Masterarbeit

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Systemic Treatment of Advanced Basal Cell Carcinoma With Drugs That Target The Hedgehog Pathway

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Introduction

Skin cancer is currently the most common form of malignancy worldwide. Of the total of 3.5 million cases of nonmelanoma skin cancer (NMSC) diagnosed each year, 80% are basal cell carcinoma (BCC) (1). Similarly, approximately 50% of all cancers reported in the United States (US) are diagnosed as BCC (2). While BCC is a slow-growing tumor that rarely metastasizes, it frequently arises in multiples and can recur in sun-exposed areas of the skin (3). The tumors themselves can present with a variety of clinical characteristics from superficial to extensive and highly destructive lesions (3).

Although 0.8% are locally advanced and 0.4% are metastatic at the time of diagnosis, most BCCs follow a benign course (1). Nonetheless, these tumors can result in extensive morbidity via the local invasion of the skin and tissue destruction (3). Likewise, while the mortality associated with BCC is negligible, this condition can result in significant morbidity, especially in cases in which the tumor remains untreated for a long period (2). The incidence of BCC is much higher in fair-skinned individuals with high cumulative ultraviolet (UV) susceptibility factors, including light eyes, light hair color, and the inability to tan (1). While 80% of all BCC lesions first appear on the face, head, and neck, 15% appear on the trunk and extremities, and rarely on the genital mucosa (2). Locally advanced BCC (LaBCC) can result in disfigurement and loss of function after its excision (2). BCC is diagnosed most frequently in individuals who are >50 years of age with a male-to-female ratio of 2:1. BCC can develop in individuals <40 years of age who present with a specific genetic predisposition (4). BCC will ultimately develop in 33–39% of all Caucasian men and 23–28% of all Caucasian women (1). The incidence of all forms of NMSC is directly related to increased exposure to intense solar UVB radiation (1).

Exposure to UVA and UVB radiation promotes the development of skin cancer by directly damaging cells via induction of DNA mutations (pyrimidine substitutions) and oxidative stress as well as depletion of energy which reduces the effectiveness of DNA repair. UV radiation can also activate local inflammatory processes and suppress anti-tumor immunity (2).

The primary treatment goal for localized BCC is the complete eradication of the tumor while preserving critical cosmetic and functional aspects (2). Local treatment options include surgical excision, Microscopic Oriented Histographic (MOH) surgery, and superficial modalities, including liquid nitrogen, topical imiquimod (5%), and 5-fluorouracil (5%). Imiquimod and 5-fluorouracil are immune response modifiers used alone or in combination with photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) or its methyl ester (MaL). (3) The five-year response rates for these modalities include 97.7% for surgery, 92–97% for PDT (4), and 82.5% for topical imiquimod (3). Surgery is used most frequently as a primary treatment for localized BCC and is most likely to be curative in this circumstance (2). In cases in which the prognosis of the BCC worsens, if the medical treatment is delayed, if BCC recurs after treatment, or if it presents as locally advanced or metastatic disease, surgical treatment may not be curative and systemic therapies should be considered (3,5).

The Hedgehog (HH) pathway is a highly-conserved signaling mechanism that promotes organogenesis, maintenance of stem cells, and tissue regeneration and repair. In skin tissue, the HH pathway is responsible for maintaining the stem cell population and controlling the development of hair follicles and sebaceous glands. (4). Aberrant activation of this pathway drives the pathogenesis of BCC via its contributions to tumor initiation, progression, and relapse. Previous systemic treatments available for these patients were limited to chemotherapy, which presented with life-threatening adverse events (AEs), particularly in elderly patients (4). Systemic treatments that inhibit HH pathway signaling have been approved for the treatment of LaBCC and metastatic BCC (MBCC). These treatments have shown promising results albeit with several prominent AEs that led to the discontinuation of therapy.

In this thesis, I will explore drug therapy, specifically, the use of HH pathway inhibitors (HHPIs) for the treatment of BCC. I will focus on their effectiveness, tolerability, resistance, disease recurrence, and methods that might be introduced to maximize their efficacy. The review will begin with a brief discussion of the clinical subtypes of BCC and diagnosis based on dermoscopy, followed by the use of reflectance confocal microscopy (RCM) and optical coherence tomography (OCT), and histopathology for disease diagnosis.

Clinical subtypes of BCC

There are several distinct clinical subtypes of BCC (2). The main clinical subtypes of BCC include nodular, superficial, and morpheaform variants. Pigmented BCC lesions contain variable amounts of melanin. lesions are typically detected on the face and the hairy skin of the upper and lower limbs and rarely on the genital mucosa.

Nodular BCC

Nodular is the most common subtype, representing 50–79% of all BCC lesions. These lesions appear as shiny, pink or flesh-colored papules or nodules with surface telangiectasia. Nodular BCC lesions may enlarge and become ulcerated, although a sharp border is typically maintained. Nodular BCC lesions are detected most frequently on the face, nose, cheeks, nasolabial folds, and eyelids. Nodular BCC might be suspected in all cases of non-healing, crusting lesions with recurrent bleeding. Pigmented BCC is detected most frequently in darker-skinned individuals (2),

Superficial BCC

Superficial BCC is the second most common clinical subtype, accounting for 15% of all diagnosed lesions. Superficial BCC appears as sharply circumscribed pink to red scaly papules, patches, or thin plaques. Regression of these lesions results in atrophic and hypopigmented areas (2). These lesions may also contain telangiectasia; multiple lesions may be present, and they are detected more frequently on the skin of the shoulders, chest, and back (2). A pigmented variant of superficial BCC has been characterized that appears on the trunk and extremities and in a younger patient cohort (2,5). These lesions are frequently multifocal and may undergo incomplete excision (2). Some of the superficial BCC lesions can evolve into nodular BCC over time (2)).

Morpheaform BCC

This subtype accounts for up 10% of all cases of BCC (2). The name “morpheaform” is based on its clinical resemblance to indurated plaque lesions characteristic of localized scleroderma (2).

Morpheaform BCC lesions are white or flesh-colored and include areas of induration and ill-defined borders that resemble scar tissue or plaque. The surface of this lesion is smooth, but it can

undergo ulceration and erosion forming a crust or a nodule. Telangiectasia may also be present. This subtype of BCC is typically more aggressive and results in extensive local destruction (5).

Giant BCC

One percent of these lesions present with a diameter of 5 cm and are classified as giant BCC. This form of BCC is highly destructive and characterized by deep invasion, a high rate of post-surgical recurrence, and poor prognosis (6).

It is critical to recognize that several subtypes can co-exist in a single lesion. These mixed tumors account for 40% of all BCCs.

Dermoscopic diagnosis of BCC

Dermoscopy is a non-invasive technique that is used *in vivo* to improve the early diagnosis of both melanoma and NMSC lesions. The use of this method increases the accuracy of the diagnosis of both pigmented and non-pigmented BCC lesions by 95–99% (2). Participants at the first dermoscopy consensus meeting (in Hamburg, Germany in 1990) recognized arborizing vessels as one of the main structures in BCC, a finding with high diagnostic accuracy and a positive predictive value of 94.1% (7).

BCC lesions identified by dermoscopy can be classified into one of three categories (7,8): vascular structures, pigment-related structures, and nonvascular/non-pigmented structures.

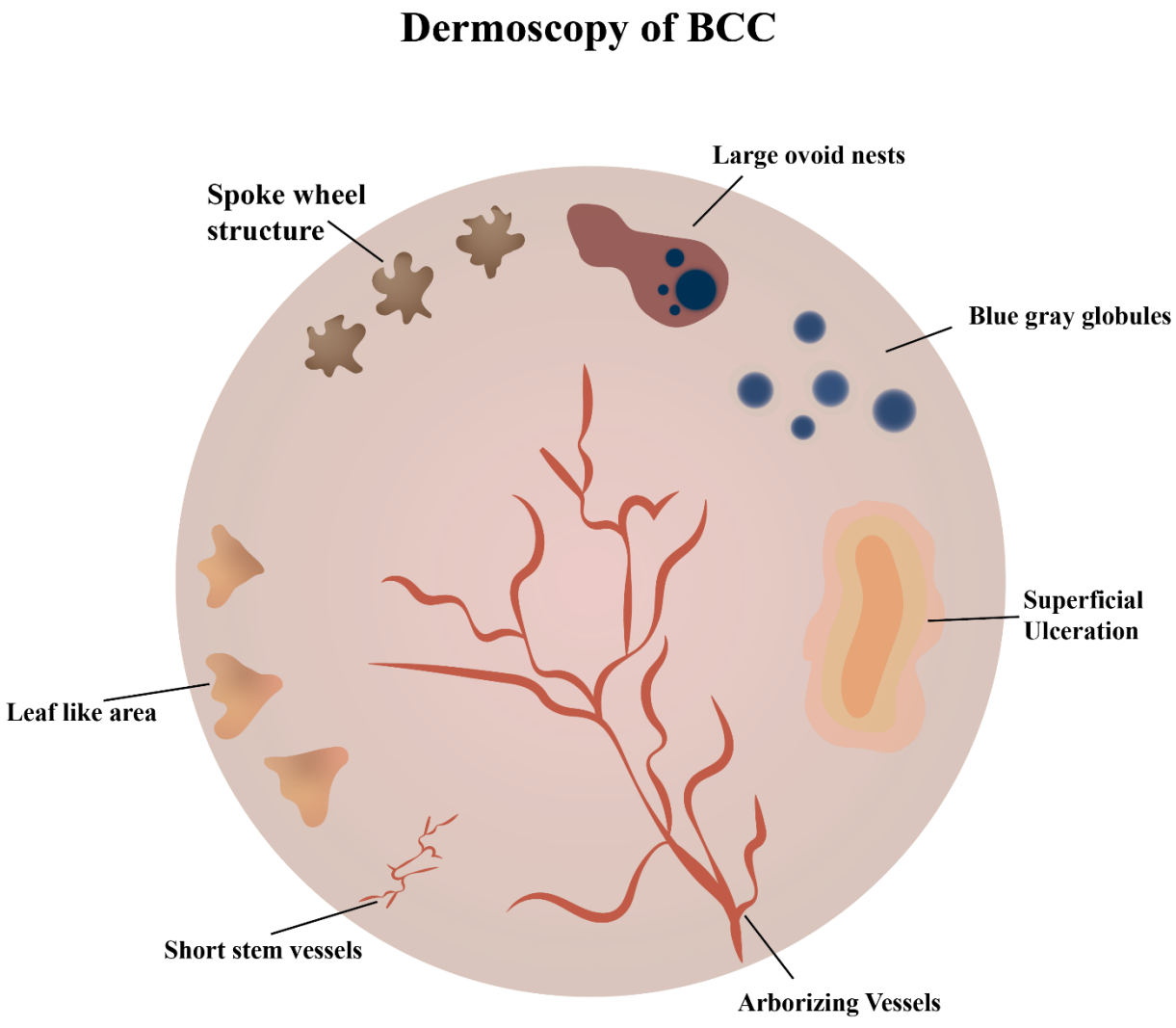
Vascular structures are arborizing vessels with short, fine telangiectasia. Arborizing vessels have large diameters and are bright red. These vessels branch irregularly into fine terminal capillaries that are longer than 1 mm (7). These vessels are located superficially just beneath the epidermis and are clearly visible (8). By contrast, short fine telangiectasias are vessels with smaller diameters of less than 1 mm in length with few to no branches (8). As noted above, the detection of these vessels is a finding with high diagnostic accuracy and a positive predictive value of 94.1%. Other vascular structures detected in BCC lesions are also found in other tumor types, including hairpin, glomerular, dotted vessels, and comma vessels (8). However, the polymorphic pattern that is characteristic of amelanotic/hypomelanotic melanoma and squamous cell carcinoma is observed only rarely in BCC.

