Basal cell carcinoma and basal cell nevus syndrome

BASAL CELL CARCINOMA AND BASAL CELL NEVUS SYNDROME

OPTIMIZING TREATMENT AND CARE

BABETTE VERKOUTEREN

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Chapter 1

General introduction

GENERAL INTRODUCTION

This thesis evaluates the treatment and care for patients with sporadic or advanced basal cell carcinoma (BCC), basal cell nevus syndrome, and patients with high-frequency BCC These specific clinical forms of BCC and the different treatment modalities are described in detail below.

Basal cell carcinoma

Epidemiology

BCC is the most common malignancy in the Caucasian population and its incidence is still rising.[1, 2] In the Netherlands, one in five to six people will develop at least one BCC during his or her life.[1] The 5-year cumulative risk of developing a subsequent BCC is approximately 30%.[3] The most rapid increase in incidence of BCC is seen in women under the age of 40 years.[2] The most important risk factor for the development of BCC is exposure to ultraviolet radiation (UVR).[4] UVR exposure causes DNA damage (C:T or CC:TT transitions) in general but also in tumour suppressor and proto-oncogenes which can eventually lead to uncontrolled cell growth and the development of a BCC. Other risk factors include exposure to ionizing radiation or carcinogenic arsenic, an immune-compromised condition, higher age, male sex, light hair, blue eyes, fair skin colour and other specific genetic factors.[4] BCC development can also be the result of an inherited disorder. This may lead to a skin cancer syndrome, of which basal cell nevus syndrome (BCNS) is the most common.[5] Other skin cancer syndromes are xeroderma pigmentosum (types A to G), Bazex-Dupré-Christol syndrome and Rombo syndrome.[5]

Pathogenesis

BCCs are skin tumours that resemble basal cell keratinocytes of the epidermis and hair follicle epithelium. The origin of BCC-initiating cells has not been completely elucidated to this day. Most theories attribute a role to both interfollicular epithelial and hair follicle stem cells.[6, 7] BCCs are reported to be one of the most highly mutated tumours in solid cancers.[8] The main driver event of BCC development is the activation of the sonic hedgehog signalling (SHH) pathway, involved in embryogenic development and tumorigenesis (Figure 1).[9] The activation of the SHH pathway, due to the binding of a hedgehog ligand, releases the inhibition of patched-1 (PTCH1) on the protein smoothened (SMO). SMO then signals downstream resulting in activation of the glioma associated oncogene (GLI) family of transcription factors (Figure 1). GLI transcription factors are partly inhibited by suppression of fused (SUFU). The GLI family transcription factors induce

proliferation, suppression of apoptosis, angiogenesis and eventually tumour formation.[9] Driver mutations in the SHH pathway are found in approximately 85% of all sporadic BCCs (*PTCH1* in 73%, *SMO* in 20% and SUFU in 8%).[10] Besides mutations in the SHH pathway, additional mutations in *TP53*, *H/N/K-RAS*, *PPP6C*, *PIK3CA*, *STK19*, *ERBB2*, *PTPN14*, *LATS1*, *MYCN*, *RB1* and *FBXW7* can be part of the mutation burden, driving the development of BCCs.[10, 11]

