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REVIEW ARTICLE

# Keratinocytic skin cancers—Update on the molecular biology

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## Abstract

Although much attention has been devoted to a detailed genomic exposition of cutaneous melanoma, other nonmelanoma skin cancers have also recently been subjected to similar analytical scrutiny. Chief among these are the most common malignancies worldwide: basal cell carcinomas and cutaneous squamous cell carcinomas. In this review, the authors summarize their latest knowledge about the molecular pathways and therapeutic opportunities attendant to these keratinocytic skin cancers.

### Plain Language Summary

- The most common cancers in the United States arise from skin cells called keratinocytes.
- Although these tumors are not formally tracked by the National Cancer Institute, it is estimated that there are millions of skin cancers called basal cell carcinomas and squamous cell carcinomas.
- This article reviews the current recent genetic insights into these tumors and therapeutic opportunities.

**KEYWORDS**
basal cell carcinoma, cutaneous squamous cell carcinoma, genetics, p53, patched

## INTRODUCTION

Keratinocytic skin cancers (KCs) are the most common cancer worldwide, with an estimated incidence of 6.3 million cases in 2019.[1] Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) account for 95% of all KCs and are the focus of this review. BCC is the most common type of KC, with an estimated incidence of 2 million cases in the United States alone.[2] cSCC, conversely, represents nearly one third of all KCs, with an estimated incidence of 1 million cases in the United States.[3] Global analysis of all cancer deaths[1] attributed 56,000 deaths to KCs in 2019, similar to the mortality attributed to melanoma (62,000 annual deaths worldwide in 2019), although the mortality rate of melanoma is substantially higher. Although research in KCs has historically lagged behind that in melanoma, rapid advances have been made recently in our understanding of the pathogenesis of KCs and have led to new therapeutic strategies. In this review, we summarize our latest knowledge about the molecular pathways and therapeutic opportunities attendant to these KCs.

### Clinical subtypes of basal cell carcinoma

BCCs are typically subdivided according to their clinical morphology. The most common subtype is the nodular BCC. It is characterized by a pearly skin nodule with telangiectasias (Figure 1A) and accounts for the majority of all BCCs. Histologically, nodular aggregates of basaloid cells are arranged in palisades at the tumor periphery with clear retraction from the surrounding stroma. Superficial BCC, conversely, is characterized by an indurated pink plaque (Figure 1B). Histologically, superficial BCCs exhibit multiple tumor foci that extend superficially, as the name suggests, from the epidermis to the upper dermis. A more surgically challenging subtype of BCC is the infiltrative subtype because it has ill defined tumor margins (Figure 1C).

 

Histologically, strings of tumor cells invade the surrounding tissue within infiltrative BCCs. Other rare subtypes of BCCs include fibroepithelioma of Pinkus, basosquamous BCCs, and metatypical BCCs.

## Molecular biology of basal cell carcinoma

The development of BCCs, like many other cancers, involves an interplay of inherited genetic susceptibility and sporadic somatic mutations. The former includes genetic syndromes and single nucleotide variants that increase an individual's susceptibility to develop BCCs, but the latter is typically needed to induce cancer development. In a rare disorder that is inherited in an autosomal dominant fashion called basal cell nevus syndrome (BCNS), or Gorlin syndrome, one of the most defining features is the development of numerous BCCs at an early onset (median age, 25 years), mainly on sun-exposed areas. It was the family-based linkage studies of BCNS kindreds that led to the discovery of the critical oncogenic pathway underlying BCC development.[4–6] The most common mutations attributed to BCNS are germline loss-of-function mutations in *PTCH1*,[7] which encodes a transmembrane receptor that inhibits signals driven along the hedgehog (HH) pathway. The sonic HH (SHH) signaling pathway is crucial for determining tissue patterning and cell fate during embryo development.[8] When the HH ligand binds to a transmembrane receptor complex formed by Smoothened (SMO) and PTCH, the HH signaling pathway is activated. In normal conditions, SMO is inhibited by PTCH, and no signal is transmitted to the nucleus for transcription (Figure 2A). When the HH ligand, SHH, binds to PTCH (or when PTCH is mutated; Figure 2B), inhibition of SMO is disabled, resulting in upregulation of the downstream signaling cascade by several proteins, such as suppressor of fused (SUFU), releasing GLI transcription factors. These factors, in turn, trigger the transcription of genes implicated in tumorigenesis and angiogenesis.[9]