Pigment-related structures contain melanin (7). Blue-grey ovoid nests are the largest of these structures (7); they exhibit full or near-confluence with well-circumscribed pigmented ovoid or elongated structures that are larger than globules (8) and not directly connected to the pigmented tumor body (8. These structures have been described in all subtypes except superficial BCC (8). Similarly, blue-grey globules and dots have features that are analogous to the aforementioned blue-grey ovoid nests, although they are smaller in size (8) and present as numerous loosely-arranged round-to-oval well-circumscribed structures that are smaller, sharper, and more focused (2,8). Other pigment-related structures include focus dots, which are loosely arranged and well-defined small grey sharply-focused globules or dots (2). These structures consist of free pigment and are deposited along the dermo-epidermal junction and/or in association with melanophages in the papillary or reticular dermis (8). Maple leaf-like areas are bulbous extensions connected to a base region that is usually brown to grey-blue (2). These structures, which do not arise from the pigmented network or adjacent pigmented regions, typically form a leaf-like pattern at the periphery of the tumor (8). Histopathological examination of these areas suggests that they are multifocal tumor nests that contain pigment aggregates that are connected by lobular extensions localized in the epidermis and papillary dermis. This structure is a unique marker of BCC, most notably the superficial type (8). Spoke wheel areas are well-circumscribed radial projections that are connected at a darker central axis (8). These structures, which are usually tan but sometimes blue or grey with a dark brown, black, or blue axis, are detected only rarely by dermoscopy but are highly specific for BCC (7), particularly the superficial subtype (8). Finally, concentric structures are defined as irregularly-shaped globule-like structures that exhibit different colors with darker central areas that are blue-grey, brown, and black. These structures, which may be precursors or a variation of the spoke wheel area described above (2), are also detected more frequently in superficial BCC.

Of note, heavily-pigmented BCC may present with dermoscopic features that are associated with melanocytic lesions such as brown globules, peppering, or a blue-white veil. These findings may complicate the differential diagnosis of this disease (8).

Nonvascular/non-pigmented structures include ulcerations, which are shallow erosions of the epidermis that reach the dermis and may be covered with coagulated blood or a serous crust (2) and are detected as structureless orange-red to black-red regions by dermoscopy (8). Multiple small erosions (i.e., those smaller than an ulceration) are detected as small brown-red to brownish-yellow crusts. By contrast, shiny white-to-red structureless areas represent diffuse dermal fibrosis or fibrotic tumor stroma (2) while shiny white streaks or crystalline detected by polarized light as orthogonal short, thick crossing lines represent dermal fibrosis (2). Detection of shiny white-to-red structureless areas with white streaks is highly suggestive of BCC.

Figure 1. Typical findings observed in BCC by dermoscopy



Main subtypes of BCC diagnosed by dermoscopy

Lesions characteristic of **superficial BCC** typically exhibit multiple small erosions, superficial fine telangiectasia, and shiny white or red structureless areas. Pigmented lesions contain both spoke wheel and maple leaf-like structures (7). By contrast, arborizing vessels are among the main typical features of **nodular BCC**. The presence of large, blue-grey ovoid nests, multiple blue-grey dots, globules, and ulcerations together with arborizing vessels suggest a high risk of local recurrence (8).

Morpheaform BCC typically exhibits more ulceration together with fine, arborizing vessels, pink-white areas, and multiple blue-grey dots and globules. The linear branched vessels characteristic of this BCC subtype are finer more scattered with fewer ramifications than the arborizing vessels characteristic of nodular lesions.

Lesions characteristic of **infiltrative BCC** exhibit ulceration, a mix of arborizing and superficial fine telangiectasia, short shiny white streaks, and red-to-white structureless areas. The presence of multiple aggregated yellow-to-white globules has been associated with high risk in both morpheaform and infiltrative BCC (8).

Fibroepithelioma of Pinkus, which is an uncommon variant of BCC, is characterized by fine arborizing vessels in the center of the lesion with dotted vessels at the periphery on a white-pinkish background. White streaks, a brown-gray structureless area, and blue-grey dots are detected, together with a negative pigment or white network (8).

Basosquamous carcinoma, which is an aggressive tumor with features of both BCC and squamous cell carcinoma (SCC), exhibits the vascular pattern characteristic of BCC and dermoscopic features (e.g., keratinization) suggestive of SCC. Truncated vessels and multiple blue-grey globules have also been reported in micronodular BCC lesions (8).

Table 1. Dermoscopic findings characteristic of different BCC subtypes

Superficial BCC	Nodular BCC	Morphea form BCC	Infiltrative BCC	Fibroepithelioma of Pinkus	Basosquamous Carcinoma	Micronodular BCC
Small Erosions	Arborizing vessels	Fine ulceration	Ulceration	Fine arborizing vessels in the center	Vascular pattern of BCC	Truncated vessels
Fine telangiectasia	Large blue-grey ovoid nests	Arborizing vessels	Arborizing vessels and fine telangiectasia	Dotted vessels in the periphery on a white pinkish background	Keratinization Superficial scales, keratin masses; ulceration with bloody crust	Multiple blue-grey globules
Shiny white or red structureless area	Multiple grey dots	Pink, white area	Shiny white structures; short white and red streaks	White streaks brown, grey structureless area; blue grey dots		
Spoke wheel and maple leaf in pigmented BBB	Ulcerations	Multiple blue-grey dots and globules	White structureless area	Negative pigment (white) network		

Diagnosis of BCC using Reflectance Confocal Microscopy (RCM)

RCM is an optical imaging technique that provides a horizontal view of the skin from the surface up to the superficial dermis using a laser source that provides near infra-red monographic light (830 nm) to penetrate the tissue and illuminate a single point (9). Various microscopic elements in the skin tissue reflect light with unique indices; for example, both melanin and keratin exhibit comparatively high reflective indices. This non-invasive method is in frequent use as a means to diagnose skin cancer.

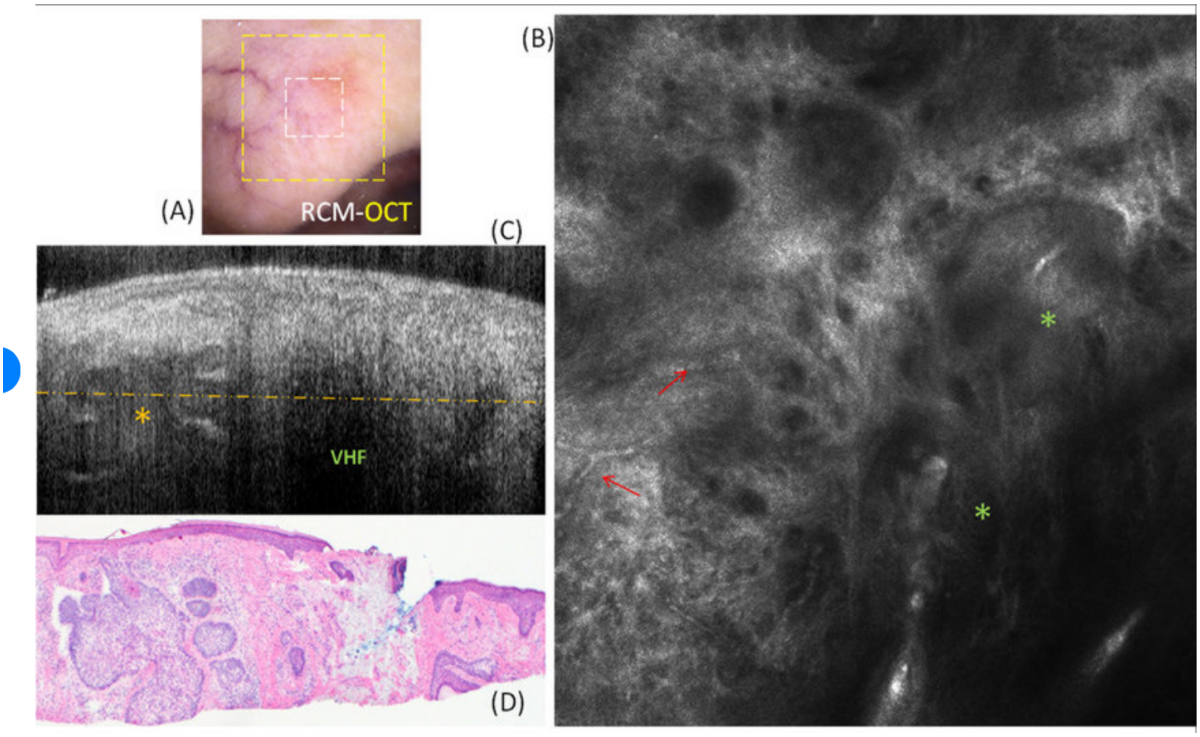
BCC is diagnosed using RCM based on parameters that emerge in the superficial dermis and/or the dermal-epidermal junction (DEJ) (2). Among these findings are dark silhouettes representing hyporeflective areas at the level of the superficial dermis or DEJ that are outlined by bright collagen bundles. Other findings include bright tumor islands that present as round-to-oval, cord-like, or lobulated bright structures that are demarcated by a surrounding dark cleft as well as cleft-

like dark spaces (black areas shaped like clefts or slits) that separate the bright tumors islands from the dermis. Dendritic cells are also detected by this method, and present as delicate cellular structures within bright tumor islands localized within the epidermis. BCC lesions also present with oval-to-stellate bright structures without nuclei and dilated vessels.

Diagnosis of BCC using Optical Coherence Tomography (OCT)

OCT is another non-invasive method that can be used to diagnose BCC via infrared light projected onto the skin that produces an image based on the sum of light reflection (9). This method can be used together with histopathologic findings to differentiate superficial from non-superficial BCC as well as non-BCC lesions. BCC-specific features resulting from OCT include a dark rim, bright perineural stroma, protrusion into the upper dermis, and signal-poor ovoid structures; protrusion into the upper dermis is a specific finding characteristic of superficial BCC. (10)

Figure 2. Diagnosis of BCC with Dermoscopy, OCT, RCM, and histopathology



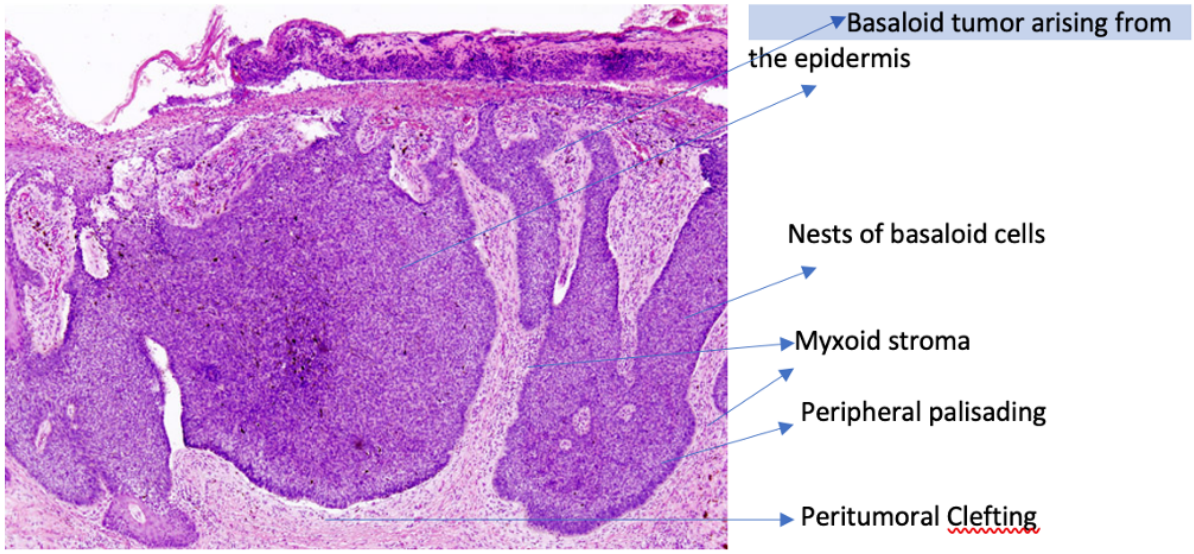
Basal cell carcinoma-OCT helps detect residual BCC tumor in previously biopsied lesion (A) dermoscopy image, (B) en face RCM image, (C) orthogonal OCT image, (D) histopathology This case is of an 89 yo woman with slightly hyperpigmented papule with fine branching vessels on nasal ala. RCM at the level of the dermis shows branching horizontal vessels with leukocyte trafficking (red arrows) above an area with numerous small blood vessels between bright interweaving collagen bundles, which are distinct from background dermal collagen. Numerous adnexal structures (green asterisk) along with structures suspicious for tumor are observed. OCT shows a gray, branching tumor lobule with dark peritumoral rim in the dermis (orange asterisk), along with structures suggestive of vellus hair follicles (VHF) and some prominent vessels. Histology confirms the presence of tumor lobules in the dermis around the scar tissue.

https://www.researchgate.net/figure/Basal-cell-carcinoma-OCT-helps-detect-residual-BCC-tumor-in-previously-biopsied-lesion_fig2_283022028

Histopathology of BCC

BCC is a basaloid epithelial tumor that arises from epidermal tissue (11,12). The epithelium takes the form of a palisade with a cleft separating it from the adjacent tumor stroma. The nuclei are crowded in the central region, and scattered mitotic figures and necrotic bodies are in evidence. BCC can be differentiated from other basaloid tumors based on the presence of mucinous stroma. Some BCC lesions present with foci of regression that are identified as areas of eosinophilic stroma that lack basaloid nests (11,12). Mitotic figures and perineural growth and invasion are features that are indicative of aggressive disease (12).

