences. This study further describes the interaction of cirRNA with the OncomiR-1 cluster. Differentially expressed cirRNAs with microRNA response elements (MREs) for members of OncomiR-1, including hsa-miR-19b-1 and hsa-miR-92, were among the top 10 cirRNAs identified. Differentially expressed long noncoding RNA (lncRNAs) in BCCs including oncogenic and epidermis specific ones such as CASC15 or ANRIL have also been identified.

6 | INTERVENTIONS

6.1 | SMO antagonists

Cyclopamine was the classic SHH inhibitor found to antagonize SMO and to prevent BCC development in a murine model of UVB carcinogenesis in our laboratory. Its toxic effects, poor oral bioavailability along with solubility, and stability issues led to the development of vismodegib (GDC-0449), a second generation cyclopamine derivative, which also directly binds SMO at the same location where cyclopamine binds. Similar to cyclopamine, vismodegib exposure during organogenesis is associated with embryo fetal death and congenital birth defects. It is currently approved to treat adults with metastatic or recurrent BCC who are not eligible for surgery or radiation therapy. According to a recent meta-analysis examining the clinical response to vismodegib, more than a quarter of the patients discontinued therapy due to adverse events such as muscle spasms, alopecia, dysgeusia, and amenorrhea. SM inhibitors such as sonidegib (LDE225) and itraconazole are in phase II clinical trials to treat advanced or metastatic BCC. Other experimental agents in Phase I clinical trials are saridegib, TAK-441 XL-139 (NCT00670189), and LEQ506 (NCT01106508) (Table 1) (Fig. 1).

Itraconazole, a systemic antifungal, is a HH signaling antagonist that inhibits SMO in a manner that is distinct from cyclopamine and vismodegib and prevents the ciliary accumulation of SMO. Posaconazole, a second generation triazole anti-fungal agent that inhibits HH signaling by a mechanism similar to itraconazole, exhibits activity against drug resistant SMO mutants. It has been proposed to be a better chemopreventative drug for suppressing BCC growth as it does not significantly affect the expression/activity of drug metabolizing cytochrome P450s as compared to itraconazole, which manifests drug-drug interactions.

A preclinical study examining the topical efficacy of SMO antagonists using a depilated mouse model concluded that even a single application of LDE-225 was sufficient to inhibit GLI and PTCH1 expression, making it the most desirable agent for topical therapy. A phase II trial showed that topical LDE-225 in NBCCs patients was well-tolerated and caused BCC regression (Table 1).

6.2 | Resistance to SMO-targeted therapy

Despite the high efficacy of vismodegib, certain tumors exhibit increased potential for drug resistance and are more capable of evading SMO inhibitor therapy. In a retrospective medical chart review, out of 28 patients with metastatic and locally advanced BCCs treated with vismodegib, 21% regrew at least one tumor during treatment within a mean time of 56
Primary resistance in these trials was defined by BCC tumors that do not respond to therapy, possibly due to variant genes downstream of SMO. In other cases, patients with advanced BCCs that initially respond to SMO inhibitor treatment, develop secondary resistance to therapy, which is attributed to SMO mutations in the drug-binding domain.

Atwood et al and Sharpe et al by comparing DNA sequences from sporadic BCCs, Gorlin syndrome patients, vismodegib sensitive and resistant tumors demonstrated that the majority of vismodegib resistance in BCC cells is caused by mutations in SMO. In these studies, SMO variants are found in 15–33% of untreated BCCs and in 69–77% of resistant tumors following therapy. The SMO mutations that occurred in or proximal to the drug binding pocket were only present in resistant BCCs implying that such mutations are specifically selected for during therapy. Some of the drug-binding site mutations include of D473, H231, W281, Q477, V321, I408, and C469. In contrast, SMO mutations distal to the drug binding domain were found in both untreated and SMO inhibitor resistant tumors suggesting their inherent role as oncodrivers. Mutations outside of the drug-binding site (such as T241M, A459V, L412F, S533N, and W535L) destabilize SMO, reduce affinity for the antagonist, and increase basal activity of SMO. Mutation W535 interacts with V321, L412F, and F460L to constitutively activate SMO. SMO mutant cells resistant to vismodegib continued to proliferate when treated with other chemically distinct SMO antagonists. This underlying cross-resistance between different inhibitors indicated that combination therapy utilizing multiple SMO antagonists may not be highly effective against emerging resistance. Interestingly in some cases, resistant tumors without SMO mutations are still associated with increased HH induced GLI expression. For this reason, multiple hereditary infundibulocystic basal cell carcinoma (MHIBC), which is associated with a germline SUFU mutation, does not respond to SMO inhibitors that target upstream of the mutated SUFU gene in this case. Sharpe et al found a loss-of-function mutation in a PI3K pathway regulator, phosphatase and tensin homologue (PTEN), which has been previously implicated in lowering response to vismodegib treatment in MB models. Highly vismodegib-resistant tumors, with or without SMO mutations, may be better treated through combination therapies with agents that directly target GLI downstream of SMO.