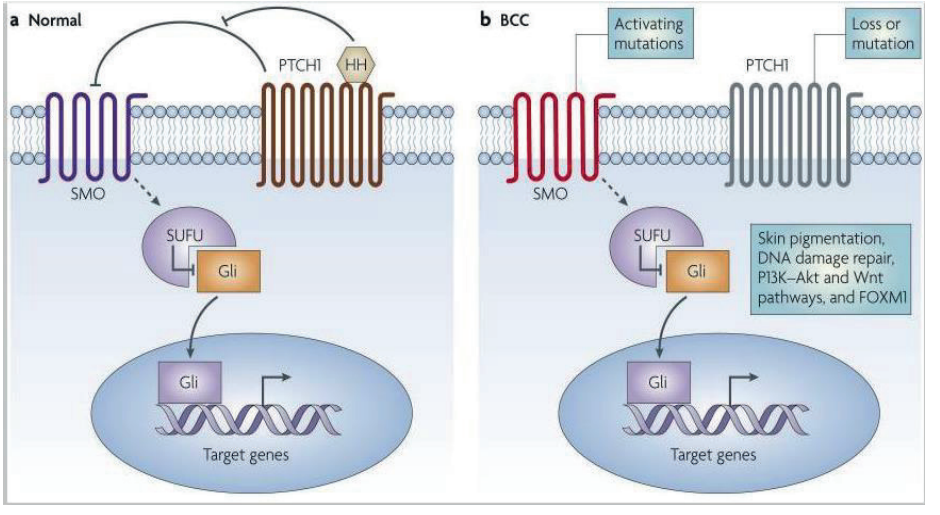

Figure 1. Hedgehog pathway in embryogenic development (a) and in basal cell carcinoma development (b).[9]

Clinical presentation and diagnosis

Clinical presentation of BCCs vary from thin, superficial erythematous patches with some scaling to erythematous, shiny papules with telangiectasias. The borders of a BCC can be well- or ill-defined. A trained dermatologist can generally diagnose a BCC based on macroscopic features combined with dermoscopy. The sensitivity and specificity for diagnosing a BCC based on clinical examination and dermoscopy are 85% and 98.2% respectively.[12] Most BCCs will be biopsied for definitive diagnosis and histologic subtyping.[13] A simplified histologic classification distinguishes three major histopathological subtypes: superficial, nodular and infiltrative BCC (Figure 2).[14] Differentiation between the histologic subtype is important since treatment options differ for each histologic subtype. The most common histologic subtype is nodular BCC, representing 40.6-57.1% of all BCCs, followed by superficial (19.5-30.7%) and infiltrative BCC (5-10%).[15] Superficial BCCs are histologically characterized by basaloid cell nests attached to

the epidermis, whilst the basaloid cell nests in nodular BCCs invade the dermis.[14] Infiltrative BCCs consist of layers of basaloid cells invading collagenised stroma.[14] A combination of multiple histological subtypes can also be found within one BCC.[14] The most aggressive subtype determines the therapeutic strategy.