Figure 3. BCC Histopathology



https://commons.wikimedia.org/wiki/File:Basal_cell_carcinoma_histopathology_(1).jpg

The role of Hedgehog (HH) signaling pathways in the pathogenesis of BCC

BCC can develop as a direct result of interactions between genetic and environmental factors (2). Among the characterized genetic factors, patients diagnosed with basal cell nevus syndrome (BCNS, also known as Gorlin syndrome) develop BCC in early life together with other tumors, including medulloblastoma. This autosomal dominant syndrome results from a mutation in *PTCH1*, which is a gene found on chromosome 9q22 that encodes the protein known as patched homolog 1 (3). Other genes, including *SMO* (Smoothened, Frizzled Class Receptor) and *GLI* (GLI family zinc finger 1) have been identified as contributing to a sporadic form of BCC.

Wieschaus and Nusslein-Volhard were the first to describe *Patched* and *Hedgehog (HH)* genes and to characterize their roles in regulating cellular differentiation, polarity, and proliferation and promoting normal development in the fruit fly, *Drosophila melanogaster* (13). Molecular alterations in HH pathway components were identified in 90% of these patients. Aberrant signal activation via has been identified as a critical feature leading to the development of BCC in Gorlin syndrome as well as in the related sporadic forms of BCC.

Genes contributing to the HH pathway

The canonical HH signaling pathway includes several key components (3,4):

Extracellular ligands: Sonic HH, Indian HH, and Desert HH.

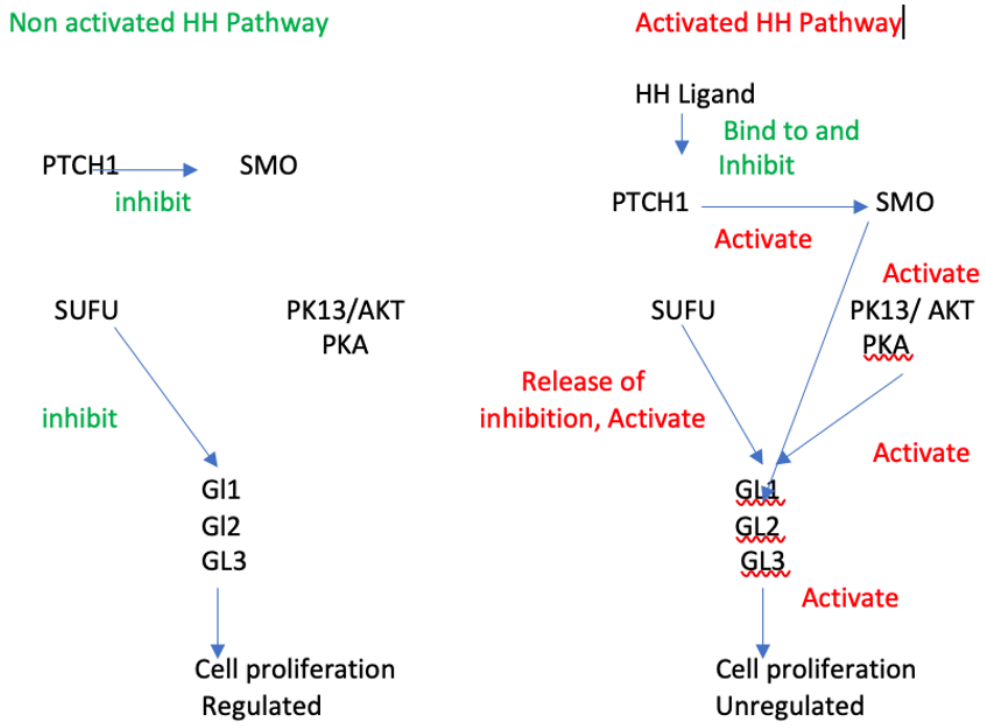
Transmembrane receptors: *PTCH1*, and *PTCH2*. PTCH1 is a 12-pass membrane receptor that negatively regulates HH pathway signaling. As noted above, mutations in the gene encoding PTCH1 have been identified in patients diagnosed with Gorlin syndrome.

G-protein coupled receptor: *SMO* (i.e., Smoothened), which encodes a seven-pass transmembrane receptor (1).

Transcription factors: *GLI1*, *GLI2*, and *GLI3*.

The HH pathway is activated when one of the extracellular HH ligands binds to PTCH1 on the target tumor cell membrane. Interaction with the HH ligand suppresses PTCH1 activity and facilitates the activation and release of SMO. Activated SMO migrates to the primary cilium, which is a highly specialized microtubule-based organelle that acts as a sensor for extracellular signals. Once reaching its target, SMO drives a signaling cascade that leads to the activation of GLI family transcription factors and their translocation in full-length form from the cytoplasm to the nucleus where they initiate transcription of HH target genes that regulate self-renewal, cell fate, survival, and angiogenesis. This pathway also includes a negative feedback loop that autoregulates HH-mediated signaling via modulation of PTCH1. Mutations found at any level of the HH signaling pathway (i.e., in the genes encoding PTCH1, SMO, or suppressor of the fused protein [SUFU]) can result in the aberrant activation of GLI factors (3). Individuals diagnosed with sporadic BCC carry inactivating point mutations in the *PTCH1* gene, including those leading to fewer copies due to loss of heterogeneity (LOH) as well as copy-neutral LOH (due to uniparental disomy). Somatic mutations in *PTCH1* have been reported in 11% to 75% of patients with this diagnosis (3). These mutations result in the activation of GLI1 in the absence of HH ligand binding or the actions of PTCH1 and SMO. GLI1 activity is positively regulated by numerous intracellular mediators, including KRAS, TGF-beta, P13K-AKT, and PKC-alpha, and negatively regulated by P53, PKA, and PKC-omega-3 (2).

Figure 4. The Hedgehog (HH) Pathway



BCC-associated mutation patterns are consistent with mechanisms involving UV-induced DNA damage; approximately half of these mutations involve UV-associated C-T and tandem CC-TT transversions. Solar UVA and UVB rays mutagenize DNA via the induction of base-pair pyrimidine substitutions. and can also damage cells via the generation of oxidative stress and impaired energy metabolism that together reduce the effectiveness of DNA repair (3). These factors can also result in the activation of local inflammatory processes and the suppression of anti-tumor immunity (2).

Other genes and pathways, including specific tumors suppressor genes, also contribute to the regulation and modulation of HH pathway signaling:

***TP53*:** Inactivation of the tumor suppressor gene, *TP53* is the second-most frequent event associated with the development of BCC. Inactivation of *TP53* results in cell cycle arrest and activation of programmed cell death, or apoptosis. TP53 protein is stabilized by phosphorylation and can alter the expression of several different sets of downstream target genes including those that cause cell cycle arrest. Loss of TP53 leads to upregulation of HH pathway activity by

increasing the expression of SMO and creating an environment in which keratinocytes are more receptive to X-ray-induced BCC. TP53 inactivation has been reported in as many as 50% of all human cancers, including BCC. Most of the TP53 mutations associated with BCC are C-T transitions together with a high frequency of CC-TT double transitions that are indicative of UV-induced DNA damage. Interestingly, fewer TP53 mutations were identified in patients diagnosed with BCC who used sunscreen compared to those who did not (3).

***Hippo-YAP* and *WNT* signaling genes:** The Hippo pathway plays an important role in the control of organ size; deregulation of this pathway contributes to tumorigenesis (2,3). The Hippo signaling axis includes a series of kinases and a cascade of phosphorylation events that ultimately lead to the inactivation of the Yes-associated protein (YAP). Inactivation of YAP (notably, the major downstream effector, YAP1, leads to dysregulated cell proliferation and apoptosis (3). Kinases MST1/2 and LAST1/2 are among the core components of the mammalian Hippo signaling pathway where they regulate hair follicle growth and promote skin proliferation (2). High concentrations of YAP1 in the nucleus lead to a massive expansion of proliferative basal epidermal cells (2).

The WNT signaling pathway plays a critical role in embryonic development and hair follicle growth and contributes via cross-talk with the HH pathway. While WNT signaling initiates hair bud formation, HH pathways promote the proliferation of follicle epithelium to form a mature follicle. WNT activation has been identified in BCC cells, including those that overexpress both canonical and non-canonical WNTs as well as beta-catenin-associated stabilization mutations. Thirty percent of all BCCs exhibit nuclear localization of beta-catenin, which is a critical component of the canonical WNT signaling pathway and is required for the activation of HH-driven development of BCC (2).

Aberrant activation of HH signaling activation induces GLI-mediated transcriptional activation of WNT. BCC relapse following the discontinuation of the HH pathway inhibitors (to be discussed below) can be explained by the persistence of tumor cells with residual WNT signaling activity. In these cases, residual BCC might be eliminated by the use of agents capable of inhibiting both WNT and HH pathways (2).

***MYCN/FBXW7* genes:** The MYC family of transcriptional activators contribute to the regulation of embryonic development as well as cell growth, proliferation, differentiation, and apoptosis. Missense mutations in *MYCN* have been identified in 30% of all BCCs. MYC has been identified as a potential downstream effector with the capacity to regulate HH pathways (2).

***NOTCH* gene:** NOTCH is a target of TP53 and a key regulator of epidermal proliferation. In normal keratinocytes, NOTCH activation results in loss of contact with the basal membrane. While NOTCH inhibition promotes tumor persistence, PTCH1 and NOTCH activation are sufficient to induce regression of established lesions (2).

Telomerase reverse transcriptase (*TERT*) promoter: *TERT* promoter mutations are detected at a comparatively high frequency in BCC. Mutations in genes encoding *PTCH1* and *TP53* as well as in the diphthamide biosynthesis (*DPH)3* promoter are detected more frequently in BCC cells with *TERT* promoter mutations. Cancer cells with TERT promoter mutations are frequently more lethal due to their propensity to undergo metastasis. Similarly, the slow responses of some BCCs to treatment with SMO inhibitors may be due to their *TERT* and *TP53* promoter mutations (2).

***DPH3-OXNAD1* bidirectional promoter:** Non-coding mutations in the bidirectional promoter of the *DPH3* and the oxidoreductase NAD p-binding domain containing 1(*OXNAD1*) genes have been reported in BCC, notably those associated with UV damage. The *DPH3* gene is a critical component of the diphthamide biosynthesis pathway and has a tumor suppressor role (2).

Ppp6c* and *STK19: Mutations in these genes are detected at high frequency and have been reported in 15% and 10% of patients diagnosed with BCC, respectively (2). *PPP6C* encodes a phosphatase that regulates cell cycle progression, while *STK19* encodes a kinase involved in transcriptional regulation (2).

Noncoding (nc) RNAs: MicroRNAs (miRNAs) are small regulatory RNAs that modulate cell function at transcriptional, post-transcriptional, and epigenetic levels. and modulate several processes; miRNAs are frequently dysregulated in cancer cells. Altered levels of miRNA

expression have been identified in BCCs. Likewise, expression levels of genes that regulate miRNA production (e.g., *DROSHA*, *DGCR8*, *AGO1*, *AGO2*, *PACT*, and *TARBP*) are significantly higher in BCCs compared to healthy control tissue. Collectively, these findings suggest a role for ncRNAs in promoting tumor growth and progression (2).

Hedgehog Pathway Inhibitors (HHPIs)

HH pathway inhibitors (HHPIs) are of profound importance for the systemic treatment of LaBCC and MBCC (2).

Vismodegib and Sonidegib are orally-administered HHPIs that were approved in 2012 by the United States Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) for the treatment of BCC that is not amenable to surgery or radiotherapy. These drugs were also approved for use in cases of recurrent BCC in which a cure was deemed unlikely and/or when surgery may result in major deformities (5).