Genome-wide association studies involving Europeans in Iceland and the United States have identified 33 loci associated with increased susceptibility to BCC development, accounting for approximately 11% of the heritability of BCC.[10] These include, among others, gene polymorphisms affecting tumor suppressor genes (including *TP53*, *CDKN2A*, and *CDKN2B*), epidermal differentiation and cytoskeleton organization (including *KRT5*), NOTCH signaling


**FIGURE 1** Clinical representation of subtypes of basal cell carcinomas, including (A) nodular basal cell carcinoma, (B) superficial basal cell carcinoma, and (C) infiltrative basal cell carcinoma

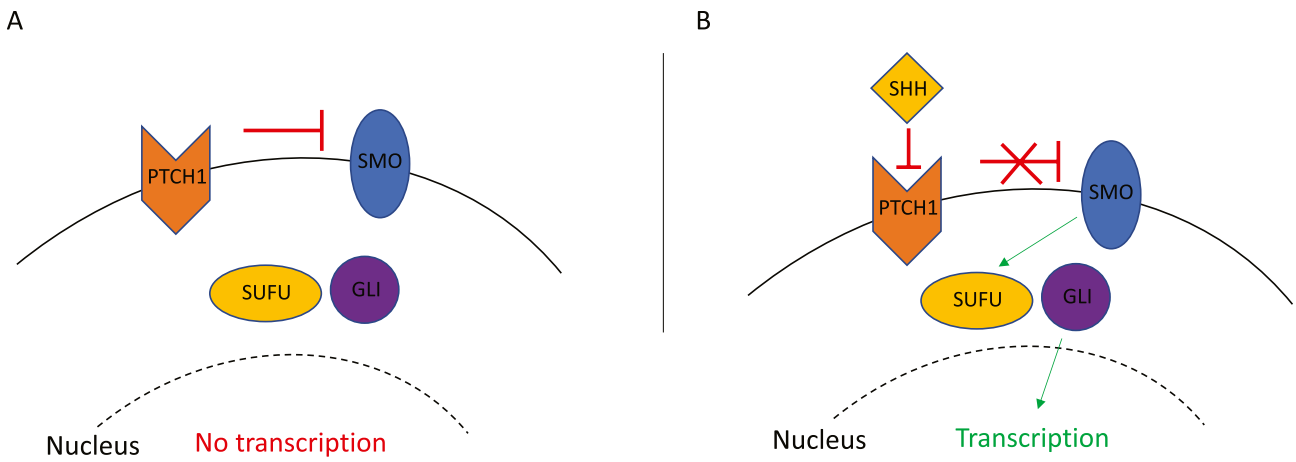


**FIGURE 2** Schematic representation of the hedgehog signaling pathway: (A) In normal conditions, SMO is inhibited by PTCH1, and no signal is transmitted to the nucleus for transcription. (B) In the presence of either of its ligands (sonic hedgehog [SHH] or mutated PTCH1 [PTCH*]), inhibition of SMO is disabled, promoting the transcription of HH target genes by activation of SUFU/GLI. HH indicates hedgehog; SMO, smoothened; SUFU, suppressor of fused; TMB, tumor mutational burden.

(including *FOXP1* and *IRF4*), telomere maintenance (including *TERT* and *OBFC1*), and DNA repair (including *XPA*, *MUS81*, and *NABP2*).