6.3 | SMO antagonists and the development of squamous cell carcinoma

Findings in a case control study showed that patients exposed to vismodegib have an increased risk for the development of a non-BCC malignancy, especially SCC with a hazard ratio of 8.12 (95% CI, 3.89–16.97; P < 0.001). Additionally, in medulloblastoma models, the RAS/MAPK pathway offers an alternative to promote tumor growth by bypassing SHH signaling. Mutations in RAS/MAPK that likely develop following treatment with SMO inhibitors circumvent canonical HH inhibition, promote resistance and alter the subsequent tumor characteristics. Typically, canonical HH signaling inhibits ERK activation in the RAS/ MAPK pathway, leading to basaloid expression and SCC suppression. Noncanonical GLI activation promotes proliferation and the squamous cell phenotype in RAS mutated BCC by stimulating the EGFR-MEK-ERK axis. Hence, the inhibition of canonical HH signaling through SMO antagonists and the subsequent compensatory...
upregulation of the noncanonical pathway promotes the development of SCCs in BCC patients with concurrent mutations in the RAS/MAPK pathway. Similarly, RAS mutations in melanoma patients treated with BRAF inhibitors have been associated with the development of SCC, which is also associated with the concurrent upregulation of MAPK signaling.

### 6.4 | GLI antagonists

In the model systems, tumor development may be efficaciously targeted by directly inhibiting GLI downstream of SMO. Agents that selectively target the transcription activities of GLI1/2 may make the most effective inhibitors. GLI antagonists such as GANT56 and GANT61 have been shown to mediate GLI-mediated gene activation. However, they have not been tested in models of BCCs. In other models where GLI expression is found to be augmented, GANT 61, a GLI 1/2 inhibitor, inhibits proliferation of GLI over-expressing cancer cells such as those derived from rhabdomyosarcoma, osteosarcoma, osteosarcoma, neuroblastoma, and ovarian cancer. GANT61 more effectively suppressed HH signaling in neuroblastoma cells as compared to SMO blockers. Similarly, GANT61 was able to inhibit breast cancer survival, mRNA expression of GLI1, nuclear translocation of GLI1, ERK1/2, MAPK signaling, and EGFR expression more so than SMO antagonist. GANT61 also inhibited rhabdomyosarcoma tumor proliferation by 50% in mouse models and significantly diminished Akt/mTOR signaling. Additionally, GANT61 increased apoptosis in pancreatic cancer stem cells by activating caspase-3 as well as suppressed EMT by increasing E-cadherin expression and inhibiting EMT regulating transcription factors snail, slug, and zeb1 (Fig. 1).

Inhibition of DYRKIB (dual-specificity-phosphorylation-regulated kinase 1B), a positive regulator of HH/GLI signaling downstream of SMO, reduces GLI signaling in SMO resistant BCC cells. More recently, arsenic trioxide, which impedes HH signaling by blocking ciliary accumulation of GLI, reduces BCC development. The anti-tumor activity of arsenic trioxide also occurs through the inactivation of NOTCH1 targets, BCL2 and NF-kB.

### 6.5 | Bromodomain inhibition

Bromo and extra C-terminal (BET) domain family are composed of multiple members (BRD2, BRD3, BRD4, BRDT, and others) that bind to acetylated histones to induce transcription. These proteins induce transcription by binding to N-e-acetyllysines on histone tails and forming critical complexes. The BET protein, BRD4, can directly bind to GLI1 and GLI2 promoter sites to induce transcription. Despite SMO inhibitor resistance, the BET inhibitor, JQ1, decreases tumor cell growth by reversing the occupancy of BRD4 at the GLI promoter site in medulloblastoma, atypical rhabdoid tumor, and BCC cells. BET inhibition through JQ1 was also efficacious against SMO resistance mechanisms including mutations of SMO, SUFU, or amplification of GLI2 or MYC (Fig. 1).