Vismodegib

Vismodegib is approved for the treatment of LaBCC and MBCC; Sonidegib is approved for the treatment of LaBCC only (2). Vismodegib was the first HHPI that was evaluated in clinical trials in patients with advanced BCC, including patients diagnosed with Gorlin syndrome who may require prolonged treatment (14). Both drugs target the HH pathway and function as SMO inhibitors, thereby preventing the signaling cascade and maintaining baseline suppression of the GLI transcription factors (2). Vismodegib and Sonidegib have both cytostatic and cytotoxic actions on target tumor cells (2).

The safety and effectiveness of Vismodegib for the treatment of patients diagnosed with BCC was evaluated as part of the ERIVANCE trial, which was a phase II multi-center non-randomized cohort trial. The 104 patients enrolled in this study were treated with 150 mg of Vismodegib once daily. Patients remained on this protocol unless there was evidence of disease progression, unacceptable toxicity, or another reason requiring withdrawal from the study (14). The primary endpoint, an objective response rate, was evaluated at nine months by an independent review facility (IRF). The IRF-reported overall response rates (ORRs; i.e., the percentages of individuals

in given treatment groups who exhibited a partial (PR) or complete response (CR) over a given period of time) of 30% in patients diagnosed with MBCC and 43% in patients who were diagnosed with LaBCC (6). By contrast, the ORRs determined by the investigators were 45% for MBCC and 60% for LaBCC. The investigator-assessed median duration of response (DOR) was 12.9 months (range, 0.7–47.8 months) for those diagnosed with MBCC and 12.7 months (range, 1.1–47.8 months) among those in the LaBCC cohort (14).

The secondary end-points of this study included investigator-assessed ORRs and DORs evaluated by the IRF as well as progression-free survival (PFS), overall survival (OS), and changes reported from the day 1 baseline, including patient-reported symptoms, safety, and absence of residual BCC in those presenting with LaBCC (14).

These results were assessed further by the IRF after 12 months of additional follow-up. At this later time point, the ORR increased from 30% to 33.3% in patients diagnosed with MBCC and from 43% to 47.6% in patients with LaBCC. Among these, 33.3% of the patients diagnosed with MBCC and 25.4% of the patients diagnosed with LaBCC exhibited a partial response; 22.2% of the latter group exhibited a CR to this drug regimen. The median DOR in patients with LaBCC increased from 7.6 to 9.5 months. No new safety concerns emerged during the extended duration of treatment (15).

Follow-up at 39 months revealed that 8% of the patients remained on the treatment protocol; 66% remained in the surviving follow-up group, while treatment had been discontinued in others due to disease progression (27.9%), patient decision to stop the treatment (26%), or AEs (21.2%).

Table 2. The ERIVANCE Trial: 9 – 39 months of follow-up from a phase 2 study conducted to evaluate the efficacy and safety of Vismodegib in patients diagnosed with LaBCC and MBCC performed by an IRF (13,14).

Independent Review Facility	9 months		12 months		39 months	
	MBCC	LaBCC	MBCC	LaBCC	MBCC	LaBCC
Objective Response Rate (ORR)	30%	42.9%	33%	47.6%	48.5%	60%
Complete Response (CR) Rate	0	20.6%	0	22.2%	0	52.6%
Partial Response (PR) Rate	30.3%	33.3%	22.2%	25.4%	32%	47.3%
Stable Disease (SD)	63.6%	38.1%	60.6%	34.9%	67%	38.1%
Median Progression Free Survival (PFS; months)	9.5	11.3	9.5	9.5	9.3	12.9
Survival			78.7%	92.3%	62.3%	85.5%

The overall median relative dose intensity received by patients undergoing treatment was 97.4%, determined at 98.9% and 96.6% for patients diagnosed with MBCC and LaBCC, respectively. The investigator-assessed ORRs were comparable across the histologic subtypes (scored by an independent pathologist) at 53.8% for infiltrative LaBCC and 85.7% for MBCC (12). Investigator-assessed ORRs were also evaluated for patients who had missed no Vismodegib doses and compared to those of patients who had missed up to 33% of the Vismodegib doses; these differences were 60% *versus* 43.5% among those in the MBCC cohort and 58.3% *versus* 63.3% among those diagnosed with LaBCC (14).

The STEVIE trial provided further evidence for the effectiveness of Vismodegib and offered more information on the potential adverse effects of drug therapy in an open-label trial involving 36 centers in 167 cities worldwide (16). Of note, the safety and efficacy of Vismodegib were

evaluated in this trial specifically in settings that were representative of those found in routine clinical practice. While the primary end-point was safety, the assessment also included treatment-emergent (TE) AEs which were defined as AEs that occurred from the first day of the treatment until 30 days after the final drug dose. AEs were those defined by the US National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4. Secondary end-points also included investigator-assessed objective responses according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Among the findings from this study, the investigator-assessed ORRs were 68.5% for patients diagnosed with LaBCC and 36.9% for those diagnosed with MBCC (15). These results were consistent with those obtained from the ERIVANCE trial. Of note, patients diagnosed with Gorlin syndrome exhibited a CR of 45.1% to Vismodegib compared to those who did not carry a diagnosis of this syndrome (CR, 28.2%).

Overall, the results of this trial confirmed that Vismodegib treatment resulted in a high response rate with a manageable and consistent safety profile in clinical practice that was consistent with that resulting from the ERIVANCE trial (16).

Sonidegib

Sonidegib is a second orally-administered HHPI that has been approved for the treatment of MBCC and LaBCC (2). The results of the BOLT trial performed in 2015 demonstrated the efficacy and safety profile of this drug (16). In this study, Sonidegib was administered to patients at either 200 mg or 800 mg per day. Treatment continued for 42 months or until there was evidence of disease progression or unacceptable toxicity/death, study termination, or withdrawal of consent. The primary end-point of this study was the ORR as determined by central review at baseline, at weeks 5, 9, and 17, and then every 8 or 12 weeks during one or two years to follow. The safety end-points included monitoring and reporting adverse events.

A central review revealed a 56% ORR among those receiving 200 mg daily Sonidegib for LaBCC and 8% for those diagnosed with MBCC. By contrast, a 46.1% ORR was observed among those with LaBCC and 17% for those with MBCC in the 800 mg Sonidegib group. The median duration of exposure to Sonidegib was 11.1 months for the 200 mg group and 6.6 months for those in the 800 mg group (17).

A phase II trial of topical Sonidegib resulted in its approval for patients diagnosed with Gorlin syndrome. Of the 13 patients enrolled in this study, three exhibited a CR, 9 exhibited a PR, and only one patient exhibited no clinical response (17).

Trial data focused on a direct comparison of Vismodegib and Sonidegib for the treatment of BCC are not available (2). However, indirect comparisons of the impact of Sonidegib (from the BOLT trial) and Vismodegib (from the ERIVANCE trial) were considered to be appropriate as both studies enrolled patients with the same baseline characteristics and both featured an independent review of the ORRs as a primary endpoint (17).

Investigator-assessed (RECIST) ORRs to Vismodegib were 47.6% at the 21-month follow-up. Of these, 22% and 25.4% of the study participants exhibited a CR and a PR, respectively. By contrast, RECIST-type ORRs to Sonidegib were 60.6% at the 18-month follow-up, including 21.2% of the participants exhibiting a CR and 39.4% exhibiting a PR (17).

A higher percentage of participants treated with Vismodegib experienced progressive disease compared to those treated with Sonidegib, at 12.7% and 1.5%, respectively (2).

Table 3. Comparison of the responses to treatment with Vismodegib and Sonidegib

	Vismodegib at 21 months		**Sonidegib at 18 months**	
	MBCC	**LaBCC**	**MBCC**	**LaBCC**
Overall Response Rate (ORR)	47.6%	60%	200 mg 8% 800 mg 17%	56% 46.1%
Adverse Effects (AEs)				10% or fewer
Complete Response (CR)		22.5%		21.2%
Stable Disease (SD)		34.9%		30.3%
Progressive Disease		12.7%		1.5%
Disease Control			200 mg 92% 800 mg 92%	91% 82%

Safety and adverse events associated with HHPIs

Patient safety was a critical parameter evaluated in both the ERIVANCE and STEVIE trials (14,16).

Table 4. Adverse events (AEs) reported in patients with BCC participating in one of the two major trials of vismodegib.

Trial name	ERIVANCE Study	STEVIE Trial
Muscle spasms	71.2%	62%
Alopecia	66.3%	62%
Dysgeusia	55.8%	54%
Weight loss	51.1%	33%
Fatigue	43%	27%
Nausea	32.2%	13%
Decreased appetite	28%	25%
Diarrhea	27%	1.7%

Other AEs reported included irregular menses and amenorrhea (33%), headache (5%), Anemia (10%), Arthralgias (4.9%), and constipation (3.8%) (14). Most of these AEs were grade 1 or grade 2 in severity. However, 55.8% of the participants reported grade 3 AEs. The most frequently reported grade 3 TEAEs were weight loss (8.7%) and muscle spasms (5.8%), as well as fatigue, decreased appetite, and diarrhea. Squamous cell carcinoma (SCC) developed in less than 5% of the study participants.

While 34.6% of the study participants reported one or more severe adverse events (SAEs), only 8.7% were directly related to Vismodegib treatment. Among these SAEs were pneumonia and syncope (2.9%), hip fracture and death (2.9%), heart failure, cellulitis, gastrointestinal hemorrhage, SCC, pulmonary embolism and deep venous thrombosis (1.9%), and dehydration (6. 14–16). Elevated serum levels of creatinine phosphokinase (CPK) were reported for 60% of study patients (17). The cumulative and chronic nature of these adverse events resulted in the discontinuation of therapy by ~31% of the study participants (15). Furthermore, these studies

revealed that treatment with Hedgehog pathway inhibitors increases the risk of developing other skin malignancies. Two cases of rapidly-growing melanoma were reported in patients that underwent 8–9 months of treatment (18).

Incidence of TEAEs based on the duration of treatment

The incidence of TEAEs was higher in patients with more than 12 months of Vismodegib treatment compared to those who were exposed to this drug for shorter periods of time. Among these TEAEs, patients who underwent Vismodegib treatment for more than 12 months reported higher rates of muscle spasms. Alopecia, dysgeusia, weight loss, fatigue, and nausea were reported more frequently among patients who were initially treated with Vismodegib for fewer than 12 months but continued with a prolonged treatment regimen. However, the risk of developing new AEs was reduced after the completion of the first year of treatment (13). The incidence of grade 3 AEs in this cohort was the same as that identified in patients who underwent Vismodegib treatment for 12 months or fewer (15).

Reversibility of AEs after discontinuation of Vismodegib in the STEVIE trial: 12-month follow-up

Analysis of the study data revealed that 97% of participants initially developed more than one ongoing AE. The percentage of patients reporting AEs decreased during the follow-up period to 92% at one month, 74.8% at three months, 59% at six months, 49% at nine months, and 45.5% at 12 months (13). The most common adverse event, which was muscle spasms, typically resolved in 1–3 months. By contrast, while ageusia, dysgeusia, and alopecia resolved in six months, weight loss required 12 months for full resolution. TEAEs were no different in the cohort diagnosed with Gorlin syndrome compared to those with other forms of BCC (16).

The persistence of AEs beyond six months might be related to the ongoing detection of Vismodegib in circulation. Vismodegib has a comparatively long half-life in circulation as a protein-bound drug; this finding may also relate to the time required to resolve pathophysiologic damage to a homeostatic process, for example, the kinetics of hair growth (16). Nonetheless, results from the BOLT trial led to the conclusion that most AEs were tolerable and reversible and could be managed with reductions or interruptions in the drug dose (16).

AEs reported in response to treatment with Sonidegib

Among the AEs reported, 1.3% of the patients treated with 200 mg Sonidegib per day and 3.3% of the patients treated with 800 mg per day developed rhabdomyolysis (17). While grade 1 or 2 AEs were reported for the 200 mg per day group, those reported for the 800 mg group were grade 3 or 4. Serious AEs were reported in 5% of the patients treated with 200 mg Sonidegib per day and 17.3% of those treated with 800 mg per day. The development of grade 4 AEs led to discontinuation of treatment in 11% of the patients who were undergoing treatment with 200 mg Sonidegib per day and in 14.7% of the patients treated with 800 mg per day. Most of the AEs were muscle spasms reported in 54% and 69.3% of the patients receiving 200 and 800 mg Sonidegib, respectively. Alopecia was reported in 49% of the participants in the 200 mg group and 58% of those in the 800 mg group. Grade 3 to 4 AEs included elevated levels of serum CK and lipase in 6% of the participants in the 200 mg group and 13.3% of those in the 800 mg group.

