Basal cell carcinoma (BCC) of the skin, the most common malignancy in individuals of mixed European descent, is increasing in incidence due to an aging population and sun exposure habits. The realization that aberrant activation of Hedgehog signaling is a pathognomonic feature of BCC development has opened the way for exciting progress toward understanding BCC biology and translation of this knowledge to the clinic. Genetic mouse models closely mimicking human BCCs have provided answers about the tumor cell of origin, and inhibition of Hedgehog signaling is emerging as a potentially useful targeted therapy for patients with advanced or multiple BCCs that have hitherto lacked effective treatment.

Introduction

Description of basal cell carcinoma. In 1827 Arthur Jacob termed the skin tumor that we now call basal cell carcinoma (BCC) “Ulcer rodens” (1). In 1900, Krompecher described BCC as a malignant, locally invasive, and destructive cancer and named it “Carcinoma epitheliare adenoideae”; he then went on to pioneer the classification of skin tumors using histogenetic principles, three years later coining the term “Basalzellenkrebs” (2, 3), a term indicating that the tumor originated in the basal layer of the epidermis or hair follicle (HF). In contrast, in 1910, Mallory used the term “hair matrix tumor” to specify the follicular origin of BCC (4), illustrating the long-standing controversy and uncertainty about the cellular origin of BCC. The locally aggressive, but overall rather benign, course of the disease, with metastasis being largely absent, was similarly noted early on and spurred the debate as to whether BCC could be considered a truly malignant cancer or a “semi-malignant” tumor. The WHO classification has retained the name “BCC” since 1974 (5). BCC is the most common human cancer and accounts for about two-thirds of all skin cancers in patients of mixed European descent. In the US this corresponds to approximately one million cases per year (6, 7).

Clinical appearance of human BCC and related tumors. The incidence of BCC is strongly associated with exposure to UV radiation; tumors develop primarily on the sun-exposed skin of elderly individuals with fair skin phototypes, are rarely found on palmo-plantar surfaces or in children, and never appear on the mucosa. Additional established risk factors include ionizing radiation (IR), arsenic, and immune suppression (8, 9). Clinically, BCCs appear as pearly and telangiectatic papules or nodules with or without ulceration, or as indurated, erythematous, or ulcerated patches with a discrete papular border, and may be pigmented.

Morphologically, BCC encompasses a group of epithelial intradermal tumors characterized by a primary cellular component that resembles the undifferentiated basal cells of the epidermis and its appendages. These basaloid cells are often arranged in palisades at the tumor periphery, are separated from the surrounding stroma by optically empty spaces, and form nodules, bands, or strings, with some continuity with the overlying epithelium in most cases. Visible desmosomal intercellular structures are absent, and the tumor cells have little cytoplasm and show chromatin-rich nuclei with frequent mitoses when compared with normal skin; however, they are often apoptotic, consistent with slow tumor growth (10).

BCCs display different morphological growth patterns: superficial, nodular, micronodular, infiltrating, sclerosing, and fibroepithelial (Figure 1). Nodular BCCs in particular may resemble adenocarcinomas or, in some regions, squamous cell carcinoma, as these BCCs demonstrate a variety of types of differentiation including basosquamous or metatypical, cystic, adenoid, pigmented, and infundibulocystic differentiation.

The diversity in the phenotypic appearance of BCCs indicates that the cell of origin may be a stem or progenitor cell. Moreover, these observations raise the question as to whether BCC is a monoclonal tumor or whether it is the result of field cancerization. Studies investigating clonal patterns of X chromosome inactivation suggest that the majority of BCCs do represent monoclonal tumors and that anatomically distinct BCCs may sometimes share the same cellular origin (11, 12).

Genetics of BCC development. Major advances in our understanding of the molecular changes leading to BCC formation have come from studies of patients with a hereditary predisposition to BCC development. As early as 1894, Jarisch and White described patients with features typical of the autosomally inherited syndrome (13, 14) now known as basal cell nevus syndrome (BCNS, also known as Gorlin syndrome), which was later described in detail by Robert Gorlin and others (15, 16). The birth incidence of BCNS in the United Kingdom is 1 in 19,000 (17); BCNS patients typically develop numerous BCCs starting at a young age and are prone to developing other tumors including medulloblastomas. Soon after the cloning of the Hedgehog (Hh) receptor Patched 1 (PTCH1) as the BCNS disease gene (18, 19), it became clear that a majority, if not all, of sporadic BCCs show abnormal activation of the Hh pathway (9, 20), ascribing constitutive activation of the Hh signaling pathway (Figure 2 and reviewed in ref. 21) as a prerequisite for the development of a BCC.