Studies using multiple myeloma models have established that bromodomain proteins facilitate c-MYC dependent transcription. Targeting c-MYC driven transcription through
JQ1 provides a therapeutic opportunity to inhibit c-MYC driven medulloblastoma and neuroblastoma. A recent report, describing that JQ1 inhibits neuroblastoma tumor growth and induce apoptosis by altering MYCN driven transcription, suggests that BET inhibitors may have the potential to target multiple MYC family members. As described previously in this review, MYCN has also been identified as a potential driver in BCC development. Hence, BET inhibitors may lead to decreased BCC tumor viability by down regulating MYCN transcription.

A recent study demonstrated that BRD4 inhibitors exert their cytotoxic effect by triggering BAX/BAK dependent intrinsic apoptosis. For this reason, in the absence of pro-apoptotic proteins, BAX and BAK, malignant hemopoietic cells remained resistant to the effects of JQ1, both in vitro and in vivo. BIM, a BH3-only protein, is an inducer of mitochondrial apoptosis that inhibits antiapoptotic BCL2 proteins and activates pro-apoptotic proteins BAX and BAK. JQ1 treatment partly increased BIM levels by suppressing miR-17-92 cluster, a negative regulator of BIM. BET inhibition is able to suppress miR-17-92, a transcriptional target of c-myc, even in the absence of c-MYC suggesting that JQ1 may counter tumorigenesis by directly altering mRNA expression associated with BCC.

A recent study showed that BRD4 is expressed throughout the brain and promotes the transcription of critical genes and synaptic proteins regulating learning and memory. Thus, JQ1 inhibition of BRD4 resulted in deficits in memory consolidation and also decreased the seizure threshold in mice. On the other hand, a more recent study showed the potential of JQ1 to actually reduce neuroinflammation in mice models of Alzheimer’s disease. Additional research to elucidate any potential neurological impairments will need to be considered while evaluating the therapeutic benefits and possible toxicity of JQ1 therapy in the treatment of BCC. Further investigation of GLI inhibition by targeting BRD4 in BCC would be a worthwhile approach at least in a combinatorial setting.

### 6.6 Other epigenetic inhibitors

Various epigenetic regulators mediating HH signaling are found mutated in the progression of neoplastic growth. It is known that promoter hypermethylation of key players that promote the SHH and WNT pathways drive BCC growth. The acetylation of GLI1 and GLI2 inhibits their recruitment to target promoter site. Upon activation, upregulated histone deacetylase 1 and 2 (HDAC1 and 2) deacetylates GLI 1 and 2 and enhances their functions. HDAC inhibitors have shown efficacy in targeting various malignancies in clinical and preclinical trials; such as T-cell lymphoma, multiple myeloma, pancreatic, breast, cervical, ovarian thyroid, and nonsmall cell lung cancer. HDAC6, which is overexpressed in murine medulloblastoma cells, induces maximal HH activation by stabilizing GLI3 and enhancing GLI2 expression. HDAC6 blockade by antagonists tabacin, CAY10603, and ACY-1215 reduced tumor growth in vitro and in vivo in allograft models. Compound NL-103, which contains both the structural elements of vismodegib and the general HDAC inhibitor vorinostat, was able to overcome SMO resistance by concurrently inhibiting HDAC function and HH signaling. EZH2, a histone methyltransferase of the polycomb repressive complex 2, methylates lysine 27 on histone H3 and is associated with tumor suppressor silencing in medulloblastoma. EZH2 expression is also elevated in aggressive
BCC subtypes and correlates with the proliferation marker Ki67.\textsuperscript{129} EZH2 may be a potential target to inhibit BCC progression. Moreover, promoter hypermethylation of important regulators in the SHH and WNT pathways contributes to BCC tumor promotion and inhibition of such key players may lead to novel treatment options\textsuperscript{132} (Fig. 1).