Table 5. Adverse events reported in patients with BCC undergoing treatment with Sonidegib (200 or 800 mg).

	Sonidegib 200 mg	Sonidegib 800 mg
Muscle spasms	54%	69%
Alopecia	49%	58%
Elevated creatine kinase (CK) and lipase	6%	13.3%
Pneumonia	3%	4%
Requiring discontinuation of treatment	11%	14.7%
Rhabdomyolysis	1.3%	3.3%

Overall, treatment with Sonidegib resulted in fewer (10%) AEs than were observed in response to vismodegib. AEs reported in patients treated with Sonidegib also developed later than those reported in response to vismodegib. Sonidegib is also the only HHPI that indicates parameters for dose modification, including a reduction from daily dosing to 200 mg every other day (2).

Management of the TEAEs

Management strategies for TEAEs were developed to facilitate the continuation of treatment (12).

The following measures were proven to be effective:
Muscle spasms that developed in response to drug treatment were managed with calcium channel blockers (13) or L carnitine (400 mg 3–4/day (26). These drugs are well-tolerated and can be initiated at the start of treatment as a prophylactic measure (33). Quinine and cyclobenzaprine were used to treat muscle spasms. Nausea and constipation were treated with prochlorperazine or the serotonin reuptake inhibitor ondansetron (Zofran) (33). Diarrhea was treated with metoclopramide (14).

A dietitian should be consulted for food substitution strategies that might be used to treat dysgeusia, loss of appetite, and weight loss of more than 5% of the original body weight. No pharmacological methods may be needed; patients may respond to fluid hydration and gentle exercise. Patients should be counseled regarding the possibility of hair loss before the start of treatment (19).

Treatment interruption for short periods is a common practice that permits patients to recover from adverse events before continuing treatment. These strategies reduce the likelihood of developing chronic TEAEs (20).

Many study participants discontinued treatment with Vismodegib and Sonidegib. Fifty percent of the patients treated with Vismodegib ultimately discontinued treatment, including 21.2% because of AEs and 26% based on a patient decision. By contrast, 30% of the patients treated with Sonidegib discontinued therapy because of AEs, including 10% based on a patient's decision and 13% on the recommendation of a physician (2). Overall, Sonidegib treatment resulted in a 10% lower incidence of most AEs compared to treatment with vismodegib. Similarly, patients treated with Sonidegib develop AEs later during therapy than was observed for those treated with Vismodegib (2).

Of note, the patients who continued to receive Vismodegib at 150 mg per week as maintenance therapy for one year following complete remission of LaBCC experienced no disease recurrence. By contrast, 26.6% of patients who discontinued the drug did experience disease recurrence. Low maintenance doses of Vismodegib were shown to be effective at eliminating the possibility of tumor recurrence as well as reducing the severity of common AEs (20).

Vismodegib was introduced as a neoadjuvant therapy before surgery in cases of BCC of the scalp and face that may be inoperable or operable with a high likelihood of functional or major aesthetic sequelae. This strategy resulted in a 27% reduction in surgery-associated defects; more than 50% of the patients receiving Vismodegib as neoadjuvant therapy exhibited a biopsy-proven complete response (21).

Vismodegib has also been used as an adjuvant to radiotherapy. This strategy resulted in improved therapeutic responses and prolonged survival with no increase in unanticipated adverse events. Vismodegib adjuvant therapy was used successfully for the treatment of periocular BCC (21). Vismodegib therapy was also found to increase the radiation sensitivity of both BCC and SCC (22).

Monitoring patients before, during, and after administration of HHPIs

Baseline staging should be performed to classify BCC as locally advanced or metastatic before any and all drug treatment (13,21,23). On average, most patients undergo 6 – 12 months of HHPI treatment (i.e., 12 months for Vismodegib and 11 months for Sonidegib). These baseline staging measurements include clinical assessments and photography of the visible sections of the lesion, physical examination of lymph nodes, and magnetic resonance imaging (MRI) or computed tomography (CT) performed with measurement components. Imaging performed for staging regional and distant metastasis may require more vigorous and individualized monitoring to facilitate tracking in cases of progression of the disease; these complications can also be monitored by assessing ulceration, pain, enlargement of the original lesion, the development of new lesions, and metastases. Symptomatic elevations in serum CK may require an adjustment to weekly Sonidegib therapy until this condition resolves to baseline levels. By contrast, grade 2 – 4 serum CK elevations without renal impairment might require drug interruption. Special considerations

may be required for elderly patients with complicated comorbidities, patients managed with medications that interact with these drugs, and patients with metastatic BCC. A geriatrician and multi-disciplinary approach will be important when caring for frail patients (23).

Sonidegib should be discontinued in patients that develop renal impairment and might be avoided in frail older patients with impaired hepatic metabolism and/or who are undergoing treatment with theophylline, levothyroxine, statins, valsartan, amoxicillin-clavulanic acid, acetaminophen, aspirin, naproxen, amlodipine, or metformin (13- 22).

According to the 8th TNM (T, primary tumor; N, regional lymph node; M, distant metastasis) classification used by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) staging system, a physical examination and imaging are used to determine nodal involvement (N) and distant metastasis (M). The specific regional lymph nodes to be assessed depend on the location of the primary tumor. BCC of the head and neck will require staging based on a physical examination and imaging to document the TNM stage. BCCs located in regions other than the eyelids, head, neck, perineum, vulva, or penis require a physical examination only to determine the primary tumor category (T). Cervical lymph nodes require an examination in all cases of BCC of the head and neck. For BCC of the eyelids, an examination should focus on the preauricular, submandibular, and cervical nodes (23).

Tumor responses to HHPI treatment should include a clinical assessment of the externally visible sections of the tumor followed by imaging to assess the infiltrative component to determine the full dimensions of the lesion. Imaging should be performed every three months in patients diagnosed with either MBCC or LaBCC to assess any underlying infiltration greater than 10 mm. Imaging modalities include MRI and CT which can be used to evaluate the tumor response according to RECIST v. 1.1 for solid tumors, specifically the information that discusses the assessment of the tumor response with imaging for measurements of the target lesions (23).

Clinical follow-up will include a total skin examination for the detection of new cancers or metastases. Clinical follow-up should also focus on potential AEs, including patient weight and

serum CK levels for all patients who had undergone treatment with vismodegib, as well as any patients presenting as symptomatic (21).

Evaluation of tumor responses according to RECIST v.1.1

The following parameters were assessed:

Complete response (CR): Disappearance of all target lesions all target or non-target pathological lymph nodes, with a reduction in short axis to <10 mm.

Partial response (PR): >30% decrease in the sum diameters of all target lesions with reference to the sum of all baseline diameters.

Progressive disease (PD): >20% increase in the sum of the diameters of all target lesions with reference to the smallest baseline sum, an increase of 5 mm or more, and/or the appearance of one or more lesions.

Stable disease (SD): Change in tumor size that does not qualify as a partial response or disease progression with reference to the smallest diameter at baseline.

The externally visible components of the tumor are assessed by standard and annotated color photography. The World Health Organization (WHO) guidelines define a partial response as a 50% reduction in the sum of the perpendicular diameters of the target lesions and progressive disease as a 25% increase in new lesions. Likewise, stable disease is defined as no partial response and no disease progression. The BOLT study reported their findings using the WHO criteria. By contrast, the ERIVANCE, STEVIE, and the US Expanded Access Study (EAS) trials utilized the RECIST definitions. Using this system, a PR is defined as a ≥30% reduction in the sum of the longest dimensions of the externally visible components of the target lesion; PD is defined as a 25% increase or the development of new lesions, with stable disease presenting as neither PR nor PD.

RECIST criteria can be used to assess the measurable component of the tumor together with imaging. A tumor biopsy can be performed during the treatment when PD is suspected and inflammation or granuloma formation needs to be ruled out. This information is particularly helpful if a patient wishes to discontinue treatment due to personal concerns or adverse events (23).

Non-invasive monitoring of BCC treated with HHPIs

Clinical and histopathological responses to Vismodegib therapy can be detected by scar formation that develops as the BCC lesion undergoes involution (22). Ulceration and eschar formation may be evident early in the clinical course of Vismodegib therapy. There may also be histologic evidence of necrosis in response to therapy that continues up to 35 days after treatment.
Vismodegib also reduces the rate of tumor cell proliferation and decreases the overall expression of mitotic markers (24).

Vismodegib did not induce direct cell death but instead induces stromal alterations that lead to reductions in tumor growth. Tumor tissues develop pyknotic nuclei, karyorrhexis, neutrophil recruitment, and amorphous, eosinophilic hyalinization that collectively suggest cellular necrosis. While fibrosis and hyalinization are detected in response to therapy, the fibroplasia is disorganized and haphazard similar to what is typically observed in a dermatofibroma rather than what typically develops in normal scar tissue. At this time, the mechanism of vismodegib-elicited regression of BCC and the associated scar formation remains unclear (24).

Increased plasma cell infiltrates with lympho-histotiocytes were observed in BCC lesions on the chest, in contrast to typical head and neck BCCs which typically contain plasma cells only. Inhibition of the HH pathway induces an immune response that includes the production of cytokines and chemokines and recruitment of the cytotoxic T cells. The impact of HH pathway signaling on B cell recruitment remains unknown (24).

The histological finding of tumors in patients treated with Vismodegib correlate directly with the tumor response (24).

Total body mapping (TBM) with video-dermoscopy

Video-dermoscopy has been used extensively to monitor tumor responses to HHPIs. TBM studies were performed before, during, and after the completion of a course of Sonidegib treatment. Tumors in patients exhibiting a PR exhibited reductions of some features while others persisted. By contrast, none of the original dermoscopic features detected at baseline remains in patients

exhibiting a CR, while some baseline features persisted in patients who experienced a PR to Sonidegib therapy (25).

Monitoring responses to HHPIs by RCM and high definition (HD)-OCT

Tumor regression was evaluated by clinical photography and radiography scans as well as by non-invasive imaging techniques such as Reflectance Confocal Microscopy (RCM) and High Definition-Optical Coherence Tomography (HD-OCT.) Many of these lesions decreased in size or completely disappeared in response to HHPI treatment. Interestingly, half of all tumors evaluated in patients who exhibited a clinical CR retained some tumor residue, including pseudocysts (empty tumor nests) and widespread fibrosis (coarse bright fibers) that were confirmed by histopathologic analysis (26).

Rechallenge with HHPIs

Regrowth of tumor tissue after an initial positive response while remaining on HHPI treatment is defined as a secondary or acquired response. Secondary resistance has been reported in up to 20% of all patients diagnosed with advanced BCC while undergoing the first course of continuous HHPI treatment (22). Secondary resistance may be diagnosed by a clinical examination and imaging to determine possible tumor regrowth. A biopsy should be performed if ulceration or pain develops at the site of the lesion (22).

Secondary resistance is frequently caused by acquired mutations in the *SMO* gene. If this is the case, HHPI treatment should be discontinued (22). Secondary resistance can extend to include all drugs in a given class; similar resistance patterns have been reported in patients treated with Vismodegib and Sonidegib (22).

Disease recurrence after discontinuation of HHPI treatment is not considered to be a form of drug resistance; thus, rechallenge with the same HHPI can be attempted. Relapse after discontinuation of Vismodegib discontinuation may be due to the persistence of a slowly cycling Wnt-signaling tumor cell population; these tumor cells are capable of reactivating the HH pathway and resuming growth once no longer exposed to this drug (22). For patients who experienced a recurrence of

BCC six months after drug discontinuation; retreatment with Vismodegib led to tumor regression and a marked clinical response (22).

Prolonged administration of Vismodegib at low doses can serve as maintenance therapy after CR from LaBCC; this strategy was highly effective at blocking tumor recurrence and reducing the severity of common AEs with minimal toxicity (20). The results of an observational retrospective study conducted at the nonmelanoma skin cancer unit of the University of Federico II (Naples) revealed that patients maintained on a low dose of Vismodegib (150 mg once weekly) exhibited no recurrence after CR. By contrast, 26.6% of the patients who discontinued the drug after CR and who were not provided with a maintenance dose reported disease recurrence (20).