Other genetic syndromes such as Bazex-Dupré-Christol syndrome (22), Rombo syndrome (23), cartilage-hair hypoplasia (CHH) (24), and xeroderma pigmentosum (XP) (25) are associated with a high risk for BCC (reviewed in ref. 26), illustrating the

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involvement of additional genetic factors and pathways such as DNA repair (XP) and telomere maintenance (CHH).

Crosstalk between Hh and other molecular signaling pathways in BCC. The Wnt pathway has a well-established role in normal HF development and cycling, and both human and mouse BCCs have increased levels of β-catenin, a critical mediator of Wnt signaling (27, 28). In accordance with these observations, overexpression of the potent Wnt antagonist, Dkk1, in mouse epidermis resulted in the inhibition of benign Hh-driven hamartomas, showing that active Wnt signaling is required for their growth (29).

In line with its importance in epidermal development, the EGFR/MEK/ERK pathway has been shown to modulate GLI-dependent transcription in human keratinocytes (30) and to synergistically induce oncogenic transformation of human keratinocytes (31). Additionally, the tumor suppressor p53 may influence BCC development. The complete loss of p53 was shown to result in upregulated expression of the Hh pathway mediator smoothened (Smo) in the interfollicular epidermis (IFE) in mice, thereby making these keratinocytes receptive to BCC induction (32).

Finally, the correct cellular context is important for the persistent growth of BCCs, and epithelial-stromal interactions play a role in creating a favorable microenvironment. Stromal cells, isolated from human BCCs, express high levels of gremlin 1, which antagonizes the pro-differentiation factors BMP2 and BMP4, synthesized in the BCC tumor cells, thereby sustaining tumor growth (33).

Modeling the disease

Genetic mouse models represent a major advance in cancer research as they provide the possibility of studying tumors in the context of the entire organism. The study of BCC tumorigenesis in mice has been hindered by the inexplicable failure of mutagenic chemicals and UV or IR to induce BCCs (34). However, genetically engineered mouse models for BCC provide the option to investigate the molecular mechanisms of BCC formation and progression and the identification of the cells of origin. Due to the obligate dependency of BCCs on activated Hh signaling, all current BCC mouse models target different components of the Hh signal transduction pathway, and these are summarized in Tables 1 and 2.

In this context it is important to note that tumors developing in the mouse may not always fully mimic human BCCs but represent various stages in a spectrum of benign to malignant Hh pathway–induced BCC-like tumors, likely reflecting a similar variation in humans, where benign hamartomas and BCCs appear to be driven by different levels of Hh pathway activation (35–38).

Cell of origin and morphological diversity of BCC. Stem and progenitor cells are thought to be the most probable sources of tumor initiation due to their longevity and ability to self-renew (39, 40). In the skin, several populations of cells with stem cell (SC) properties have been discovered; however, defined SC markers are, as yet, limited to the HF (Figure 3; reviewed in refs. 41, 42). A collection of recent publications describes the use of mouse genetics to identify BCC cells of origin using Cre-mediated cell-specific targeting, either by lineage tracing, which involves the genetic labeling of cells, or by the activation of oncogenic Hh signaling in distinct skin cell populations (Figure 3). The identification of tumor cells of origin and, equally importantly, cells that cannot initiate tumorigenesis, will make it possible to pinpoint molecular mechanisms that either predispose or protect a cell from oncogenic transformation.

In the first publication to address the cellular origin of BCC, Youssef et al. used mice conditionally expressing SmoM2 (43), a constitutively active variant of Smo (44). When SmoM2 expression was activated in different cell compartments in the epidermis,
including HF SCs (Table 2), only cells originating in the IFE and the upper infundibulum produced full-blown BCCs. Importantly, the IFE-derived tumors also exhibited an HF-like protein expression pattern, demonstrating that the biochemical and morphological characterization of tumor cells may be misleading when used to identify the cellular origin of cancers (29, 43).

These same mouse models (employing conditional expression of SmoM2 in K14- or K15-positive cells; Table 2) were used to study the effect of wound healing on BCC development (45). It was previously reported that active wound repair recruits HF cells for re-epithelialization (46–48). In K15-SmoM2 skin, which targets SmoM2 expression mostly to the HFs, the HFs exhibited only occasional basaloid lesions in the bulge and the hair germ (HG) (43, 49).