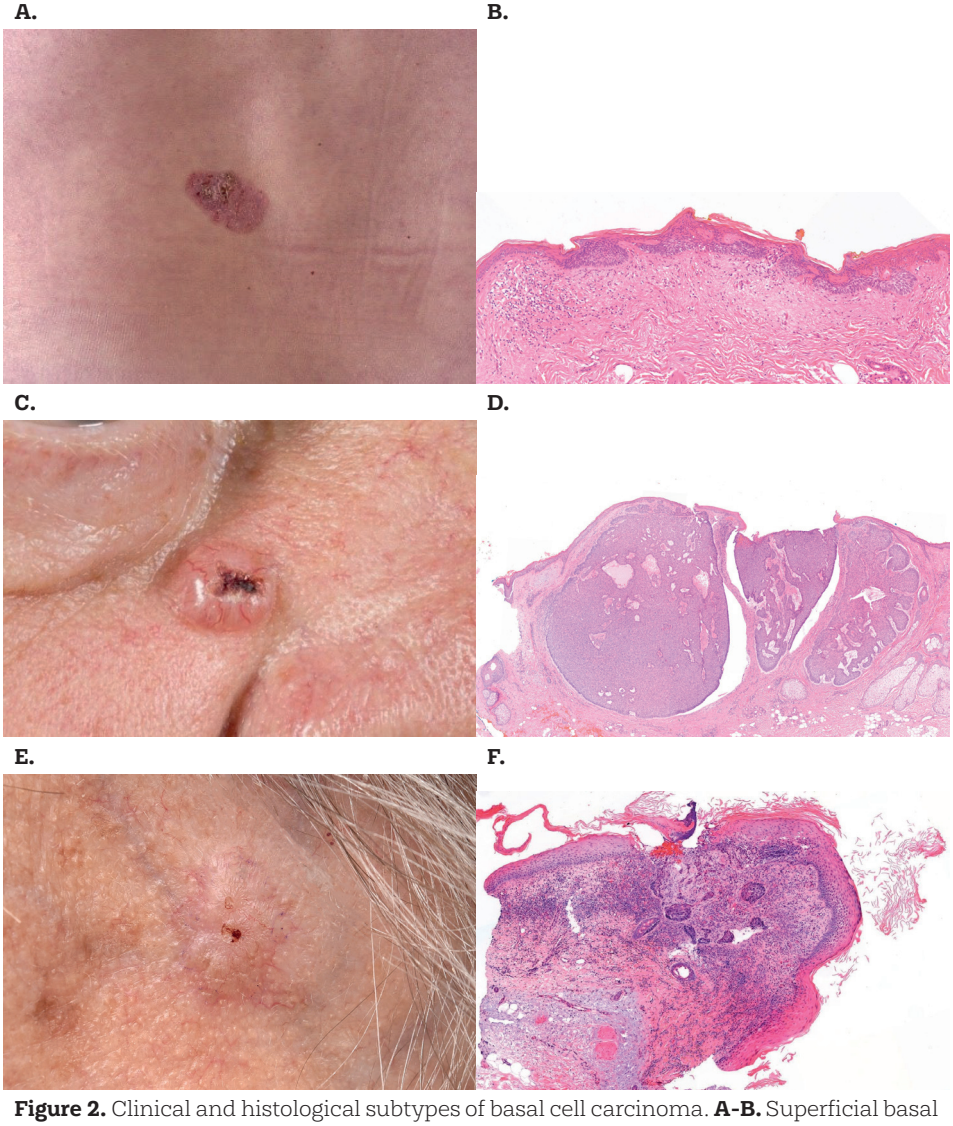

Figure 2. Clinical and histological subtypes of basal cell carcinoma. **A-B.** Superficial basal cell carcinoma, **C-D.** Nodular basal cell carcinoma, **E-F.** Infiltrative basal cell carcinoma.

Advanced basal cell carcinoma

If a BCC is left untreated, it can develop into a locally destructive tumour (Figure 3). There is no consensus on the definition of locally advanced BCC, but in different guidelines a definition for laBCC has been proposed.[16, 17] The European BCC guidelines state: 'although not clearly defined, the word 'advanced' usually implies that (1) there has been a long history without treatment or with repeated failures of treatments and recurrences, (2) there is extensive tissue destruction in the surrounding anatomical area and (3) it has become difficult or impossible to cure the tumour through standard surgery (unresectable) or through radiotherapy.[18] Metastasized BCC is defined as a BCC with regional nodal invasion or distant metastasis.[19] The incidence of metastasized BCC is estimated to be only 0.0028-0.055% of all BCCs.[20] Reports in literature of mBCC consist of case reports, case series and retrospective cohort studies; but molecular analysis of the metastases to prove the origin of the basal cell carcinoma is lacking.[21] The most common metastatic sites are regional lymph nodes, lungs, and bones.[21] Risk factors for mBCC are tumours located in the head/neck area, tumour diameter above four centimetres and tumour depth beyond the subcutaneous fat.[22] MBCC has a poor prognosis with a median survival of 10 months (range, 0.5-108.0 months) after diagnosis.[23]

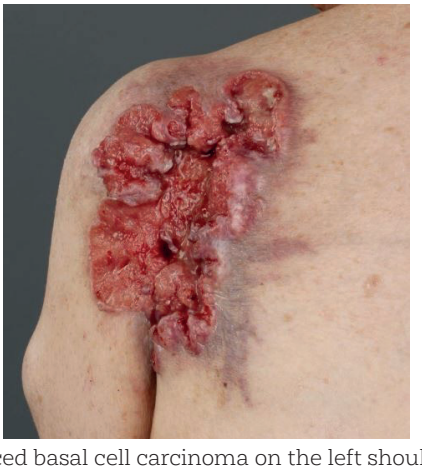

Figure 3. Locally advanced basal cell carcinoma on the left shoulder.