The use of Patidegib to treat BCC

Patidegib (34) is an HHPI that has been approved by the EMA and FDA as an orphan drug designed for the treatment of Gorlin syndrome (also known as NBCS). Patidegib was introduced orally in a Phase I trial at 20 mg/day for NCBS patients who were Vismodegib naïve and those who progressed despite adequate vismodegib. Among the results, Patidegib was more effective in drug-naïve patients than in patients whose disease progressed while in treatment with Vismodegib with a toxicity profile that was similar to other drugs in the HHPI class (27). Of note, patients diagnosed with Gorlin syndrome with MBCC respond differently than those diagnosed with simple cutaneous forms of BCC. Individuals with metastatic disease progressed further while on this drug and frequently developed new metastases (27).

A phase II trial has been initiated to evaluate the use of topical 2% and 4% Patidegib applied once or twice daily in patients with stage I Gorlin syndrome. This treatment strategy resulted in a 51.29% and 26.563% reduction in disease from baseline levels in response to topical administration of 2% and 4% Patidegib gel, respectively (27).

The molecular efficacy of Patidegib was determined based on the percentage changes from the baseline of expression of HH pathway signaling target genes as well as mRNA encoding GLI1 at the end of the treatment. The most prominent changes were obtained in response to the

administration of Patidegib (2%) gel once daily which resulted in a 56.3% reduction in the expression of GLI1 mRNA (27).

In a phase III trial, Patidegib (2%) was used to treat patients with Gorlin syndrome with a focus on reducing the tumor burden in those with persistent BCC. The results of this trial revealed that Patidegib was effective in patients with Gorlin syndrome as well as those who did not carry this diagnosis but experienced frequent BCCs (27).

Patients with vismodegib-resistant BCC did not respond to Sonidegib and displayed ongoing resistance and tumor progression in response to other SMO inhibitors. In other words, there is significant cross-resistance among the drugs of the same class. Thus, HHPIs are not indicated in patients demonstrating resistance to a single drug; other treatments should be considered. One advantage of topical Patidegib is that it is unlikely to cause systemic AEs and thus facilitates improved management of these somewhat fragile patients. Topical and systemic forms of Patidegib were safe, tolerable, and effective in drug-naïve patients with stage 1, 2, or 3 cutaneous BCC including those diagnosed in patients who do not have Gorlin syndrome. However, this drug had no impact on patients with stage 4 disease and those who were not drug-naïve at the outset of the study (27).

Targeting HH signaling pathways with bromodomain extra-terminal motif inhibitors

The bromodomain extra-terminal motif (BET) protein family includes numerous members (BRD2, BRD3, BRD4, and BRDt, among others) that bind to acetylated histones and induce gene transcription (1) specifically via interactions with N3-acetyl lysines that result in the formation of complexes on histone tails. For example, BRD4 binds directly to the promoters of GLI1 and GLI2 and regulates the transcription of these HH pathway components. Bromodomain extra-terminal motif (BET) inhibitors such as JQ1 can limit tumor growth by reversing BRD4 binding at the GLI promoter in BCC, medulloblastoma, and atypical rhabdoid tumor cells. JQ1-mediated BET inhibition is an effective means of counteracting SMO-dependent drug resistance that develops in response to mutations in *SMO* and/or *SUFU* or amplification of *GLI2* or *MYC*. (1)

Other epigenetic regulators of the HH pathway

The various epigenetic regulators that mediate signaling via the HH pathway undergo mutation during tumor growth and development of tumor. For example, promoter hypermethylation is a key element promoting HH and WNT signaling (1).

Gene expression patterns associated with resistance to HHPIs.

The results of several studies revealed that BCC responses to Vismodegib treatment can be predicted by evaluating the expression of HH pathway genes both before and after treatment. For example, in a study of 40 HH signaling pathways genes, 16 underwent significant changes from before to after treatment. Categorization of LaBCC patients into those responding with a partial as opposed to a CR revealed that expression of a single gene – Growth Arrest Specific (GAS) 1– predicted the tumor response (28).

Localized expression of BCC genes was evaluated in the head and neck, which are sites of substantial sun exposure, compared to the trunk, which experiences only minimal sun exposure. This study revealed three differentially-expressed HH pathway genes including *Gli3*, *PRKACG* (protein kinase cAMP-dependent type 1 regulatory submit beta), and WNT2 (wingless integrated 2).

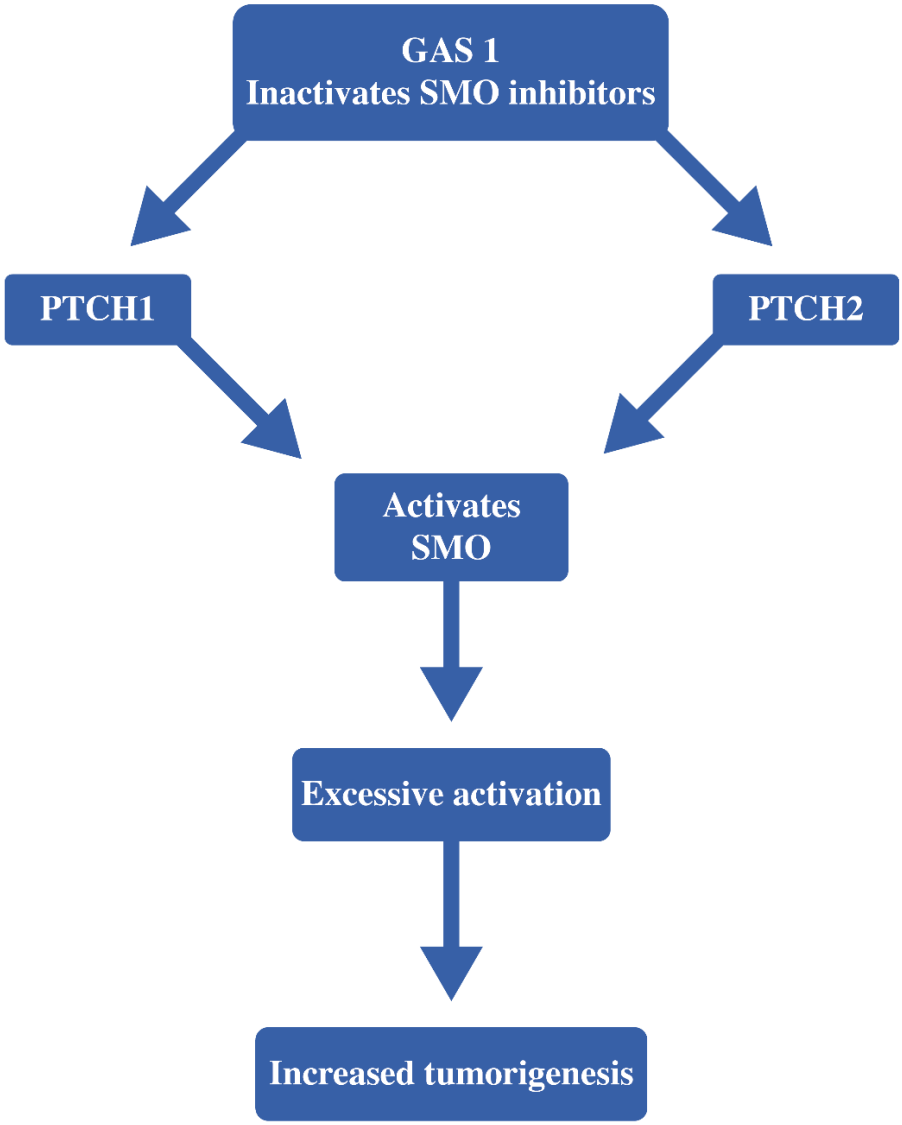
The results of nanostring Ncounter analysis revealed that administration of Vismodegib resulted in a significant decrease in the expression of HH pathway genes found downstream of the SMO receptor. Because this tool can generate information on simultaneous gene expression, it may be very useful for evaluating gene expression and determining tumor responses to treatment. It can also be used to identify genes that are overexpressed in resistant tumors that might be targeted using second-line therapy. Of a series of potential targets, GAS 1 was the only gene that exhibited a significant change in expression from baseline and could differentiate between a complete and a partial response. Other genes differentially regulated under these circumstances include GLIS2, which is a member of a subfamily of GLI transcription factors with a strong impact on cancer, and PRKCG, which is a member of the protein kinase c family with the capacity to regulate tumor cell differentiation and proliferation. Differential expression of GAS 1, GLIS2, and PRKG1 were observed before treatment when comparing locally advanced *versus* localized BCC (28). GAS 1

is a negative regulator of the HH pathway in BCC and also prevents tumorigenesis and metastasis of colon and gastric cancer. GAS 1 also suppresses tumor growth and spread via its capacity to promote negative regulation of aerobic glycolysis. Several groups have reported that GAS 1 is expressed at higher levels in partially treatment-responsive tumors compared to those that are completely responsive; the elevated pre-treatment levels of GAS 1 detected in partially-responsive LaBCC suggest that this property renders these tumors more aggressive and that GAS 1 levels may correspond directly with resistance to systemic treatment. Thus, baseline levels of GAS 1 expression may help to predict the responses of LaBCC to systemic treatment with vismodegib.

GAS 1 modulates signaling in concert with the actions of two additional cell surface proteins, including cell adhesion molecule (cAMP)-related downregulated by oncogenes (CDON) and brother of CDON (BOC). GAS 1 is the only one of these three proteins expressed only in vertebrates. However, all three of these proteins function as coreceptors for the HH ligand; they interact with the PTCH1 protein on the cell surface, increase its affinity for the HH ligand, and amplify signaling to activate the HH pathway and promote tumorigenesis (28).

GAS 1 may also interact with PTCH2. GAS 1 overexpression leads to the inactivation of SMO inhibition of PTCH2, demonstrates increased affinity for HH ligand, and has an enhanced capacity to activate SMO. The resulting activation of the SMO receptors, together with mutations in PTCH1 or SMO genes resulting from vismodegib, collectively results in increased tumor resistance and prolonged tumor survival. Baseline GAS 1 levels have proven to be extremely important for predicting treatment resistance in patients diagnosed with LaBCC (28).

Figure 5. Aberrant activation of the HH pathway



GAS1 Growth arrest specific 1

PCTH1 Patched 1

PTCH2 Patched 2

SMO G-protein-coupled smoothened transmembrane receptor

Vismodegib inhibits the HH pathway at points that are downstream of GAS 1; thus, the impact of this drug is not altered by the GAS 1 overexpression. However, overexpression of GAS 1 potentiates the pathway and provides it with the capacity to overcome Vismodegib inhibition by releasing more SMO from PTCH1. GAS 1 can also have a mild impact on SMO (28).

Overexpression of *WNT2* and *PRKAR1B* and underexpression of *GLI3* have also been reported in this context. As mentioned above, the WNT pathway plays an important role in embryonic development. Overexpression of genes involved in the WNT pathway promotes the proliferation, migration, and invasion of tumor cells and is a prominent characteristic of BCC and other types of cancers, including melanoma and SCC of the head and neck (28).

Histologic and genetic analysis can be used to guide disease management, avoid TEAEs associated with Vismodegib therapy, and reduce the likelihood of treatment resistance. GAS 1 serves as a target for studies focused on treatment resistance that develops in response to Vismodegib (28). However, it is critical to recognize that not all subtypes of BCC behave identically. A histopathological and genetic investigation of cases of primary resistance may be used to direct more effective treatment strategies (31).

Gene mutations and resistance to HHPIs

Primary drug resistance is defined as stable or progressive disease in which the tumor exhibited no response to treatment. Primary resistance has been reported in 50% of patients undergoing drug treatment. Secondary resistance defines tumor recurrence and progressive disease after an initial positive response to drug treatment. Secondary resistance has been reported in 20% of patients undergoing drug treatment (29). Clinical examination and imaging studies are needed to identify patients falling into one of these two resistance categories (29).

Resistance to HHPIs occurs most frequently as a result of mutation(s) that develop in *SMO* (30). Primary resistance has been attributed to mutations such as *SMO* G497W, while secondary resistance has been related to both pre and post-treatment mutations of *PTCH1* as well as *SMO* D473Y.