Although germline polymorphisms increase an individual's susceptibility to developing BCCs, subsequent sporadic somatic mutations are required for cancer to develop. Indeed, BCC is characterized by the highest tumor mutational burden among all cancers, having >65 mutations per megabase.[11] The single most important driver of this strikingly high mutational burden in BCC is exposure to ultraviolet (UV) radiation. In approximately 90% of sporadic BCCs, molecular alterations in the HH pathway are observed[12] (Figure 3), the most frequent being loss-of-function somatic mutations in *PTCH1* (up to approximately 75%). Fifty percent of *PTCH1* mutations were UV-signature mutations,[12] underscoring the causative role of UV radiation in BCC development. Activating mutations of SMO were reported in 10%–20% of BCCs, whereas loss-of-function variants of SUFU were detected in 8% of BCCs.[11] In >50% of all sporadic BCCs, mutations in the *TP53* gene have been detected.[13] Approximately 66% of these mutations were at nine mutational hotspots and were UV fingerprint mutations.[14] *TP53* encodes p53 phosphoprotein, whose functions include DNA repair, cell cycle control, and induction of apoptosis. Notably, patients with Li-Fraumeni syndrome (loss of p53) do not have a higher propensity to develop BCCs, indicating that further molecular alterations are necessary for BCCs to develop. In a recent study that included 293 BCCs, mutations in *PTCH1*, *SMO*, and *TP53* were identified in 73%, 20%, and 61% of BCCs, respectively.[11] Importantly, that study also reported driver mutations in other cancer-related genes in 85% of BCCs. *NRAS*, *KRAS*, or *HRAS* mutations were identified in 2% of the tumors. Recurrent mutations in the Hippo-YAP pathway genes *LATS1*, *LATS2*, and *PTPN14* were identified in 16%, 12%, and 23%, respectively. The Hippo-YAP pathway is a crucial regulator of tissue hemostasis involved in controlling cell proliferation and promoting apoptosis.[15] MST1 and MST2 phosphorylate and activate LATS1 and LATS2, which then, in turn, phosphorylate and activate the YAP/TAZ transcription coactivators, thereby preventing transcription. Other mutations significantly associated with BCC development include *MYCN* (30%), *PPP6C* (15%), *RB1* (8%), *FBXW7* (5%), and *ERBB2* (4%).[11] Other genes with identified mutations, but at a lower frequency, include *CASP8*, *NOTCH1/NOTC2*, *ARID1A*, *RAC1*, and *CSMD1/CSMD2*.[11] It is important to note that, although cancer genes often harbor mutations, only a fraction of these mutations are driver mutations that provide a selective advantage to cell growth, thereby driving the cell lineage to cancer. Additional studies are needed to investigate whether these genes are drivers or secondary passenger mutations in BCC development. Delineating driver mutations from passenger events is challenging, especially in noncoding parts of the genome, for several reasons, including the finding that noncoding regions are incompletely annotated, and these regions generally function within complex regulatory networks.[16]

## Emerging treatment strategies for basal cell carcinoma

Although surgical removal alongside destructive modalities, such as electrodessication and curettage, or topical therapies, such as imiquimod, 5-fluorouracil, remain the most common treatment strategy for BCCs, increasing understanding of key molecular pathways essential for BCC development has led to the development of molecular targeted therapies as well as immunotherapy.

## Inhibitors of the hedgehog pathway

Given the crucial role of the HH pathway in BCC development, several therapies targeting this pathway have been developed. The


**FIGURE 3** Mutational landscape of basal cell carcinomas (generated from cBioPortal; www.cbioportal.org). *N* = 293 samples from one study (Bonilla et al., 2016[11]).

first SMO inhibitor studied was cyclopamine, which led to a reduction in murine BCCs by 90%, but it was recognized as a nonviable clinical therapy because of significant side effects, including weight loss and death in mice.[17] The search for new compounds subsequently led to the development of vismodegib and sonidegib, both oral SMO antagonists, which are now approved by the US Food and Drug Administration (FDA) for the treatment of locally advanced or metastatic BCCs and for patients who are not candidates for surgical resection or radiotherapy. In the largest randomized controlled trial to date using vismodegib, STEVIE (ClinicalTrials.gov identifier 01367665),[18] which included 1215 patients with BCCs, the overall response rate was 68.5% for locally advanced BCCs and 36.9% for metastatic BCCs. Unfortunately, however, 31% of patients discontinued treatment because of adverse events. Similar efficacy and a similar side-effect profile were reported with the use of sonidegib in patients with locally advanced and metastatic BCCs.[19] Given the significant adverse effects reported with oral SMO antagonists, topical HH inhibitors have been developed with the hope of increased tolerability. Patidegib, a topical HH inhibitor, has been shown to reduce disease burden in patients with BCNS in an early report (ClinicalTrials.gov identifier NCT02762084). In addition, itraconazole, an antifungal agent, has been identified as a HH inhibitor, although early trials of oral as well as topical itraconazole have demonstrated mixed results.[20]