Intriguingly, during wound healing, the HF SmoM2-expressing cells are mobilized to the IFE, where they drive BCC formation and form tumors (45). Why does SmoM2 induce tumors in the IFE upon wounding but not in the intact bulge or HG? One possible explanation is that the bulge microenvironment suppresses SmoM2-mediated oncogenesis, as Gli1 and Gli2 mRNAs, which encode Hh pathway effector proteins downstream of Smo, are upregulated in IFE-associated tumors but not in HFs (45).

Our group has also addressed the question of the BCC cell of origin and the effect of wounding on tumor growth (ref. 50 and Table 2). Overexpression of human GLI1 under the control of the K5 promoter resulted in BCC formation, preferentially in the IFE, but also in the HF. Lineage-tracing of Lgr5+ SCs, which give rise to the bulge and HG, showed that in this BCC model, HF- and IFE-associated lesions had distinct cells of origin, as no Lgr5+-labeled lesions were found in the IFE. However, Lgr5-labeled HF cells were able to give rise to BCCs in the IFE upon wounding, in line with the study by Wong et al. (45). In the Lgr5Cre-Ptch1fl/fl mouse model, Ptch1 deletion in Lgr5-expressing HF SCs resulted in the formation of locally restricted basaloid proliferations in the lower part of the HF. In wounded skin, Ptch1-deficient Lgr5+ cells were again recruited to the wound sites in the IFE, where they induced de novo basaloid lesions (50). Thus, the concept that wounding is an important factor in tumor development, postulated 150 years ago (52), also appears applicable to BCC development.

Despite similar molecular pathogenesis, a considerable morphological heterogeneity exists among BCCs (53). How do these morphological differences occur? By comparing two different BCC models, Grachtchouk et al. found that BK5-GLI2 mice (54) with strong Hh signaling developed full-featured BCCs, while the weaker Hh signal in ΔK5-SMO-M2 mice resulted in follicular hamartomas (35). Furthermore, a recent, more detailed study from the same group revealed that activated SmoA1 expression in the HF does not give rise to BCC-like lesions in the HF and that low levels of GLI2ΔN expression throughout the basal compartment do not lead to nodular BCCs in the HF (in contrast to the model with high GLI2ΔN expression), but to slow-growing basaloid follicular hamartomas (50).
artomas resembling the tumors found in ΔBK5-SMO-M2 mice (ref. 37 and Table 2). These observations support the suggestion that, downstream of Ptc1, the level of Hh pathway activation, rather than the exact molecular target, is crucial in determining the BCC subtype. It is worth noting that high levels of GLI2AN expression in the bulge and HG rapidly lead to nodular tumors, most likely initiated in the lower bulge and the HG (37), and the HG may also be the source for tumor-initiating cells in Lgr5Cre-Ptc1hi−/− mice (50). The lower bulge and HG harbor cells with active Hh signaling in telogen (55, 56), which may represent a cell population that is preferentially susceptible to Hh pathway–driven tumorigenesis.

The influence of the hair cycle phase on BCC growth is also important, and the authors of three studies have presented direct evidence that BCC development occurs preferentially, but not exclusively, during anagen phase (37, 57, 58). One reason may be that cells located in the outer root sheath (ORS) of anagen HF can give rise directly to nodular BCCs, supporting the idea that this compartment contains cells capable of transformation by oncogenic Hh signaling and, therefore, provides an expanded pool of potential tumor progenitors.

Together, the results obtained using mouse models to study BCC development have so far revealed that oncogenic Hh signaling can drive BCC-like tumor formation in several different epithelial progenitor populations in skin, although the morphology and the final outcome of BCC development are influenced by the cell of origin, the mutated Hh pathway member, and the strength of oncogenic Hh signaling.

### New therapies

Highly efficient treatment modalities such as surgery that aims at complete extirpation, radiotherapy, curettage, cryotherapy, photodynamic therapy, and topical applications of imiquimod or 5-fluorouracil are available and effective for the great majority of BCC patients (9, 59). However, the occurrence of locally aggressive and invasive tumors, a bleak prognosis upon metastatic spread, a significant rate of recurrence often associated with increased aggressiveness, as well as the multitude of tumors appearing in high-risk populations such as BCNS patients, provide compelling reasons to search for new preventive and therapeutic avenues (60).

Hh signaling as a target for new BCC therapies. The first evidence that the Hh signaling pathway is sensitive to inhibition by small molecules stemmed from the observation of cyclopia in lambs, induced by the maternal ingestion of corn lilies (Veratrum californicum) (61), followed by the demonstration that the active compound, cyclopamine, inhibits Hh signaling (62) and binds to SMO (63). Initial studies showed that, in addition to Hh inhibition in various in vitro systems, the oral administration of cyclopamine reduced the growth and development of BCCs in Ptc1hi−/− mice exposed to UV irradiation (64), and its topical application to human BCCs can induce regression (65).