Basal cell nevus syndrome

Basal cell nevus syndrome (BCNS) (OMIM #109400), also known as Gorlin(-Goltz) syndrome, is a rare, autosomal dominant disorder, with a broad variety of symptoms.[24] The major dermatologic problem is the high number of BCCs in these

patients, of which the first can already develop in childhood. Standard treatment options for BCC are not always suitable due to these high numbers of BCCs. Non-dermatological symptoms consist mainly of odontogenic keratocysts of the jaw, bone development disorders, cardiac and ovarian fibromas, and medulloblastoma. Diagnosis of BCNS is based on a combination of major and minor criteria, with or without genetic confirmation (Table 1).[24] A broad spectrum of other symptoms and tumours has also been reported in BCNS patients, such as renal dysfunction and meningiomas. The combination of the high number of BCCs requiring subsequent treatments and all other symptoms can lead to a decreased quality of life in patients with BCNS.[25-27]

Table 1. Diagnostic criteria for basal cell nevus syndrome by Bree et al.[24]

Major criteria	Minor criteria
Multiple BCCs or one BCC in a person younger than 20 years	Bifid, fused or splayed ribs
Odontogenic keratocysts	Other specific skeletal and radiologic abnormalities (i.e. pectus excavatum, scoliosis, hemivertebrae, Sprengel's deformity, syndactyly of digits, bony bridging of the sella turcica, flame-shaped lucencies of phalanges)
Palmar or plantar pits	Macrocephaly
Lamellar calcification of the falx cerebri	Cleft lip or palate
Medulloblastoma in early childhood	Ovarian or cardiac fibroma
First-degree relative with BCNS	Lymphomesenteric cysts
	Ocular anomalies (i.e. congenital cataract, coloboma, glaucoma, hypertelorism)

BCC, basal cell carcinoma; BCNS, basal cell nevus syndrome.
Two major criteria, one major criteria and two minor criteria, or one major criteria and genetic confirmation are required for diagnosis.

In approximately 85% of patients a germline mutation in the tumour suppressor gene *PTCH1* and in 5% of patients a mutation in the tumour suppressor gene *SUFU* can be found.[28, 29] According to the two-hit hypothesis, a mutation needs to be accompanied by a second hit in the wild type allele of a tumour suppressor gene in order to result in a loss of functionality and subsequent induction of tumorigenesis.[30] In several BCNS-related tumours such as odontogenic keratocysts and BCCs, *PTCH1* mutation with loss of heterozygosity has been described. However, for other tumours that have been reported in patients with BCNS, this molecular mechanism has not been elucidated. Two cohorts investigated

genotype-phenotype relations between *PTCH1* and *SUFU* mutations and BCNS symptoms.[29, 31] One cohort found that patients with a germline mutation in *SUFU* (n=9) have a higher risk of developing a medulloblastoma in childhood, but this has not yet been confirmed in other cohorts.[29] Furthermore, odontogenic keratocysts of the jaw were not seen in the 9 patients with a SUFU mutation.[29] Differences in phenotypes between *PTCH1* and *SUFU* mutations might implicate different follow-up and screening schedules for these patients. The most recent guideline for patients with BCNS was published in 2011.[24] At that time genetic testing did not play a major role in diagnosing patients, nor were phenotype-genotype relations taken into consideration. There is a need for an updated guideline, that also considers genetic testing and genotype-phenotype relations.

High-frequency basal cell carcinoma

Besides patients with BCNS, there is also a category of patients who develop an unusually high number of BCCs without an identified genetic cause. These patients are referred to as high-frequency BCC (HF-BCC) patients. Several criteria for HF-BCC have been proposed or used in literature. Patients are defined as HF-BCC patients when they have six BCCs at once[32], developed nine or more BCCs in three years,[33, 34] or developed at least six BCCs in ten years.[35] Depending on this definition, the estimated prevalence of patients with HF-BCC varies between 49 to 51 per 100.000 in patients >18 years old, in Denmark and the United States respectively.[33, 34] In these patients, multiple required treatments might also lead to a high disease burden.