Another important limitation to the use of HH inhibitor, aside from its side-effect profile, is the development of acquired resistance. Acquired mutations in *SMO*[21]; amplification of downstream genes, such as *GLI2*[22]; and activation of regulators of GLI, such as atypical protein kinase Cτ/λ, have been implicated as mechanisms underlying resistance. A rational approach to overcome resistance, therefore, is to target the transcription factors downstream of SMO. For example, BCC cells show high expression of c-JUN, and its expression is regulated by GLI1. In a phase 1 study that included nine patients, it was demonstrated that intralesional delivery of a DNAzyme targeting the messenger RNA of c-JUN was able to reduce the histologic depth of five of nine nodular BCCs.[23]

## Immunotherapy

Given the very high tumor mutational burden in BCCs (which likely represents high levels of tumor neoantigens that may be targets for the immune system), there is an increasing interest in using immunotherapy for HH inhibitor-refractory or inhibitor-intolerant patients with BCCs. A phase 2 clinical trial recently reported an objective response rate (ORR) of 31% with cemiplimab (antiprogramed cell death receptor 1 [anti-PD1] antibody) in patients who had locally advanced BCCs[24] with an acceptable safety profile. A phase 2 study investigating the use of cemiplimab in patients with metastatic BCC (ClinicalTrials.gov identifier NCT03132636) and a phase 2 clinical trial investigating the use of combination immunotherapy (CTLA-4/PD-1 combination) for patients with locally advanced unresectable or metastatic BCCs (ClinicalTrials.gov identifier NCT03521830) are currently in progress.

In addition to investigating the effects of combining different immunotherapies, the possible effect of combining immunotherapy (pembrolizumab, an anti-PD1 antibody) with an HH inhibitor has been investigated. For example, in a study that included 16 patients with metastatic or unresectable BCC after prior HH inhibitor therapy, the ORR was 44% for the monotherapy group and 29% for the combination therapy group, with no clear evidence that combining the two agents resulted in improved efficacy.[25]

## Clinical subtypes of cutaneous squamous cell carcinoma

Clinically, cSCCs manifest as a spectrum ranging from actinic keratosis (AK) as precancerous lesions to in situ squamous cell carcinoma (SCCIS), which then progresses to invasive carcinoma (Figure 4). AKs (Figure 4A) are characterized by pink-to-red hyperkeratotic papules. Histologically, the key feature of an AK is the disrupted epidermal differentiation, leading to disorganized growth in the upper epidermis with a thickened layer of stratum corneum as well as corneocytes with retained nuclei (also known as parakeratosis). SCCIS and invasive cSCC are skin-colored papules or plaques with or without central


**FIGURE 4** Clinical representation of cutaneous squamous cell carcinomas and its precursors, including (A) actinic keratosis, (B) squamous carcinoma in situ, and (C) invasive squamous cell carcinoma

ulceration (Figure 4B,C). Histologically, invasive cSCC is characterized by tumor keratinocytes with prominent, atypical, hyperchromatic nuclei that are found beyond the basement membrane.

## Molecular biology of cutaneous squamous cell carcinoma

Unlike BCCs, with the pivotal HH pathway driving their tumorigenesis, the pathogenesis of cSCCs follows a distinct path because the majority of cSCCs arise from precursor (precancerous) lesions.[26] The concept of field cancerization posits that mutations in individual keratinocytes (caused by exposure to UV radiation in the majority of cases) are selected over time.[27] Further genetic as well as epigenetic changes in these mutated subclinical clones of keratinocytes subsequently lead to the development of AKs, then to SCCIS, ultimately resulting in polyclonal, invasive cSCCs.[28] This process of accumulation of genetic mutations leading to the development of precursor and malignant lesions is accelerated when intrinsic defenses are compromised (such as those on immunosuppression after organ transplantation[29] or those with heritable deficiencies in the DNA repair machinery, such as xeroderma pigmentosum[30]). The increased prevalence of cSCCs in immunosuppressed patients also suggests a possible viral etiology. Available evidence indicates that human papillomavirus (HPV), especially beta-HPVs, may act as a co-carcinogen together with UV radiation by the amplification of UV radiation-induced DNA breaks and somatic mutations.[31,32] A recent systematic review[33] suggested that beta-HPV may play a role in the initiation of oncogenesis, but not in tumor promotion or maintenance, based on findings that not all cSCC neoplastic cells contain a copy of viral DNA, and viral loads were higher in AKs than in invasive cSCCs.[34]