Sirtunins (SIRT), NADH-dependent deacetylase, are involved with DNA repair and offer protection against DNA damage and oxidative stress.\textsuperscript{140} Reduced expression of mRNA levels of two SIRT family members (SIRT 2 and 3) has been found in BCCs as compared to nontumoral skin.\textsuperscript{141} SIRT3, which has been shown to affect NOTCH levels in gastric tumors, may likely affect NOTCH expression in BCCs too and also affect HH signaling in the primary cilia.\textsuperscript{141,142}

6.7 | Immunotherapy

Immunotherapy is currently considered to be an effective modality to manage neoplastic growth. Recently, regressing tumors were shown to have increased infiltration of CD3\textsuperscript{+}CD4\textsuperscript{+} T-cells and an altered cytokine profile as compared to nonregressing BCCs, suggesting that immunotherapy may be a valuable therapeutic modality for this neoplasm.\textsuperscript{143} Initial efforts to apply immunotherapy to this context included of sensitization with dinitrobenzene and microbial allergens in combination with cytokines.\textsuperscript{144,145} A recent study attempted to assess the effectiveness of intrallesional candida antigen in treating BCCs by stimulating an immune response.\textsuperscript{146} While such treatments have shown some potential, they have not been further explored due to lower efficacy as compared to the gold standard.\textsuperscript{147} Treatment of BCC with IFN alpha has been shown to stimulate IL2 and inhibit IL10, leading to tumor regression.\textsuperscript{148} This treatment modality requires multiple intrallesional injections and can cause flu-like symptoms.\textsuperscript{149}

Topical immune response modifier imiquimod 5% cream, which stimulates a cytokine response and activates Toll like receptors (TLR7/8), is a promising option.\textsuperscript{150} The resulting innate immune response enhances IFN, TNF, IL1, IL12 and further stimulates an acquired immune response through the activation of TH1 cells.\textsuperscript{150} IFN alpha mediates apoptosis by inhibiting the RAS-ERK pathway induced by HH signaling.\textsuperscript{151} Additionally, IFN causes BCC cells to express CD95 receptor so that CD95 receptor CD95 ligand interaction can induce apoptosis.\textsuperscript{152} Imiquimod has also been shown to inhibit GLI transcription through adenosine receptors (ADORAs) that are known to regulate proliferation, cell death, and signaling throughout the body.\textsuperscript{152} ADORA promotes PKA activity leading to the phosphorylation of GLI and the selection of the repressor form of GLI.\textsuperscript{152} Imiquimod has been shown to stimulate p53-induced apoptosis through increased ROS production and stimulation of the ATM/ATR.\textsuperscript{153} However, this mechanism may not operate in BCCs carrying mutant p53. Tumor cells exhibit decreased BCL2 expression following treatment with Imiquimod, making them vulnerable to apoptosis.\textsuperscript{150} Treatment with imiquimod results in a massive increase in macrophages surrounding and infiltrating tumor islets.\textsuperscript{150} Since BCC tumor cells do not express MHC class I molecules and subsequently lack infiltration by CD8 cells, local imiquimod treatment has been shown to upregulate MHC I on tumor cells along with a surge in CD8 cells.\textsuperscript{154} Imiquimod also leads to keratinocytes differentiation by activating the NOTCH pathway through the upregulation of JAGGED1.\textsuperscript{155} Multiple phase
III clinical trials have shown the utility of imiquimod in the initial and long-term clearance of superficial BCCs along with favorable cosmetic outcomes.\textsuperscript{156–159} Adverse reactions associated with imiquimod include cutaneous psoriasiform eruptions, and oral ulcerations.\textsuperscript{160–162}

Peripheral tissues and tumor cells express PDL-1 which binds with the PD-1 receptor on T cells to deliver an inhibitory signal that suppresses T cell proliferation and TCR mediated activation of IL2.\textsuperscript{163} Immune checkpoint blockade with antibodies targeting PD-1 and its ligand PD-L1 enhances the anti-tumor immune response mediated by T cells and counters the immune-suppressive microenvironment in tumors.\textsuperscript{164} Blockade of the PD-1 pathways with humanized anti-PD-1 monoclonal antibody, pembrolizumab, has shown a modest tumor response rate in patients with advanced melanoma.\textsuperscript{165} A recently published case report shared a case of patient with metastatic BCC who was treated with pembrolizumab.\textsuperscript{166} Following the cessation of therapy, metastatic lung lesions stabilized.\textsuperscript{166} A phase I clinical trial is currently studying if pembrolizumab can be used with or without vismodegib to treat metastatic or unresectable BCCs (NCT02690948). Macular popular rash, pruritus, lichenoid dermatitis or psoriasis are immune related adverse events observed following anti-PD-1/ PDL1 therapy.\textsuperscript{167}