Treatment modalities for basal cell carcinoma

Surgical excision and non-invasive treatment modalities

The gold standard for BCC treatment is surgical excision, with a clinical safety margin between three and five millimetres, depending on tumour size and histologic subtype. Recurrence rates 5 years after surgical excision range from 2-8%.[36] The main advantage of surgical excision is the histopathologic confirmation of tumour free margins. Disadvantages include the possibility of complications, such as an infection or bleeding, and the development of a scar. BCCs at difficult to treat locations, such as the H-zone of the face, require Mohs micrographic surgery, which directly provides histologic confirmation of tumour free margins.[13, 18] For low-risk small BCCs of the superficial and nodular subtype, non-invasive treatments such as imiquimod 5% cream, 5-fluorouracil cream, photodynamic therapy, curettage and cryosurgery are available, of which imiquimod 5% cream is proven to be the most effective in terms of a long term complete clearance rate.[37] Since part of this thesis focusses mainly on treatment with imiquimod 5% cream, only this non-invasive treatment will be explained in more detail below.

Imiquimod

Imiquimod primarily exerts its effect by activating toll-like receptor 7 (TLR7) and potentially toll-like receptor 8 (TLR8), which are both located on the X-chromosome.[38-40] TLR7 and TLR8 are pathogen-recognition receptors located on endosomes in the cytoplasm of immune cells. Both receptors recognize pathogen-associated molecular patterns and play a bridging role between innate and adaptive immunity. Imiquimod ligates to TLR7 on immune cells and initiates downstream activation of the transcription mediator NF-kB via the myeloid differentiation factor 88 (MyD88).[38-40] Consequently, this induces an inflammatory cascade with maturation and secretion of various pro-inflammatory cytokines including interleukins (IL; 1β, IL1RA, IL6, IL10, IL12), interferons (IFN; alpha and gamma) and tumour necrosis factor alpha (TNF-alpha). The production of immune mediators induced by imiquimod stimulate a T-helper 1 immune response, resulting in antitumour activity.[40] Additionally, TLR7/8 independent mechanisms of imiquimod in BCC have been described, involving direct pro-apoptotic effects via adenosine receptor (ADORA) signalling and caspase activation.[38]

Imiquimod is commonly prescribed in superficial BCC and in a large randomized controlled trial (RCT) the probability of tumour-free survival at 5 years was 80.5% (95% CI: 74.0-85.6) for patients treated with imiquimod.[41] One RCT investigated the efficacy of imiquimod (12 weeks) compared to surgical excision in superficial and nodular BCC. Complete clearance rates after 3 years of follow-up were 84% for imiquimod and 98% for surgical excision (relative risk 0.84, 98% CI 0.78-0.91, p<0.0001).[42,43] Sinx et al. performed an RCT comparing surgery with imiquimod (6 weeks) preceded by superficial curettage in nodular BCC. This showed that after 1 year, 86.3% of BCCs had complete clearance in the imiquimod group.[44] Currently it is unknown why approximately 15-20% of BCCs do not respond to imiquimod treatment.

Imiquimod is available in different concentrations, such as 1%, 2.5%, 3.75% and 5%. The European Agency of Medicine (EMA) approved imiquimod 5% cream (Aldara®) for the treatment of small superficial BCC (once daily for five days per week, for six consecutive weeks). Side effects of imiquimod 5% cream mainly consist of local skin reactions such as erythema, scaling, itching, burning, pain, irritation, erosion and ulceration.[45] Some patients experience flu-like symptoms, such as fever, during treatment with imiquimod 5% cream. Side effects do not usually require treatment and resolve after the treatment with imiquimod 5% cream has been discontinued.