The *SMO* G497W mutation results in protein rearrangement and partial obstruction of drug entry (30). By contrast, *SMO* D473Y has a direct impact on the binding geometry and leads to a complete disruption of the stabilizing hydrogen bond network. Thus, the G497W and D473Y *SMO* mutations represent two distinct mechanisms leading to primary and secondary resistance, respectively (30). Patients with BCC lesions that are resistant specifically to Vismodegib may also exhibit tumor progression in response to other SMO inhibitors (30). The incidence and prevalence of drug resistance are likely to increase as more patients with BCC undergo treatment with HH pathway SMO inhibitors.

Other studies uncovered a role for germline SUFU mutations in the generation of drug resistance. SUFU is a tumor suppressor and negative regulator of the HH pathway. Mutations leading to a loss of function SUFU disrupt baseline inhibition of GL1-mediated transcription which results in the overactivations of numerous target genes (31). Vismodegib is not effective in cases of SUFU-associated, multiple hereditary infundibulocystic BCC which is a rare clinicopathological variant involving a factor that is downstream of SMO in the HH pathway (31).

To date, 18 gene mutations have been associated with vismodegib-resistance (32). Patients diagnosed with BCC who carry any one of these mutations will not respond to drug treatment (28). Ninety percent of the cases of sporadic BCC lesions exhibit LOH mutations in *PTCH1*. The remaining 10% of these cases exhibit gain-of-function or activating mutations in *SMO*. Both mutations result in the uncontrolled proliferation of basal skin cells (32). Mutations leading to drug resistance typically develop within a few months after the start of treatment with an HHPI thereby limiting the drug's overall impact (28). Resistance mutations reduce the affinity for the HHPI for its molecular target; this reduction cannot be overcome by increasing the drug dose because of issues with toxicity and intolerance. In addition to normal variation, as many as 40 mutations targeting 35 amino acids were identified in *SMO* based on findings reported in the Catalogue of Somatic Mutation in Cancer (COSMIC) database (32).

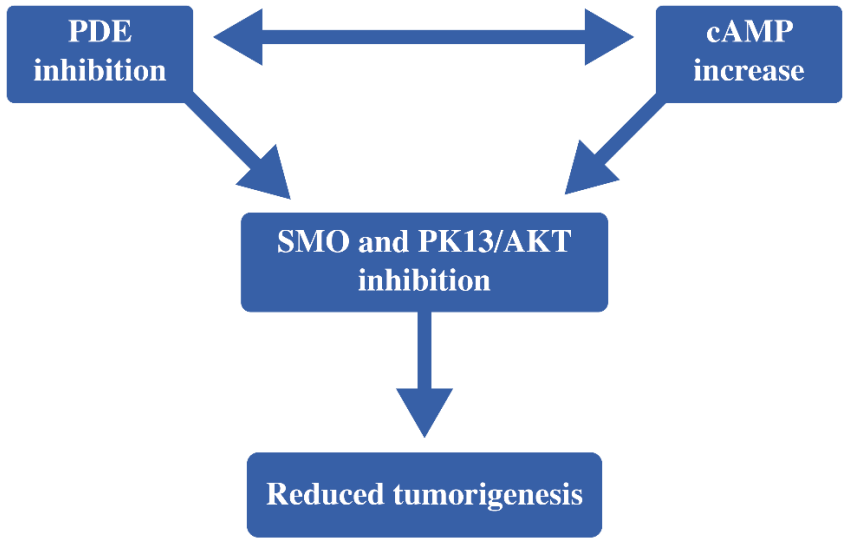
Alternative signaling and resistance mechanisms

Resistance to HHPIs can arise via secondary mechanisms involving one or more alternative pathways that lead to the blockade of the main signaling pathway and permit tumor proliferation despite drug treatment. One alternate pathway involves the intracellular kinases P13K/Akt and phosphodiesterases (PDEs). P13K/Akt signaling plays a critical role in promoting tumor proliferation in response to an increase in SMO activity. Likewise, cyclic adenosine monophosphate (cAMP) is a critical control point that might be targeted to reduce tumor growth and proliferation (32).

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that catalyze the destruction of the phosphodiester bonds characteristic of cAMP and cGMP. Once cAMP levels have increased, the SMO network becomes less complex, as both GSK3b and SUFU become less important in the process of conveying signals from SMO that induce tumor proliferation (32). PDE inhibition increases the concentrations of free cAMP and reduces tumor proliferation (17). The results of a network control analysis revealed the significance of the roles played by SMO and P13K was a function of the levels of circulating cAMP (32).

It may be possible to combat tumor proliferation via PDE inhibition, which will increase cAMP levels, together with inhibition of both SMO and the P13K/Akt pathway. The need for dual inhibition of both SMO and P13K may depend on the PDE activity. Selective and non-selective PDE inhibitors are available to increase the cAMP levels in target cells (32).
Combination therapy may be an important potential treatment for patients with BCC that has become HHPI drug-resistant (32).

Figure 6. P13K/AKT and PDEs identified as potential targets for the treatment of BCC



PDE Phosphodiesterase

cAMP Cyclic adenosine monophosphate

SMO G-protein coupled smoothed transmembrane receptor

Combination therapy for the treatment of MBCC

Combination therapy with arsenic trioxide and itraconazole (ATO-ITRA) was used successfully to overcome resistance to HHPIs in patients diagnosed with MBCC (20,28) because these drugs antagonize HH pathways at the points that are distinct from those targeted by SMO inhibitors, Patients who experienced relapses after a primary trial with SMO inhibitors were treated with intravenous arsenic trioxide and oral itraconazole. Administration of these latter drugs reduced the expression of GL11 mRNA by 75% from baseline levels after three cycles of treatment (33).

Itraconazole is an oral antifungal agent that was identified as a strong inhibitor of HH signaling. Administration of itraconazole limits tumor carcinogenesis and results in diminished expression of mRNA encoding GLI family transcription factors in both murine models and patients with non-advanced cutaneous BCC (33).

Arsenic trioxide (ATO) has been approved by the FDA for use as a treatment for acute promyelocytic leukemia. Administration of ATO inhibits signaling from pathways that are downstream from the SMO via its role in preventing ciliary trafficking and destabilizing GLI2, thus bypassing acquired mutations in SMO that are frequently observed in HHPI-resistant tumors. Among the outcomes, treatment with arsenic trioxide led to a 54% decrease in GLI mRNA, which exceeded the 50% reduction required for an anti-tumor clinical effect (20). Arsenic trioxide was administered intravenously for five days in each 28-day cycle, while itraconazole was administered orally between day 6 and day 28 of the cycle. The recommended dose of ATO is 0.3 mg/kg for five days for a total of three cycles or until disease progression or unacceptable toxicity emerges. Itraconazole (400 mg/day) was administered orally on days 6–28 to reduce the likelihood of AEs (33).

Treatment with ATO-ITRA can lead to AEs that include grade 1–2 leukopenia, increases in blood urea nitrogen and creatinine levels, transaminitis, and dyspnea. Other AEs include grade 4 leukopenia and grade 3 infection that requires antibiotic treatment. Grade 1 asymptomatic atrial flutter was documented in one case that disappeared after discontinuing treatment. Grade 1 AEs were reported in 95% of the patients treated with ATO-ITRA; 25% discontinued treatment (33).

Administration of ATO-ITRA resulted in a 75% reduction of *GL11* mRNA levels from those detected at baseline

After one month of treatment, ATO-ITRA elicited a 75% reduction in HH pathway activity; by contrast, treatment with Vismodegib resulted in 90% inhibition. Treatment with ATO-ITRA led to clinically stable disease at best; this outcome can be compared to the 30% response rate observed in response to Vismodegib in patients diagnosed with MBCC. Patients with non-MBCC treated for one month with itraconazole (400 mg/day) exhibited reduced activity (33).

The decrease in tumor size observed in vismodegib-naïve MBCC patients but not those who had undergone a prior trial of this drug may explain some of the limited response. The response to this regimen (i.e., stable disease or disease progression *versus* shrinkage of the tumor) may be due to the dosing regimen. A change to a continuous intravenous or oral administration protocol may result in more effective HH pathway inhibition and thus an improved clinical response.

Studies of combined treatment (ATO-ITRA) protocols using a murine BCC model revealed an additive HH pathway inhibitory effect. This modality should be considered given the overall reasonable response rate with fewer AEs, thus permitting the patient to undergo treatment for a prolonged period (33).

Posaconazole is a second-generation triazole antifungal drug and a structural analog of the well-established antifungal agent, itraconazole. Posaconazole has an excellent long-term safety profile with only mild side effects. Posaconazole is involved in fewer drug-drug interactions than have been reported for itraconazole and does not require dose adjustment due to mild to moderate renal or hepatic insufficiency. The combination of Posaconazole and ATO results in a durable response and does not promote drug-resistant mutations in *SMO* (34).

There are no effective treatments available for MBCC patients who experience tumor progression after treatment with vismodegib. These patients did not respond to high-dose Sonidegib (800 mg/day) and developed secondary resistance to other SMO inhibitors. The dosing schedule has no significant impact on the clinical efficacy of second-line therapies used to treat patients with drug-resistant MBCC. The poor response to these secondary inhibitors cannot be fully explained at this time, save for the possibility that the tumor may have acquired the capacity for pathway–independent growth (33).

Systemic treatment of BCC by targeting the immune system

Systemic treatments for MBCC that circumvent the AEs, resistance, and toxicity of HHPIs might focus on drugs that target the immune system. One fundamental property of the immune system is its ability to differentiate between normal and foreign cells (including cancer cells); this provides the immune system with the capacity to attack and eliminate foreign cells while leaving normal cells unharmed. However, some cancer cells are capable of bypassing these “checkpoints” and thus avoid recognition and elimination by the immune system (35).

Monoclonal antibodies have been developed that target these checkpoints. These drugs are known collectively as checkpoint inhibitors (30). While checkpoint inhibitors do not eliminate cancer

cells directly, they help the immune system to recognize and attack them. Checkpoint inhibitors that have been developed to target programmed cell death 1 (PD-1) and PD-ligand 1 (PD-L1) improve anti-tumor responses with remarkable efficacy (35).

PD-1 is a checkpoint protein expressed by T cells. PD-1 functions as an "off" switch to prevent T cells from attacking other cells in the body. Checkpoint inhibitors block PD-1 binding to PD-L1, which is a protein found on the surface of normal and cancer cells that protects them against T-cell mediated attack (35). Some cancer cells also express PD-L1 which permits them to avoid immune attacks. Thus, monoclonal antibodies that target PD-1 or PD-L1 can block this binding interaction and enhance the immune response against cancer cells (35).

Pembrolizumab (Keytruda®), nivolumab (Opdivo®), and Cemiplimab (Libtayo®) are among the inhibitors that target PD-1, while atezolizumab (Tecentriq®), avelumab (Bavencio®), and durvalumab (Imfinzi®) were designed to target PD-L1 (35).

Side effects of checkpoint inhibitors that have been reported include diarrhea, fatigue, cough, nausea, skin rash, poor appetite, and constipation, as well as muscle and joint pain. Other more serious AEs that occur less frequently are infusion/hypersensitivity reactions, including fever, chills, flushing of the face, pruritis, dry skin, dizziness, wheezing, and/or difficulty breathing. Because they eliminate the safeguard, or checkpoints on the immune system, the use of these drugs can result in aberrant immune attacks in other parts of the body. These adverse responses may be severe and in some cases life-threatening, particularly those that affect the lung, liver, intestine, hormone-producing glands, and kidneys (30). If serious AEs of this nature develop, treatment must be stopped and the patient may need high-dose of corticosteroids to suppress the immune system (35).

Nonetheless, the use of PD-1/PD-L1 inhibitors typically results in a durable response and improved survival. These drugs are well-tolerated compared to other systemic therapies and are safe to use in older frail patients. Furthermore, patients can safely discontinue the treatment without the risk of disease recurrence (35).

Clinical responses to PD-1/PD-L1 inhibition

There is substantial evidence to support the use of PD-1/PD-L1 inhibition to treat advanced BCC. As noted above, BCC frequently exhibits a UV radiation mutation signature with PD-1/PD-L1 expression on tumor cells as well as tumor-infiltrating lymphocytes and macrophages.
These findings are further supported by the observed increased incidence of BCC in patients who are immunosuppressed as well as the efficacy of the immune response modifiers (i.e., imiquimod) (36).