Sustained activation of CTLA4, a negative regulator of T cell activation, induces immune tolerance to the oncogenic antigens.\textsuperscript{168} CTLA-4 blocking antibodies such as, ipilimumab, promote T-cell recognition of tumors such as melanoma and nonsmall cell lung cancer.\textsuperscript{169,170} Treatment for recurrent nodular melanoma with ipilimumab incidentally also led to the regression of concurrent advanced BCC.\textsuperscript{171} This surprising finding suggests that impilimumab may be an effective agent to counter CTLA-4 mediated immunotherapy in BCCs.\textsuperscript{171}

The inflammatory response produced by COX-2 induced prostaglandins appears important in the development of BCC. COX-2 overexpression, induced by ROS following UV exposure could be linked with BCC tumor proliferation and promotion at least in part.\textsuperscript{172} Enhanced COX-2 expression increases anti-apoptosis, angiogenesis and tumorigenesis in BCC,\textsuperscript{173} while NSAIDs and COX-2 selective inhibitors suppress tumor progression by inhibiting acute inflammation, proliferation of keratinocytes, and activation of epithelial neutrophil infiltration.\textsuperscript{172} Celecoxib therapy has been shown to partially reduce BCC tumor burden in experimental animals and decrease the development of new BCCs in patients with Gorlin syndrome.\textsuperscript{174} Clinical trials have shown that celecoxib may be effective for the prevention of NMSC, including BCCs, in high-risk skin cancer patients.\textsuperscript{175} Result of a recent phase II clinical trial demonstrated that topical diclofenac therapy promotes tumor regression of superficial BCCs and alleviates anti-apoptotic and proliferative markers.\textsuperscript{176}

6.8 | Photodynamic therapy

Photodynamic therapy (PDT) involves the application of a photosensitizing drug, such as aminolevulinic acid (ALA) or methylaminolevulinate, to the skin.\textsuperscript{149} The accumulated photosensitizing compounds are converted to protoporphyrin IX once absorbed into the epithelium.\textsuperscript{177} Treatment efficacy using photodynamic therapy is limited by the penetration of the topical photosensitizers.\textsuperscript{149} Following its accumulation within intracellular
membranes of organelles, protoporphyrin IX is activated by visible light to produce cytotoxic ROS that results in tumor cell damage and killing.\textsuperscript{177} PDT therapy exerts its effects by directly damaging tumor cells, indirectly disrupting tumor vasculature, and activating the immune responses.\textsuperscript{178} PDT invokes an inflammatory response characterized by localized edema at the target site through the generation of damage-associated molecular patterns (DAMPs), cell death-associated molecular patterns (CDAMPS) and the stimulation of dendritic cells.\textsuperscript{179} It is typically used to treat superficial BCCs and results in favorable cosmetic outcomes.\textsuperscript{173} Various clinical studies have demonstrated the efficacy of PDT in the treatment of nodular and superficial BCCs.\textsuperscript{180–183}

Verteporfin is a second generation photosensitizer that has been approved by the FDA for the treatment of age related macular degeneration.\textsuperscript{184} Verteporfin in addition to its light sensitivity shows significant pharmacological activity. Verteporfin alone has been shown to inhibit the proliferation of hepatocellular carcinoma and retinoblastoma cells by inhibiting the YAP pathway.\textsuperscript{185,186} Verteporfin therapy leads to a decrease in YAP nuclear localization resulting and a concurrent increase in cytosolic YAP via its trapping by 14-3-3σ which may occur in a p53-dependent manner\textsuperscript{29} (Fig. 1). It is interesting to note that limiting YAP signaling in the skin increases epidermal differentiation and dampens proliferation by accelerating apoptosis. Our group is currently studying the effects of verteporfin administered as monotherapy and in combination with SMO inhibitors on BCC tumor regression (unpublished data).