Radiotherapy

Ionizing radiation has the ability to directly and indirectly damage DNA.[46] There is no international consensus on the optimal dose and fractionation schedules of radiotherapy. In low- and high-risk primary BCCs of the face, recurrence rates of 5.2% to 6.4% have been reported three and four years post-treatment respectively.[47] A retrospective cohort study of all BCCs treated with radiotherapy between 2001-2006 found efficacy rates of 97.6% and 96.9% three years post-treatment, depending on the fractionation schedule.[48] Although radiotherapy has a high efficacy rate, it is not commonly used in small BCCs, because of its disadvantages. First, patients need to visit the hospital multiple times per week, for several weeks. Second, there is no direct histologic confirmation of treatment radicality. And third, in the case of a recurrence, the BCC cannot be treated with radiotherapy again, and radiation-induced tissue alterations hamper healing in case of surgery after radiotherapy. In general, side effects of radiotherapy are pain/discomfort, acute radiation dermatitis, dyspigmentation, telangiectasia and necrosis.[47, 49] Additional side effects can be necrosis of cartilage and a large, open defect. Despite these side effects, radiotherapy should be considered for aBCC if surgery is not feasible due to functional or mutilating consequences, or in patients who decline surgery.[50] Furthermore, radiotherapy can be used as a palliative treatment in patients with aBCC and mBCC. Radiotherapy is contraindicated in patients with BCNS as they have an increased risk of developing multiple BCCs within the area of radiation, as a result of the direct and indirect DNA damage.[51]

Chemotherapy

Treatment of mBCC with conventional chemotherapy has been addressed in a few case reports and case series.[21] Chemotherapy regimens that have been used are mostly platinum-based, and include cisplatin, bleomycin and carboplatin.[23] Side effects are numerous and include nephrotoxicity, myelosupression, neurotoxicity, anaphylaxis, cytopenias, hepatotoxicity, ototoxicity, cardiotoxicity, nausea and vomiting, diarrhoea, mucositis, stomatitis, pain, alopecia, anorexia, cachexia and asthenia.[52] Unfortunately, reported response rates are low and the duration of response is short.[23]

Hedgehog pathway inhibitors

In 2013, the first hedgehog pathway inhibitor (HPI), vismodegib, was approved by the European Medicines Agency (EMA) for the treatment of aBCC and mBCC. Vismodegib and sonidegib are oral HPIs specifically intervening in the sonic hedgehog signalling pathway, by inhibiting the oncogenic protein SMO. According to the Dutch guidelines, "HPIs should be considered for treatment in adult patients

with aBCC or mBCC, where it is estimated that conventional treatments, such as surgery and radiotherapy, are insufficiently effective or encounter objections."[50] Similar statements are also incorporated in international guidelines.[13,18] Sonidegib 200mg/day has been approved for the treatment of laBCC and vismodegib 150mg/day for both laBCC and mBCC by the EMA and United States Food and Drug Administration (FDA). The efficacy has been determined in two, industry-driven trials for vismodegib and in one industry-driven trial for sonidegib.[53-55] The investigator-assessed overall response rate (ORR) of vismodegib in laBCC was 60.3% (95% CI: 47.2-71.7) after 39 months in the first trial and 68.5% (95% CI: 65.7-71.3) after 19 months in the second trial.[56, 57] In sonidegib the central review objective response rate was 56.1% after 42 months.[58] In mBCC the ORR of vismodegib ranged from 36.9-48.5% after 19 and 39 months respectively.[56, 57] The development of resistance to SMO inhibitors occurs in approximately 20% of aBCCs.[59] SMO variants impair binding of vismodegib to the SMO protein and are therefore responsible for development of resistance.[60, 61] SMO variants can be present in the tumour before therapy either as activating SMO variants driving tumour growth or can clonally expand during treatment.[60] Both SMO inhibitors have many side effects in almost all patients including muscle spasms, dysgeusia, weight loss, alopecia and fatigue.[53-55] Sonidegib might have less side effects compared to vismodegib, since it is more lipophilic and has a higher volume distribution.[62] However, no head-to-head trials comparing both HPIs have been performed.[62] Real-life, clinical data for the treatment of aBCC with HPIs in large populations is lacking.