## Molecular characterization of cutaneous squamous cell carcinoma

Whole-exome sequencing and targeted sequencing of clinically unremarkable skin compared with AKs, SCCIS, and invasive cSCCs have identified several pathways that preferentially drive different stages of tumorigenesis.[35–38] A recently published meta-analysis of sequencing data covering 105 tumors indicated that mutations in genes encoding proteins involved in the p53 and NOTCH pathways were ubiquitous in cSCCs[39] (Figure 5). High numbers of UV-induced point mutations in *TP53* have been detected in early sequencing studies of human cSCCs.[42] Indeed, *TP53* mutations have been reported in 60% of AKs and in 50%–90% of cSCCs.[43] Although the association between *TP53* mutations and cSCC is clearly established, these mutations do not appear in and of themselves to be sufficient to establish cSCC. Up to 60% of cSCCs are thought to arise from AKs. Although approximately 30% of AKs spontaneously regress in 1 year, the rate of malignant transformation to cSCC for each AK is 0.025%–16.0% per year, with most studies estimating the risk at <1.0%.[37] *TP53* mutation appears to be required for progression from normal to precancerous lesions, whereas additional mutations are required for precancerous lesions to progress to invasive cSCC. Consistent with this, patients with Li-Fraumeni syndrome harboring a germline loss of *TP53* do not exhibit a significantly increased risk of developing cSCC,[44] underscoring the need for additional mutations to develop cSCC.

It is well established that the NOTCH signaling pathway is crucial for the development and maintenance of the epidermis.[45] Because of its vital regulatory role in keratinocyte differentiation, defects in NOTCH signaling have been implicated in cSCC pathogenesis. UV signature loss-of-function mutations in *NOTCH1* and *NOTCH2* have been reported in 75% of cSCCs[46] but also in clinically unremarkable skin,[47] indicating that these may be an earlier mediator involved in the transition of normal keratinocytes to a precancerous state.

The *CDKN2A* gene encodes two tumor suppressor proteins, $p16^{INK4a}$ and $p14^{ARF}$, which indirectly control the activities of the p53 protein discussed above. Mutations in the *CDKN2A* locus have been identified in 24%–45% of sporadic cSCCs.[35,46,48] In a study of human metastatic cSCCs, *CDKN2A* mutation was reported in 31% of tumors and was notably associated with tumor aggressiveness.[49] Moreover, deletion of $p16^{INK4a}$ increased the incidence of progression from AK to cSCC.[50] Given that mutations in *CDKN2A* are rare and do not appear to be under positive selection in normal skin,[38] it is