6.9 | Combination therapies

Combination therapies targeting canonical HH signaling along with key crosstalk pathways offer a synergistic strategy to curb tumor development. Treatment with gefitinib, an EGFR inhibitor, in combination with either cyclopamine or GANT61 reduced cell growth of BCC cell line more effectively than any of the agents individually.\textsuperscript{13} Cetuximab, a monoclonal antibody inhibits EGFR and has shown potential in targeting NMSC.\textsuperscript{187} It may also be evaluated for the treatment of advanced BCCs along with HH pathway inhibitors.\textsuperscript{187}

Crosstalk involving PI3K and SHH has been linked with acquired resistance to SMO inhibition.\textsuperscript{188} Thus, combination therapy involving PI3K/mTOR inhibition along with HH inhibition may be an effective therapeutic strategy. Adding a PI3K class I inhibitor to a potent SMO antagonist delayed the development of resistance in a medulloblastoma mouse model.\textsuperscript{189} Dijkstra et al\textsuperscript{190} demonstrated this therapeutic synergy when they found that SMO resistant medulloblastoma was still sensitive to PI3K inhibition. Similarly, the combined inhibition of SHH and mTOR signaling pathways together with standard care chemotherapy is capable of eliminating pancreatic cancer stem cells both in vitro and in vivo.\textsuperscript{191} Co-treatment with GANT61 and PI3K/ mTOR inhibitor, PI103, at subtoxic concentrations synergistically inhibited tumor growth in SHH-driven rhabdomyosarcoma model.\textsuperscript{192} A pilot trial to examine the efficacy of vismodegib (SMO inhibitor) and buparlisib (PI3K inhibitor) in metastatic or advanced BCCs is already underway (NCT02303041).

Chaudhary et al\textsuperscript{24} showed that the combined inhibition of SHH signaling and the inflammatory response using a COX-2 inhibitor, sulindac, and SMO antagonist,
itraconazole, respectively, in a Gorlin syndrome murine model inhibited the growth of UVB-induced BCCs by more than 90%. These authors elucidated that the p50 subunit of NF-κB engages BCL3, an atypical member of the IκB family, to mediate transcription program, which may be important in the pathogenesis of BCCs. Moreover, they also confirmed the crosstalk between SHH signaling and BCL3 pathways by demonstrating the efficacy of targeting this crosstalk in effectively eliminating BCCs.

Kim et al showed that the combination of itraconazole and arsenic trioxide inhibited HH signaling driven growth of drug resistant BCC and medulloblastoma in vivo and in vitro. A recent clinical study with five patients undergoing itraconazole and arsenic trioxide treatment led to inhibition of the HH pathway by 75% compared to baseline; however, while some patients did experience tumor arrest, none noticed tumor shrinkage. The lack of clinical efficacy in this trial may be due to suboptimal dosing, the short treatment length (approximately 3 months) and the small sample size.

PDT therapy may also induce tumor cell resistance due to the activation of NF-κB, MAPK, protein kinase B/AKT, PI3K, and COX-2 following therapy. As a result, combination therapies along with PDT may increase efficacy and counter resistance. Studies have shown that neoadjuvant PDT therapy prior to surgical excision may significantly lower tumor burden, and result in a surgical excision with histologically clear margins. Substantially improved clinical outcomes were observed when complementing PDT with imiquimod. Future studies examining the synergy between PDT and other small molecules that target various pathways associated with BCCs are needed to further explore the utility of these therapeutic modalities.