New derivatives of cyclopamine with improved pharmaceutical properties are now in clinical trials (Table 3 and ref. 66). Excellent results were obtained with the orally administered SMO inhibitor GDC-0449 (vismodegib; Table 3) in a phase I trial of patients with locally advanced or metastatic BCC (67, 68). Phase II results

### Table 1

<table>
<thead>
<tr>
<th>Cell targeting alleles</th>
<th>Effector alleles</th>
<th>Start of Hh pathway modulation</th>
<th>Additional treatment</th>
<th>Pathology</th>
<th>Reference</th>
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<tbody>
<tr>
<td>K14</td>
<td>Shh Tg</td>
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<td>hK5</td>
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<td>Trichoblastomas</td>
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<td>UV, γIR, X-ray</td>
<td>BCC-like lesions</td>
<td>91</td>
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<td>BKS</td>
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<td>E04</td>
<td>No</td>
<td>BCC, trichoepithelomas, cylindromas, trichoblastomas</td>
<td>36</td>
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<td>E04</td>
<td>No</td>
<td>BCC</td>
<td>54</td>
</tr>
<tr>
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<td>E04</td>
<td>No</td>
<td>BCC, trichoblastomas, cylindromas, basoloid follicular hamartomas</td>
<td>92</td>
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<td>ΔBK5</td>
<td>hSMO-M2 Tg</td>
<td>E04</td>
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<td>Basaloid follicular hamartomas</td>
<td>35</td>
</tr>
<tr>
<td>All</td>
<td>Ptch1f−/−</td>
<td>E04</td>
<td>No</td>
<td>Basaloid hyperproliferations</td>
<td>93</td>
</tr>
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<td>All</td>
<td>Ptch1f−/−</td>
<td>E04</td>
<td>X-ray</td>
<td>BCC (nodular and infiltrative)</td>
<td>93</td>
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<td>K5HTA (bovine)</td>
<td>TREMGLI2 Tg</td>
<td>E04</td>
<td>DOX off</td>
<td>BCC</td>
<td>75</td>
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<tr>
<td>All</td>
<td>Su102e−/−</td>
<td>E04</td>
<td>No</td>
<td>BCC, basaloid follicular hamartomas</td>
<td>94</td>
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<tr>
<td>K6a-Cre</td>
<td>Ptch1f−/−</td>
<td>E04</td>
<td>No</td>
<td>ORS hyperplasia</td>
<td>27</td>
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<td>Retinoic acidβ</td>
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<td>K14-Cre</td>
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<td>95</td>
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<tr>
<td>Mx1-Cre</td>
<td>Ptch1f−/−</td>
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<td>poly(I:C)γ</td>
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<td>95</td>
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<td>CA956-CreER</td>
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<td>P10</td>
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<td>BCC-like lesions</td>
<td>96</td>
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<td>fl-STOP-fl-SmoM2-YFPα</td>
<td>P30–P35</td>
<td>No</td>
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<td>97</td>
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<td>K14-CreERT</td>
<td>fl-STOP-fl-SmoM2-YFPαβ</td>
<td>P30–P35</td>
<td>No</td>
<td>Inhibition of BCC-like tumors</td>
<td>97</td>
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<td>K14-CreERT</td>
<td>CLEG2-condβ</td>
<td>P30–P35</td>
<td>No</td>
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<td>97</td>
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<tr>
<td>K14-CreERT</td>
<td>CLEG2-condβγ</td>
<td>P30–P35</td>
<td>No</td>
<td>Enhanced BCC-like tumors</td>
<td>97</td>
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</table>

*Effective promoter activation is dependent on temporal regulation during embryogenesis (e.g., K14 starts at E9.5). *To activate K6 expression in the IFE and ORS at P32. *To activate Mx1 promoter via interferon response. *Fusion protein of mSmoM2 with YFP inserted into the Rosa26 locus. *Additional allele is Kif3a−/−. **CLEG2 denotes Myc-tagged, constitutively active human GLI2.
### Table 2