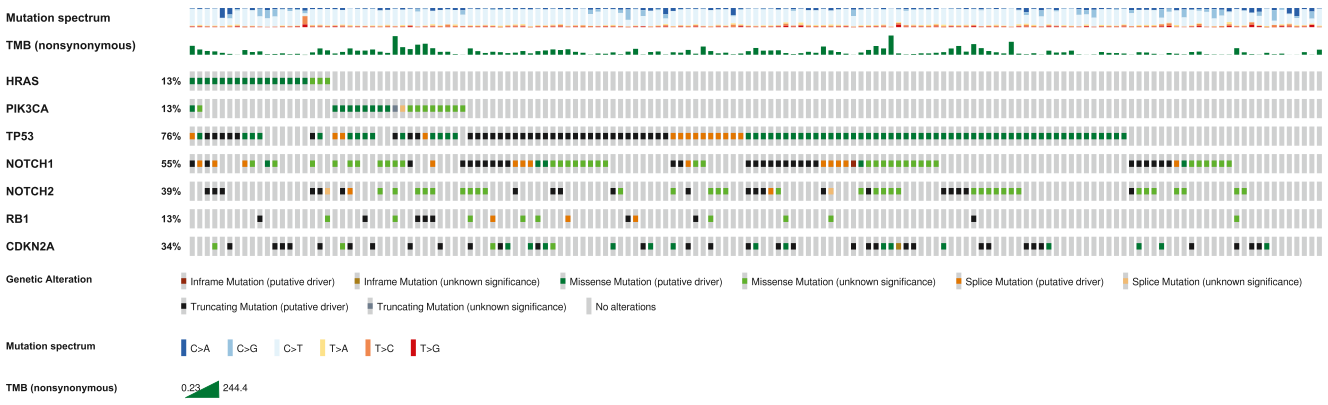


**FIGURE 5** Mutational landscape of squamous cell carcinomas (generated from cBioPortal; www.cbioportal.org). *N* = 151 samples from three studies (Chang and Shain, 2021[39]; Pickering et al., 2014[40]; and Li et al., 2015[41]). TMB indicates tumor mutational burden.

possible that altered functions of $p16^{INK4a}$ and/or $p14^{ARF}$ play an important role not only in forming precancerous lesions but also in progression from precancerous to invasive cSCCs.

The PI3K and mammalian target of rapamycin (mTOR) pathways are important regulators for a range of functions within a cell, such as cell growth and differentiation, metabolism, and survival. *PIK3CA* alterations, copy number amplifications, and/or gain-of-function hotspot mutations were identified in approximately 50% of human cSCCs in a recent sequencing study involving 10 tumor samples.[38] If confirmed in larger cohorts, this finding supports the potential use of topical tyrosine kinase inhibitors for the treatment of AKs and even cSCCs.

Activating mutations in genes of the RAS family, particularly *HRAS*, have been reported in 9% of cSCCs,[51] with incidence up to 60% of tumors developed in patients who are treated with BRAF/MEK inhibitors.[52] Additional genes with identified mutations in cSCCs include *FAT1*, *TP63*, *TGFBR1*, and *TGFBR2*.[40] Further studies are needed to determine whether they are driver or secondary passenger mutations in AKs and/or cSCCs.

## Emerging treatment strategies for cutaneous squamous cell carcinoma

Targeted therapies with HH inhibitors for BCCs were developed after discovery of the pivotal role of the HH pathway in their pathogenesis. Although the high mutational burden of cSCCs has made it difficult to similarly identify pivotal genes for cSCC tumorigenesis, there have been promising new therapeutic strategies, including targeted therapy and immunotherapy.

## Targeted therapies

### *SRC* family kinase inhibitors

It has been demonstrated that targeting *SRC* family kinases is a viable therapeutic option for AKs and cSCCs in early phase clinical trials. Topical application of tirbanibulin, an inhibitor of Src kinase with downstream activity against the PI3K pathway, has been approved for the management of AKs. Daily application of tirbanibulin 1% ointment for 5 days was recently shown to be superior to placebo for the treatment of AKs in two phase 3 trials.[53] Further studies comparing tirbanibulin with conventional topical treatments (such as 5-flurouracil) for the treatment of AKs are warranted.

### *PI3K*/mTOR inhibitors

Because upregulation of PI3K/mTOR signaling has been reported in SCCs, especially in head and neck SCC, there are currently several ongoing clinical trials to investigate PI3K/mTOR inhibitors for head and neck SCC,[54] but the number of similar studies in cSCC is limited. CLL442, a topical mTOR/PI3K inhibitor, is currently being investigated for patients with SCCIS. The initial data have reported that twice-daily application of CLL442 was safe and tolerated by the patients, but the primary study end point of lesion reduction or complete lesion clearance, unfortunately, was not met (ClinicalTrials.gov identifier NCT03333694). Given increasing evidence of the efficacy of PI3K/mTOR inhibitors in other cancers, further studies are warranted to investigate these agents in cSCCs.