7 | SUMMARY AND FUTURE PROSPECTS

Hedgehog signaling has been extensively studied as a key player in the pathogenesis of both sporadic BCCs and those linked with NBCCS. Crosstalk of the HH pathway with EGFR, TGFβ, aPKC, mTOR, PI3K, and NF-κB amongst others maximizes the GLI response that leads to BCC tumor growth and perhaps metastasis. Recent studies have identified additional mutations that take part in BCC development. For instance, aberrant activation of the oncogenic YAP promotes BCC through nuclear localization and transcriptional activation of YAP1. The ciliary accumulation of SMO, which is mediated by critical proteins and phosphoinositides, is central to the activation of GLI proteins. Primary cilia also regulate other signaling pathways that are associated with BCC, such as WNT, NOTCH, mTOR, and Hippo. Altered miRNAs expression levels are associated with BCC, suggesting the role of noncoding RNA regulation in tumor promotion. Various clinical trials have illustrated the limited efficacy of SMO inhibitors on tumor suppression (Table 1). Despite some success, resistance tends to develop following therapy with SMO antagonists paving the need for additional agents. SMO resistance can be partially overcome by targeting HH pathway downstream of SMO through agents like GANT61, DYRKIB, arsenic trioxide, and bromodomain inhibitors; although novel more effective and less toxic agents are required to achieve the desired therapeutic resistance. Exploration of epigenetic regulators that mediate HH signaling in the context of BCC genesis may provide additional options to curb tumor development. Immunotherapy through imiquimod, PD-1/ PD-L1 inhibitors, ipilimumab and
COX-2 inhibitors together and can be evaluated as valuable treatment options. Photodynamic therapy, especially through novel agents such as verteporfin, should be explored in the context of BCCs. Many of these therapeutic strategies succeed in outplaying BCCs by promoting epidermal cell differentiation and inducing p53-mediated apoptosis. Therapeutic cocktails that combine the various treatment modalities and target multiple pathways associated with BCCs may offer the unique opportunity for treating metastatic and advanced BCCs. Additionally, the finding that differentially expressed miRNAs was found in vismodegib treated BCC tissue warrants additional investigations to confirm the role noncoding RNA in promoting tumor resistance. Future studies can explore strategies to modulate miRNAs that are associated with BCC pathogenesis and resistance. Finally, the discovery of mutations beyond HH could be important to develop therapies that target the novel additional driver signaling pathways that could be central to BCC growth progression despite targeting HH.

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Abbreviations

ADORAs  adenosine receptors  
AGO1  argonaute-1  
AGO2  Argonaute-2  
ALA  aminolevulinic acid  
aPKC  atypical protein kinase C  
BCC  basal cell carcinoma  
BET  bromo and extra C-terminal  
CDAMPS  cell death-associated molecular patterns  
COX-2  cyclooxygenase-2  
CTLA-4  cytotoxic T-lymphocyte protein 4  
DAMPs  damage-associated molecular patterns  
DGCR8  DiGeorge syndrome critical region gene 8  
DYRKIB  dual-specificity-phosphorylation-regulated kinase 1B  
EGFR  epidermal growth factor receptor  
EMT  epithelial-mesenchymal transition  
ERK  extracellular signal regulated kinases
EVC  
Ellis-van Creveld syndrome

Gpr161  
G-protein coupled receptor 161

Gprasp2  
G protein-coupled receptor associated sorting protein 2

HDAC  
histone deacetylase

HH  
hedgehog

IFN  
interferon

IFT  
intraflagellar transport

IGF  
insulin like growth factor

IL  
interleukin

lncRNAs  
long noncoding RNA

Inpp5e  
phosphoinositide 5-phosphatase

IQCE  
IQ domain-containing protein E

KNSTRN  
kinetochore-localized astrin/SPAG5 binding protein

MAPK  
mitogen-activated protein kinase

MHIBCC  
multiple hereditary infundibulocystic basal cell carcinoma

MMP-9  
matrix metalloproteinase-9

MREs  
microRNA response elements

mTOR  
mammalian target of rapamycin

NBCCS  
Nevoid basal cell carcinoma syndrome

NMSC  
nonmelanoma skin cancer

NSAID  
nonsteroidal anti-inflammatory drug

PDL-1  
programmed death-ligand

PDT  
photodynamic therapy

PI(4)P  
phosphatidylinositol 4-phosphate

PI(4,5)P2  
phosphatidylinositol 4,5 phosphate 2

PKA  
protein kinase A

PTCH  
patched

RISC  
RNA-induced silencing complexes

ROS  
reactive oxygen species
SCC  squamous cell cancer
SHH  sonic hedgehog
SIRT  sirtuins
SMO  smoothened
SUFU  suppressor of fused
TGF-β  transforming growth factor beta
TLR  toll like receptors
TNF  tumor necrosis factor
UV  ultraviolet
YAP  yes-associated protein.