Mouse models used to identify the putative BCC cell of origin

<table>
<thead>
<tr>
<th>Targeting alleles</th>
<th>Effector alleles</th>
<th>Additional alleles</th>
<th>Start of Hh pathway modulation</th>
<th>Additional treatment</th>
<th>Pathology</th>
<th>BCC localization</th>
<th>Ref.</th>
</tr>
</thead>
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<td>K14-CreER</td>
<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>–</td>
<td>P23–P28</td>
<td>No</td>
<td>BCC (nodular)</td>
<td>IFE and infundibulum</td>
<td>43</td>
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<td>Shh-CreER</td>
<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>–</td>
<td>P25–P30</td>
<td>No</td>
<td>No morphological changes</td>
<td>No</td>
<td>43</td>
</tr>
<tr>
<td>K15-CreER</td>
<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>–</td>
<td>P21–P23</td>
<td>No</td>
<td>Rare hyper/dysplastic changes</td>
<td>HF</td>
<td>43</td>
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<tr>
<td>K15-CreER</td>
<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>–</td>
<td>P21–P23</td>
<td>No</td>
<td>Rare hyper/dysplastic changes</td>
<td>HF</td>
<td>43</td>
</tr>
<tr>
<td>K19-CreER</td>
<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
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<td>P28–P32</td>
<td>No</td>
<td>No hyper/dysplastic changes</td>
<td>HF</td>
<td>43</td>
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<tr>
<td>K15-CrePR1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>Ptch1&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>R26-YFP</td>
<td>7.5 weeks</td>
<td>X-ray at 8 weeks</td>
<td>BCC</td>
<td>HF</td>
<td>32</td>
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<tr>
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<td>P53&lt;sup&gt;+/–&lt;/sup&gt;</td>
<td>7.5 weeks</td>
<td>X-ray at 8 weeks</td>
<td>BCC</td>
<td>HF (enhanced)</td>
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<td>7.5 weeks</td>
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<td>BCC</td>
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<td>P53&lt;sup&gt;+/–&lt;/sup&gt;</td>
<td>7.5 weeks</td>
<td>X-ray at 8 weeks</td>
<td>BCC</td>
<td>HF (enhanced)</td>
<td>32</td>
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<td>K5tTA (bovine)</td>
<td>TREGL1 Tg</td>
<td>Lgr5-EGFP-IRES-CreERT2/ R26-LacZ&lt;sup&gt;C&lt;/sup&gt;</td>
<td>P16&lt;sup&gt;D&lt;/sup&gt;</td>
<td>DOX off</td>
<td>BCC-like lesions</td>
<td>IFE and HF</td>
<td>50</td>
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<tr>
<td>K5tTA (bovine)</td>
<td>TREGL1 Tg</td>
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<td>P16&lt;sup&gt;D&lt;/sup&gt;</td>
<td>DOX off and full-thickness wound</td>
<td>Enhanced size of BCC-like lesions</td>
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<td>50</td>
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<td>Locally restricted BCC-like lesions</td>
<td>HF</td>
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<td>P18–P24</td>
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<td>P14–P20</td>
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<td>IFE and HF</td>
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<td>R26-LacZ</td>
<td>7.5 weeks</td>
<td>Full-thickness wound, 3 days&lt;sup&gt;E&lt;/sup&gt;</td>
<td>BCC (IFE), rare small basaloid proliferations (HF)</td>
<td>IFE and HF</td>
<td>45</td>
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<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>R26-LacZ</td>
<td>7.5 weeks</td>
<td>Full-thickness wound, 5 weeks&lt;sup&gt;E&lt;/sup&gt;</td>
<td>Not tumor promoting</td>
<td>HF</td>
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<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>R26-LacZ</td>
<td>7.5 weeks</td>
<td>Anagen induction via HF depilation, 3 days&lt;sup&gt;E&lt;/sup&gt;</td>
<td>Not tumor promoting</td>
<td>HF</td>
<td>45</td>
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<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>R26-LacZ</td>
<td>7.5 weeks</td>
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<td>BCC</td>
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<td>45</td>
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<tr>
<td>K14-CreER</td>
<td>fl-STOP-fl-SmoM2-YFP&lt;sup&gt;A&lt;/sup&gt;</td>
<td>R26-LacZ</td>
<td>7.5 weeks</td>
<td>Full-thickness wound, 3 days&lt;sup&gt;E&lt;/sup&gt;</td>
<td>Not tumor initiation promoting</td>
<td>IFE</td>
<td>45</td>
</tr>
<tr>
<td>K15-CrePR1</td>
<td>tetO-GLI2ΔN Tg</td>
<td>R26-LSL-rtTA</td>
<td>7 weeks</td>
<td>High DOX dose</td>
<td>BCC (nodular)</td>
<td>HF</td>
<td>37</td>
</tr>
<tr>
<td>K15-CrePR1</td>
<td>tetO-GLI2ΔN Tg</td>
<td>R26-LSL-rtTA</td>
<td>8 weeks</td>
<td>High DOX dose and anagen</td>
<td>Acceleration of BCC</td>
<td>HF and ORS</td>
<td>37</td>
</tr>
<tr>
<td>Lgr5-EGFP-CreERT2</td>
<td>tetO-GLI2ΔN Tg</td>
<td>R26-LSL-rtTA</td>
<td>7 weeks</td>
<td>High DOX dose</td>
<td>BCC (nodular)</td>
<td>HF</td>
<td>37</td>
</tr>
<tr>
<td>Lgr5-EGFP-CreERT2</td>
<td>tetO-GLI2ΔN Tg</td>
<td>R26-LSL-rtTA</td>
<td>8 weeks</td>
<td>High DOX dose and anagen</td>
<td>Acceleration of BCC</td>
<td>HF and ORS</td>
<td>37</td>
</tr>
<tr>
<td>K14rtTA</td>
<td>tetO-GLI2ΔN Tg</td>
<td>R26-LSL-rtTA</td>
<td>–</td>
<td>High DOX dose</td>
<td>BCC (nodular, HF; superficial, IFE)</td>
<td>IFE, HF, SG</td>
<td>37</td>
</tr>
<tr>
<td>K5-CreER</td>
<td>tetO-GLI2ΔN Tg</td>
<td>R26-LSL-rtTA</td>
<td>7 weeks</td>
<td>Low DOX dose</td>
<td>Basaloid follicular hamartomas</td>
<td>IFE and possibly HF involvement</td>
<td>37</td>
</tr>
<tr>
<td>K15-CrePR1</td>
<td>SmoA1 (mouse)</td>
<td>R26-LSL-rtTA</td>
<td>7 weeks</td>
<td>High DOX dose</td>
<td>Modest hyperplasia</td>
<td>HF</td>
<td>37</td>
</tr>
</tbody>
</table>