Blockade of PD-L1 was shown to be effective for the treatment of cutaneous malignancies. Treatment protocols that include PD-L1 inhibitors yield durable responses and improve patient survival; this feature permits patients to discontinue therapy while continuing to benefit from the treatment. PD-L1 inhibitors are well-tolerated compared to many systemic treatments and can be used safely in patients who are older and frail (36). Several case reports featuring patients with advanced BCC demonstrated durable responses to this type of immune therapy (36). BCC is a UV-induced tumor with a high tumor mutation burden (TMB) that corresponds with the antitumor response. This may be explained as a high TMB can lead to an increase in neoantigen production and thus a greater chance that the tumor will be recognized and targeted by $CD8^+$ T cells (37).

Two studies that feature the use of PD-1 inhibitors

An open-label multi-center phase II non-randomized study was performed to evaluate the impact of Cemiplimab in patients with LaBCC or MBCC who were intolerant to HHPIs, experienced disease progression after HHPI therapy (37), had no objective response after nine months of treatment, or could not be treated with radiation or surgery (37). Patients were treated with Cemiplimab (350 mg) every 3 weeks for 93 weeks or until the disease progressed or unacceptable toxicity developed. The median duration of follow-up was 15 months and the median duration of exposure was 47 weeks. Among the results of this study, the ORR determined by ICR was 31%, the CR rate was 6%, and the PR rate was 26% (37). Cemiplimab was well-tolerated by patients with advanced BCC and there were no safety concerns (23). Ninety-seven percent of the patients reported one or more AEs, including fatigue (30%), diarrhea (24%), pruritis (21%), and asthenia (20%) (37). Other AEs included nausea, constipation, poor appetite, and muscle and joint pain

(31). Forty-eight percent of the patients reported grade 3–4 AEs that included hypertension (5%), colitis (5%), fatigue (4%), urinary tract infections (4%), and visual impairment (4%). Eleven percent of the patients reported serious AEs, including colitis (4%) and adrenal insufficiency (2%). Eleven percent of the study participants discontinued treatment because of AEs (37). Although there were no reports of grade 4–5 immune-related AEs and no treatment-related deaths, 13.8% of the patients developed hypothyroidism secondary to PD-1 inhibition (37).

In February 2021, Cemiplimab was approved by the FDA as second-line therapy for BCC in patients who had undergone prior therapy with an HHPI (31). Cemiplimab can be used as neoadjuvant therapy together with surgery in patients with resectable LaBCC and in patients likely to experience significant surgical morbidity and complexities (31). Cemiplimab can also be used to treat unresectable or MBCC in combination with an HHPI (37).

A proof-of-concept study was performed that featured pembrolizumab provided to patients with advanced BCC who had not undergone prior treatment with HHPIs. This was followed by a cohort study in which patients were treated with both pembrolizumab and an HHPI. Interestingly, the RR was higher among those treated with Pembrolizimzb alone (44%) compared to combined pembrolizumab and Vismodegib (29%) (38); no concerning AEs were reported. This study revealed that pembrolizumab and Vismodegib can be used together as first-line treatment for patients with BCC despite no significant evidence for either additive or synergistic effects (38). The results of other studies revealed that these drugs remain effective for long periods. For example, the median DOR reported for patients treated with pembrolizumab alone was 67.6 weeks; the DOR for pembrolizumab plus Vismodegib was 52.8 weeks (38,39).

An ongoing trial (NCT03521830) in which patients were treated with an HHPI followed by nivolumab (Opdivo®) or combination therapy with an HHPI and PD-1 inhibitor. when they progressed, they got nivolumab plus ipilimumab (Yervoy®) as a potential salvage treatment. With combination immunotherapy, after PD-1 monotherapy was not effective. Numerous ongoing efforts have been directed toward the effective use of combined immunotherapy in these challenging patients (38).

Use of PD-L1 inhibitors with adjuvant ablative fractional laser (AFL) therapy

The combination of PD-L1 inhibitors and topical ablative fractional laser (AFL) therapy is a promising alternative for the treatment of advanced BCC as together they improve the anti-tumor response (35). Local AFL exposure boosts the immune response to systemic anti-PD-1by increasing infiltration of immune cells and thus increasing survival time. This combined regimen also limits tumor growth and improves tumor clearance more effectively than either treatment alone. Treatment with this combination results in greater tumor clearance than any of the other treatment interventions that are currently available. A single exposure to AFL increases tumor clearance in patients treated with systemic PD-1 blockade in association with a potent $CD8^+$ T cell and neutrophil anti-tumor immune response and the induction of systemic anti-tumor immunity (40).

AFL had been used previously in combination with topical imiquimod to treat keratinocyte carcinoma. This combination resulted in increased tumor clearance and lymphocytic infiltration which led to the use of AFL as an adjuvant to PD-1 blockade for the treatment of BCC. In cases of untreated BCC, very few tumor-infiltrating $CD4^+$ or $CD8^+$ T lymphocytes are detected relative to the total immune cell count. Administration of AFL alone or combined with PD-1 blockade results in an increase in the absolute number of neutrophils as well as $CD4^+$ and $CD8^+$ T cells infiltrating the tumor tissue. AFL repolarizes tumor-associated neutrophils from a pro-tumor to an antitumor phenotype. AFL-induced neutrophil infiltration also activates cytotoxic $CD8^+$ T cells and increases their anti-tumor immune responses. The proportion of neutrophils expressing major histocompatibility complex class II (MHC class II) also increased in response to AFL treatment and doubled in response to combined therapy. The increase in MHC class II expression suggests that neutrophils may present antigen and thus prime naïve $CD4^+$ T cells and thus increase the number of tumor-infiltrating T cells following AFL treatment. Furthermore, local inflammation elicited by AFL may mediate the recruitment of more T cells to the tumor microenvironment (40).

In summary, AFL treatment may change the immune status of BCC by markedly increasing the extent of immune cell infiltration from minimal levels observed in untreated tumor tissue via a substantial increase in the number of $CD8^+$ T cells and MHC class II-expressing neutrophils. Cell

recruitment boosts the efficacy of PD-1 blockade by eliciting an antigen-specific $CD8^+$ T cell-mediated immune response (40).

Vaccination strategies that target PD-1

A vaccination strategy was devised based on the successful treatment of BCC with immune checkpoint blockade antibodies that target PD-1. The IO103 vaccine is a 19 amino acid peptide designed to target both PD-1 and PD-L1. Vaccination with IO103 (NCT03042793) activated PD-L1-specific T cells and converts them into a proinflammatory and cytotoxic state that activates their capacity to clear PD-L1-positive tumor cells and their precursors (41).

A phase II study was conducted in 2018-2019 in Denmark in which nine patients diagnosed with resectable BCC underwent vaccination with IO103 together with a Montanide adjuvant. Patients were vaccinated six times for several weeks; those who responded were then provided with three additional vaccines every four weeks. The study did not include a control group. The primary endpoints of this study were clinical responses, changes in tumor size, and specific immune responses to the vaccine. Safety was evaluated as a secondary endpoint. Overall, 20% of the participants exhibited a reduction in tumor size of at least 30% in the longest diameter. Eighty percent of the participants responded with stable disease, while 70% exhibited a 70% decline in the longest tumor diameter. No tumor regression was observed in 70% of the study participants. Of note, patients with more than one tumor exhibited great variability with respect to changes in tumor size. Other effects of vaccination included the development of folliculitis decalvans, psoriasis, and porokeratosis, interestingly, all in a single patient (41).

AEs include a grade 1 reaction at the site of injection. One patient reported a decrease in vision due to wet age-related macular degeneration. Further evaluation revealed that this development was not caused by vaccination with IO103 (41).

Monitoring of the vaccine response

Vaccine-specific immune responses in peripheral blood mononuclear cells (PBMCs) were assessed by quantifying the number of interferon-gamma (IFNɣ)-positive lymphocytes by ELISspot assay at baseline and after two, six, and eight vaccinations. PBMCs from one patient

exhibited an immune response to IO103 (DFR score 29) at baseline. Baseline responses (DFR score 2) were also detected in 70% percent of the vaccinated patients. Other patients exhibited more modest albeit significant immune responses. The mean increase in the amplitude of these responses achieved statistical significance at the final timepoint in four patients who were vaccinated of the total nine patients who were enrolled in the trial (41).

Immune response in the skin infiltrating lymphocytes

Delayed-type hypersensitivity reactions (DTH) at the site of intradermal injection are typically manifested by induration at the site of the injection. In vaccinated patients, these lesions were twice as large as those that developed in patients that received the control injection. Skin-infiltrating lymphocytes (SKILs) identified in biopsies collected at DTH sites revealed strong reactivity against IO103 in an IFN-gamma Elispot, which is an assay that provides a direct measure of the number of T cells capable of secreting cytokines following stimulation with a specific antigen(41)

Immune response in the peripheral blood

The findings revealed no significant changes in T lymphocyte populations, notably no changes in circulating levels of $CD4^+$ or $CD8^+$ T cells (41).

Immunohistochemical evaluation of BCC biopsy specimens

Biopsy tissues from patients undergoing vaccination included tumor-infiltrating immune cells that were variably PD-L1 positive. Vaccination resulted in a 12.5% reduction in the size of the target tumors in 70% of the cases. Interestingly, the non-target tumors were also reduced in size in response to this vaccine. This may be because the act of collecting biopsy samples from a given tumor likely induces a local inflammatory response via the release of damage-associated molecular pattern molecules (DAMPs) and lead to a Th1 immune response. The act of performing a tissue biopsy may actually serve as an adjuvant to the anti-tumor vaccine (41).

Clinical evaluation

Biopsy specimens should be collected from all tumors 21 – 95 days before the first vaccination. Target tumors should be biopsied once again two to six weeks after vaccination. It is not necessary

to collect biopsy tissue from non-target tumors. Baseline measurements performed at the first vaccination, during the vaccination period, and during evaluation after completion of the vaccine series as well as any adverse effects throughout should be recorded and graded (41).

Vaccination of BCC patients with IO103 was proven to be safe and effective with few to no adverse events. Vaccination led to the induction of an immune response in 88.8% of the patients studied. Likewise, no disease progression was observed during vaccination. Responses of tumor tissues that were biopsied as well as those that were not were detected throughout (41).

The significance of immune surveillance to the control of BCC was concluded from an analysis of the profoundly increased incidence of tumors in patients who were medically immunocompromised because of recent organ transplantation. These findings might be compared to those collected from the remaining members of the population. This treatment protocol can also be used to treat other skin cancers, including melanoma (41).

Table 5. Drugs and vaccines to treat BCC and their mechanisms of action

Therapeutic agents	Mechanisms of action
Vismodegib	SMO inhibition
Sonidegib	SMO inhibition
Patidegib	SMO inhibition
Itraconazole	Limits expression of mRNA encoding GLI family transcription factors
Arsenic trioxide	Prevents downstream activation secondary to SMO by preventing ciliary trafficking and destabilizing GL2
Cemiplimab	PD1/PDL-1 inhibition
Pembrolizumab	PD1 inhibition
Peptide #IO103	Vaccine against PD-1

The overall impact of HHPIs on patient health-related quality of life (HRQOL) was assessed using the Dermatology Life Quality Index (DLQI). A high HRQOL score indicates better outcomes and is inversely proportional to the DLQI score. Follow-up of patients who exhibited complete clearance of their BCC lesions in response to Vismodegib treatment but relapsed

within six months of therapy withdrawal responded with reductions in HRQOL and increases in DLQI scores (42).

Conclusion

The availability of systemic therapies developed to treat both LaBCC and MBCC represents new milestones toward efforts to control this progressive disease. Although HHPIs are approved for these indications by both the FDA and EMA, drug use results in adverse events in nearly all patients, including some that require discontinuation of the treatment regimen. In some cases, dose reduction and/or dose interruption can be implemented to provide the patient with some time to recover and then continue treatment at a later date.

Unfortunately, patients can develop drug resistance as their tumors develop new gene mutations and alternate pathways that permit them to overcome the effects of a given HHPI. New targeted treatments designed to overcome primary drug resistance by blocking one or more alternative pathways leading to tumor proliferation are currently in development. It will be important to continue with these research efforts and to elucidate the mechanisms underlying these critical pathways so that they can be targeted effectively in clinical practice.

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