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FIGURE 1.
Molecular targets for therapy in the HH pathway web of collaboration. The HH pathway interacts with various other signaling networks that synergistically contribute to tumor development. PTCH is a 12-pass transmembrane receptor that generally represses SMO. HH binding to PTCH or inactivating PTCH mutations suppress this repressive response and allow for the translocation of SMO to the primary cilium to induce GLI transcription. The mTOR pathway helps release GLI from SUFU through S6K1 mediated GLI phosphorylation. The IGF/PI3K/AKT and EGFR/MEK/ERK pathways modulate PKA-dependent phosphorylation of GLI. In the hippo pathway, MST1/2 kinases and SAV1 form a complex to phosphorylate and activate LATS1/2 and MOB1. In turn, LATS1/2 dephosphorylates YAP/TAZ, allowing the complex to translocate the nucleus and to interact with TEAD1-4 to induce the expression of genes that promote tumor progression. The aggrandizing effect of these various pathways results in BCC development, progression, and tumor resistance. Inhibiting various players within this crosstalk may lead to effective and resistance-proof treatment modalities. SMO antagonists, crosstalk pathway inhibitors, GLI antagonists, HDAC inhibitors, bromodomain inhibitors, and verteporfin target these various pathways to curb tumor development.
## TABLE 1
Chronology of hedgehog inhibitors development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial</th>
<th>Patient population</th>
<th>Number of patients</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vismodegib</td>
<td>Phase I^1^102</td>
<td>Patients with solid tumors refractory to current therapy including basal cell cancer</td>
<td>68</td>
<td>Established an acceptable safety profile at a recommended daily dose of 150 mg/d and an effective tumor response in patients with BCC.</td>
</tr>
<tr>
<td></td>
<td>Phase II^1^114</td>
<td>Patients with Basal Cell Nevus Syndrome</td>
<td>41</td>
<td>At 1 month, vismodegib use had reduced the hedgehog target gene expression in BCCs by 90% and no residual BCC was histologically detectable in 83% of biopsy samples from clinically regressed basal cell carcinoma sites.</td>
</tr>
<tr>
<td></td>
<td>Phase II (ERIVSNCE: 12 month analysis)^1^95</td>
<td>Patients with metastatic BCC and locally advanced BCC</td>
<td>104</td>
<td>Objective response rate in patients with metastatic and locally advanced BCC was 33.3% and 46.7% respectively.</td>
</tr>
<tr>
<td></td>
<td>Phase II (ERIVSNCE 30 month analysis)^1^96</td>
<td>Patients with metastatic BCC and locally advanced BCC</td>
<td>104</td>
<td>Objective response rate by investigator review: 48.5% for metastatic BCC and 60.3% for locally advanced BCC.</td>
</tr>
<tr>
<td></td>
<td>Phase II STEVIE^1^97</td>
<td>Patients with metastatic BCC and locally advanced BCC</td>
<td>499</td>
<td>Overall response in 66.7% of patients with advanced BCC and 37.9% of patients with metastatic BCC.</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis^1^99</td>
<td>Patients with metastatic BCC and locally advanced BCC</td>
<td>744</td>
<td>Objective response for locally advanced and metastatic BCC was 64.7% and 31.1% respectively. Complete response was as 31.1% for locally advanced BCC and only 3.9% for metastatic BCC.</td>
</tr>
<tr>
<td>Sonidegib (LDE225)</td>
<td>Basal cell carcinoma outcomes with LDE 225 (BOLT) trial^100</td>
<td>Patients with metastatic BCC and locally advanced BCC</td>
<td>94</td>
<td>Objective response rate of 57.6% for locally advanced BCC and 7.7% for metastatic BCC.</td>
</tr>
<tr>
<td>Topical Sonidegib (LDE225)</td>
<td>Phase II^1^101</td>
<td>Patients with Basal Cell Nevus Syndrome</td>
<td>8</td>
<td>Out of 13 LDE225-treated BCCs: 3 showed a complete, 9 a partial, and only 1 no clinical response.</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Phase I^1^31</td>
<td>Patients with one or more BCC tumor &gt;4 mm in diameter</td>
<td>29</td>
<td>4 patients experienced partial response and four had stable disease.</td>
</tr>
<tr>
<td>Saradegib- IPI-926</td>
<td>Phase I^1^32</td>
<td>Patients with solid tumors including BCC</td>
<td>94</td>
<td>8 of 28 BCC patients naïve to previous SMO inhibitor therapy showed a response to IPI-926 at doses ≥30 mg.</td>
</tr>
<tr>
<td>TAK-441</td>
<td>Phase I^1^33</td>
<td>Patients with solid tumors including BCC</td>
<td>34</td>
<td>Best response was partial (1 patient with BCC) and stable disease (7 patients with various solid tumors).</td>
</tr>
</tbody>
</table>

BCC, basal cell carcinoma; SMO, smoothened.