SG, sebaceous gland. Preferential localization, when present, is indicated by bold font. *Fusion protein of mSmoM2 with YFP inserted into the Rosa26 locus. Only used for lineage tracing and p53 alleles; Ptch1 heterozygous in all cells and all tissues. <sup>1</sup>Cre activated at P14. <sup>2</sup>Effective GLI1 protein expression: P28. <sup>3</sup>Time after SmoM2 induction.
using GDC-0449 in patients with locally advanced or metastatic BCC (69) and in BCNS patients with multiple BCCs (70) were so promising that the Data Safety Monitoring Board recommended ending the placebo arm of the BCNS study in light of the differences between the study arms. Importantly, no resistance has so far been reported. In addition, a phase I study of IPI-926 (Table 3) given orally to patients with advanced types of BCC also resulted in a positive response rate (71).

In the studies investigating systemic treatment with SMO inhibitors, a common set of adverse effects has been observed, including muscle spasms, loss of taste (dysgeusia), hair loss, fatigue, nausea, and hyponatremia. It is likely that hair loss and altered taste, at least, are related directly to SMO inhibition, since Hh signaling is known to be active in HFs and taste buds (72, 73). One way to avoid or reduce such effects might be to use these inhibitors topically, thus limiting systemic exposure. A small, short-term study in BCNS patients with nodular and superficial BCC, employing twice-daily topical treatments of the SMO inhibitor LDE225 for four weeks, resulted in a positive response, and BCC regression correlated with a decrease in Hh target gene expression in most treated tumors (74). No treatment-related side effects were noted, consistent with low levels of systemic exposure to the inhibitor.

A potential caveat associated with the use of Hh pathway antagonists for the treatment of BCC is the possibility that, while treatment may result in a dramatic reduction in tumor mass, a small number of residual cells that are relatively insensitive to Hh signal inhibition may persist so that treatment may not be curative. The existence of such a cell population has been shown both in a mouse BCC model (75) and in a clinical trial of the SMO inhibitor LDE225 (ref. 74 and Table 3).

Another concern is the development over time of resistance to SMO inhibitors used in treatment, which may or may not involve mechanisms similar to those that render certain BCCs refractory to treatment from the start. Medulloblastoma is another tumor type in which Hh signaling is frequently activated by mutations in \[^{PTCH1}\] or \[^{SMO}\] (76). The treatment of one patient with a medulloblastoma carrying \[^{PTCH1}\] mutations with GDC-0449 led to rapid but transient tumor regression (76). Subsequently, it was found that an amino acid substitution in SMO that had no effect on Hh signaling but disrupted the ability of GDC-0449 to bind SMO and suppress this pathway was the underlying cause of the relapse (77).