Neoadjuvant treatment with a HPI, in order to shrink the tumour, has been investigated in three prospective open-label trials.[63-65] An open-label, single arm study in 11 patients with a BCC larger than 5mm investigated the change in surgical defect area from pretreatment to posttreatment. Vismodegib treatment reduced the surgical area by 27% (95% CI: -45.7 to -7.9) after a mean treatment duration of 4 months (±2).[63] After a mean follow-up of 11.5 months (range 4-12), one BCC recurred 17 months after Mohs micrographic surgery.[63] Another open-label, noncomparative study in 55 patients with laBCC of the face investigated the percentage of patients with tumour downstaging following surgical resection after neoadjuvant vismodegib.[64] After a mean treatment duration of 6.0 months (±2.3), treatment downstaging was seen in 80% (44/55) of patients (95% CI: 67-90).[64] After three years of follow-up, 36% (16/44) of patients had a known recurrence (95% CI: 22-51).[64] The third open-label trial was a 3-cohort trial that investigated the rate of complete histological clearance (CHC) of operable BCCs in the excised target site in three cohorts.[65] In cohort 1 (n=24) this was done directly after 12 weeks of

daily vismodegib treatment, in cohort 2 (n=25) this was done 24 weeks after 12 weeks of daily vismodegib treatment and in cohort 3 (n=24) this was done directly after intermittent vismodegib treatment (16 weeks of vismodegib treatment with a treatment stop of 4 weeks after the first 8 weeks).[65] CHC was seen in 42% of patients of cohort 1, 16% of patients in cohort 2 and 44% of patients in cohort 3.[65] As one trial showed, neoadjuvant vismodegib might lead to down staging of the surgical procedure.[64] There are two major issues with this strategy. First, HPIs can cause areas of growth and remission at the same time, leading to unreliable histological resection margins.[66] Second, tumour cells might remain present and induce new tumour cells after treatment discontinuation.[60,67] Therefore, in order to achieve a histological, tumour-free surgical margin, the initially affected area should be completely, surgically removed. This means that there would be no benefit in reducing the tumour size with HPIs before surgical excision of the BCC.

Off-label maintenance treatment with HPIs for multiple BCCs has also been investigated in patients with BCNS and HF-BCC.[32,68] Although response rates are high, with multiple lesions going into regression during therapy (Figure 4), oral HPIs are not suitable for long-term use, as they are accompanied by many side effects.[32,68] BCCs of patients with BCNS will re-occur at the exact same location after discontinuing treatment with oral HPIs, even if histological clearance was reached.[69] Physicians adjust dosing regimens for patients with multiple BCCs to reduce toxicity and enable long-term treatment. One RCT provided data on efficacy and safety of two different vismodegib regimens that alternated several weeks of daily vismodegib treatment with several weeks of placebo treatment.[32] Both dosing regimens showed a reduction of mean number of BCCs at week 73 compared to baseline, but long-term effects of treatment regimens are unclear. Recently, several topical HPIs, such as itraconazole gel, patidegib gel and LDE225 cream, have been developed for the treatment of multiple BCCs. The main advantage of these topical HPIs would be a lower toxicity when compared to oral HPIs while maintaining acceptable BCC clearance rates. Results of RCTs investigating these topical HPIs have to be awaited.