### Immune checkpoint inhibitors

Given the high tumor mutational burden in cSCCs, which likely results in high levels of tumor neoantigens that may be targets for the immune system, several studies have investigated the use of immunotherapy for the treatment of cSCCs. After an aggregated data analysis of 108 patients who had advanced cSCCs from a phase 1/2 clinical trial (ClinicalTrials.gov identifiers NCT02383212 and NCT02760498)[55] and phase 2 clinical trial (EudraCT identifier number R2810-ONC-1540),[56] which found a combined ORR of 47% with durable responses ≥6 months in 61% of responders, cemiplimab was approved by the FDA as the first immunotherapy in 2018 for locally advanced or metastatic cSCCs. The most recent data from the phase 2 study showed that patients who received treatment with cemiplimab had an ORR of 46% and an estimated probability of overall survival at 2 years of 73%.[57] In 2020, pembrolizumab, another anti-PD1 antibody, was approved by the FDA for the treatment of locally advanced or metastatic cSCCs after the KEYNOTE-629 study (ClinicalTrials.gov identifier NCT03284424), which demonstrated an ORR of 34%.[58]

The role of checkpoint inhibitors as adjuvant and neoadjuvant treatments for cSCC is currently being studied. Several clinical trials are currently underway to investigate the use of cemiplimab in the adjuvant (ClinicalTrials.gov identifier NCT03969004), neoadjuvant (ClinicalTrials.gov identifiers NCT03916627 and NCT04315701), and combined adjuvant/neoadjuvant (ClinicalTrials.gov identifiers NCT4428671 and NCT04632433) settings for patients with resectable or potentially resectable cSCCs. There are promising preliminary data on its use as neoadjuvant therapy before surgical resection. In a single-center phase 2 study of 20 patients with stage III or IV head and neck cSCC (ClinicalTrials.gov identifier NCT03565783)[59] who were treated with cemiplimab in a neoadjuvant fashion, the ORR was 30%, and the pathologic complete response rate was 55%. Recently published data from a phase 2, multicenter, nonrandomized study reported similarly that neoadjuvant cemiplimab was associated with a pathologic complete response in a high percentage of patients with resectable cSCC (ClinicalTrials.gov identifier NCT04154943).[60]

### Antiviral therapies

Given evidence supporting the association between beta-HPV infection and cSCC development, several studies have examined the therapeutic implications of HPV vaccination. The nine-valent

HPV vaccine is FDA approved for the prevention of genital warts and anogenital cancer caused by HPV infection. Recently, Wenande et al.[61] reported that off-label administration of a nine-valent HPV vaccine (using a three-dose schedule at 0, 2, and 6 months) to 12 immunocompetent patients who had a median AK burden of 56 AKs resulted in an average 85% reduction in AK burden at 12 months after the first dose of vaccine. Nichols et al.[62] also successfully administered a quadrivalent HPV vaccine using the same three-dose regime to two patients and observed an approximately 60% reduction in the number of new cSCCs. This is an important area of investigation because the potential benefit from the prevention of beta-HPV–related cSCC is high given the prevalence of infection and the large burden of cSCCs. In addition to using it as a preventative measure, HPV vaccine may be used as an adjuvant treatment for patients with recalcitrant cSCCs. The successful use of combined systemic and intratumoral administration of the nine-valent HPV vaccine to treat inoperable cSCC in an immunocompetent patient[63] and an SCCIS in an immunosuppressed patient[64] has been reported. Further studies with larger numbers of patients are needed to determine the efficacy of HPV vaccine as a preventative and/or therapeutic measure for cSCCs and to determine the type of vaccine that is most effective for each indication.

## CONCLUSION

During the last decade, major advances have been made in our understanding of the molecular biology of BCC and SCC. It is now established that activation of the HH pathway is the most important driving force in BCC development. This knowledge has already been translated into clinical practice because it has been demonstrated that the effective inhibition of HH signaling reduces tumor size. In cSCCs, however, the majority of mutations are in tumor suppressor genes, which has made the development of targeted therapies more challenging. However, through further understanding of the multiple genetic alterations at the molecular level, several studies are currently underway. The use of immune checkpoint inhibitors has broadened the therapeutic armamentarium offered to patients with both advanced and metastatic BCCs and cSCCs.

### AUTHOR CONTRIBUTIONS

Drs Win and Tsao cowrote the article.

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### CONFLICTS OF INTEREST

Hensin Tsao reports personal fees from UpToDate outside the submitted work and owns stock options in Epiphany Dermatology. Thet Su Win made no disclosures.

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