Studies in animal models confirmed that the development of resistance can be caused by mutations in Smo as well as by the amplification of downstream genes such as \[^{Gli2}\] and cyclin D1 (78, 79). Potential methods of overcoming such resistance involve the use of alternative SMO inhibitors (79) such as the FDA-approved antifungal drug itraconazole, which was recently found to inhibit Hh signaling by binding to SMO at a site different from cyclopamine and to delay BCC development in \[^{Ptc1^{-/-}}\] mice (80). At present, itraconazole is being evaluated as a possible treatment for BCC.
in a phase II trial (Table 3). Alternatively, blocking other signaling pathways in resistant tumors may be effective, and in preclinical studies of medulloblastomas, PI3K inhibition has emerged as a promising possibility (78, 79).

In situations in which Hh pathway activation occurs downstream of SMO, targeting the final effectors in the pathway, such as the GLI transcription factors, would be preferable. The potential viability of this strategy has been demonstrated by the identification of small molecule inhibitors acting at the level of GLI, or at alternative steps downstream, and independent of SMO (refs. 81, 82, and Table 3). Interestingly, in two studies it has been found that arsenic trioxide, in clinical use for the treatment of acute promyelocytic leukemia, can inhibit Hh signaling at the GLI protein level, although the exact mechanism remains controversial (83, 84). However, given the diverse set of targets for arsenic trioxide, it will be challenging to delineate critical targets in an in vivo setting, and existing side effects may limit its attractiveness as a treatment for BCC; curiously, arsenic exposure is also a known risk factor in BCC development (85).

Given the key role of primary cilia in the transduction of the Hh signal (Figure 2), inhibitors of ciliogenesis or ciliary function represent a further means of intervention in BCC tumorigenesis, and a small molecule blocking ciliogenesis has been identified as an Hh inhibitor (ref. 82 and Table 3). Again, the multiple cellular effects expected as a result of cilia disruption will place obstacles in the way of obtaining specificity.

Other potential new BCC therapies. Vitamin D3 has been shown to block Hh signaling in vitro and in murine BCCs in vivo, presumably at the level of SMO, in a manner independent of vitamin D receptor (VDR) activation (86, 87). The enhanced differentiation of keratinocytes induced via VDR activation is an additional and well-established effect of vitamin D3. A phase III trial combining topical vitamin D3 therapy and treatment with an anti-inflammatory agent in patients with nodular BCC has been initiated (Table 3).

Finally, topical treatment with the RARB/RARG-selective retinoid tazarotene has been shown to reduce the number and size of early BCC lesions in irradiated Pch1+/−/K14-CreERT2-pS395 mice exposed to IR (c).

To summarize, early clinical results targeting the Hh signaling pathway are very promising, especially in regard to BCC treatment and chemoprevention in BCNS patients and for the treatment of locally aggressive or metastatic BCC. However, we still do not have the answer to several important questions: (a) Will treatment truly result in the eradication of BCCs, or will dormant tumor cells remain? (b) What are the major resistance mechanisms in BCCs? (c) Why are a substantial fraction of BCCs refractory to treatment

Table 3
Emerging BCC therapies

<table>
<thead>
<tr>
<th>Agent/compound</th>
<th>Target</th>
<th>Condition/patient group studied</th>
<th>Study phase</th>
<th>ClinicalTrials.gov ID</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDC-0449A</td>
<td>SMO</td>
<td>BCNS patients</td>
<td>II</td>
<td>NCT00957229</td>
<td>70</td>
</tr>
<tr>
<td>GDC-0449</td>
<td>SMO</td>
<td>Locally advanced or metastatic BCC</td>
<td>II</td>
<td>NCT01367665</td>
<td>69</td>
</tr>
<tr>
<td>GDC-0449</td>
<td>SMO</td>
<td>Operable BCC</td>
<td>II</td>
<td>NCT01201915</td>
<td>–</td>
</tr>
<tr>
<td>GDC-0449</td>
<td>SMO</td>
<td>BCC</td>
<td>II</td>
<td>NCT00959647</td>
<td>–</td>
</tr>
<tr>
<td>GDC-0449</td>
<td>SMO</td>
<td>Advanced BCC</td>
<td>II</td>
<td>NCT00833417</td>
<td>–</td>
</tr>
<tr>
<td>GDC-0449</td>
<td>SMO</td>
<td>Locally advanced or metastatic BCC</td>
<td>II</td>
<td>NCT01162025</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>BCNS patients</td>
<td>II</td>
<td>NCT00961896</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>Advanced solid tumors (BCC, medulloblastoma)</td>
<td>I</td>
<td>NCT01208831</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>Advanced solid tumors (BCC, medulloblastoma)</td>
<td>I</td>
<td>NCT00880308</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>Sporadic superficial and nodular BCC</td>
<td>II</td>
<td>NCT01033019</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>Locally advanced or metastatic BCC</td>
<td>II</td>
<td>NCT01327053</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>BCNS patients</td>
<td>II</td>
<td>NCT01350115</td>
<td>–</td>
</tr>
<tr>
<td>LDE-225</td>
<td>SMO</td>
<td>BCNS patients</td>
<td>Preclinical</td>
<td>–</td>
<td>74</td>
</tr>
<tr>
<td>Cur61414</td>
<td>SMO</td>
<td>UV-treated Pch1+/−/ mice; Superficial and nodular BCC</td>
<td>Preclinical</td>
<td>–</td>
<td>98, 99</td>
</tr>
<tr>
<td>Cur61414</td>
<td>SMO</td>
<td>UV-treated Pch1+/−/ mice; Superficial and nodular BCC</td>
<td>Preclinical</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>IPI-926</td>
<td>SMO</td>
<td>BCC (including locally advanced or metastatic)</td>
<td>I</td>
<td>NCT00761696</td>
<td>71</td>
</tr>
<tr>
<td>BMS-833923</td>
<td>SMO</td>
<td>Advanced or metastatic BCC</td>
<td>I</td>
<td>NCT00670189</td>
<td>–</td>
</tr>
<tr>
<td>TAK-441</td>
<td>SMO</td>
<td>Advanced BCC</td>
<td>I</td>
<td>NCT01204073</td>
<td>–</td>
</tr>
<tr>
<td>Itracnazol</td>
<td>SMO</td>
<td>BCC</td>
<td>II</td>
<td>NCT01108094</td>
<td>–</td>
</tr>
<tr>
<td>Downstream Hh pathway inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GANT 58, GANT 61</td>
<td>GLI</td>
<td>NA</td>
<td>Preclinical</td>
<td>–</td>
<td>81</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>GLI</td>
<td>NA</td>
<td>Preclinical</td>
<td>–</td>
<td>83, 84</td>
</tr>
<tr>
<td>HP11/2/3</td>
<td>GLI</td>
<td>NA</td>
<td>Preclinical</td>
<td>–</td>
<td>82</td>
</tr>
<tr>
<td>HIP4</td>
<td>Cilogenesis</td>
<td>NA</td>
<td>Preclinical</td>
<td>–</td>
<td>82</td>
</tr>
<tr>
<td>Other agents</td>
<td>Vitamin D3</td>
<td>SMO/cell proliferation</td>
<td>BCC</td>
<td>III</td>
<td>NCT01358045</td>
</tr>
<tr>
<td>Tazarotene</td>
<td>RAR-β/RAR-γ</td>
<td>BCC on chest and back of BCNS patients</td>
<td>II</td>
<td>NCT00738065</td>
<td>–</td>
</tr>
<tr>
<td>Tazarotene</td>
<td>RAR-β/RAR-γ</td>
<td>BCC on face of BCNS patients</td>
<td>II</td>
<td>NCT00489086</td>
<td>–</td>
</tr>
</tbody>
</table>

*Drug name vismodegib. *BCCs failed to show signs of clinical response or significant GLI1 target gene inhibition, likely due to inadequate drug absorption.
from the start? Moreover, the adverse effects that develop with sys-
temic treatment are a significant concern, arguing for the eval-
uation of local treatment options.

Perspectives
The great advances in our understanding of BCC biology, derived
from deciphering its molecular genetics and from incisive studies
using genetic mouse models that closely mimic the human dis-
ease, have been translated rapidly into new and promising targeted
therapies. At the same time, it is important to realize that we are
only just beginning to resolve the long-standing question about
the BCC cell of origin. We know from studies in the mouse that
SCs and progenitor cells present in the HF can serve as cells of
origin, but that additional cells of origin must exist. The marked
plasticity of skin epithelial stem cell populations, as revealed upon
tissue injury, provides an additional layer of complexity.

Another challenging question begging an answer is the nature of
the genetic events that cooperate with an activated Hh signal-
ing pathway to determine BCC subtypes, ranging from the benign
dermal and nodular superficial forms to aggressive and, in rare cases,
metastatic forms. Future studies will certainly provide answers to
many of these questions, and it is to be hoped, moreover, that the
lessons learned from treating BCC with Hh pathway inhibitors
will pave the way for progress in the treatment of other tumors
that depend on the presence of active Hh signaling.

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