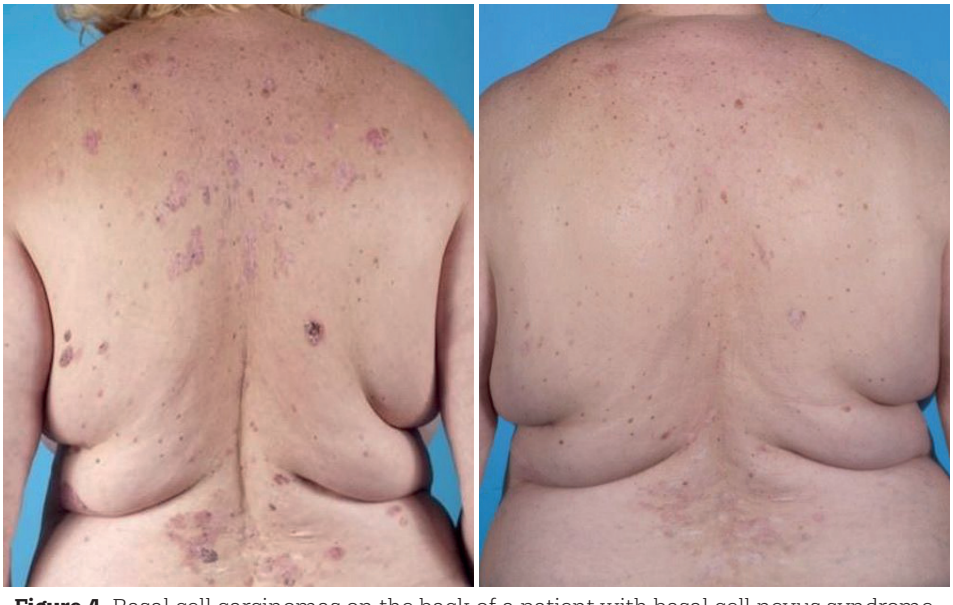

Figure 4. Basal cell carcinomas on the back of a patient with basal cell nevus syndrome, before (left) and after treatment with vismodegib (right).

AIMS AND OUTLINE OF THIS THESIS

The general aim of this thesis is to evaluate and optimize treatment and care for patients with sporadic BCC, aBCC, BCNS and HF-BCC. Therefore, the content of this thesis is subdivided into three parts: sporadic BCC, aBCC and BCNS/HF-BCC.

Chapter 2 – SPORADIC BASAL CELL CARCINOMA

The aim of chapter 2 is to evaluate the effectiveness of imiquimod 5% cream in nodular BCC and to identify clinical and histological prognostic factors that are associated with risk of treatment failure in both superficial and nodular BCC. *In chapter 2.1* a prospective non-inferiority, randomized controlled trial has been performed to compare the effectiveness of imiquimod 5% cream to surgical excision 5 years post-treatment in nodular BCC. *In chapter 2.2* clinical and histological prognostic factors associated with the risk of treatment failure after imiquimod treatment in superficial and nodular BCC are identified.

Chapter 3 – ADVANCED BASAL CELL CARCINOMA

The aim of chapter 3 is to evaluate the effectiveness of new diagnostic and therapeutic modalities for aBCC. *In chapter 3.1* we performed a retrospective cohort study to evaluate the effectiveness of vismodegib for the treatment of aBCC and BCNS patients in the Netherlands. *In chapter 3.2* we performed molecular analysis of several primary BCCs and their metastases in order to identify a clonal relationship between BCCs and their metastases and to explore which hedgehog-pathway related mutations are involved in mBCC. *In chapter 3.3* the effectiveness of PD-1 inhibitors in a case series of patients with progressive disease, after vismodegib treatment, is determined.

Chapter 4 – BASAL CELL NEVUS SYNDROME AND HIGH FREQUENCY BASAL CELL CARCINOMA

The aim of chapter 4 was to provide up-to-date evidence for diagnosis, surveillance and treatment of symptoms of patients with BCNS. *In chapter 4.1* we developed a multidisciplinary guideline using the Appraisal of Guidelines and Evaluation II, and the Grading of Recommendations, Assessment, Development and Evaluation instruments. *In chapter 4.2* a systematic review on efficacy, safety, dosing regimens, tumour resistance and reoccurrence, and quality of life concerning treatment with hedgehog pathway inhibitors, in patients with BCNS and HF-BCC, is provided. *Chapter 4.3* describes a case series of BCCs in children and adolescents with BCNS, treated with imiquimod 5% cream preceded by superficial curettage, to gain more information about the effectiveness of this treatment in this specific

population. *In chapter 4.4* we investigate whether the extracutaneous tumours of four patients with BCNS are caused by a second hit in either *PTCH1* or *SUFU*. *In chapter 4.5* the prevalence of medulloblastoma in the Dutch *PTCH1* mutation cohort is determined.

Chapter 5, 6 and 7 – General discussion and summary, Dutch summary and impact paragraph

Chapter 5 provides the general discussion and summary of this thesis. *Chapter 6* is a Dutch summary of this thesis and in *chapter 7* the impact of this thesis is discussed.

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Chapter 2

Sporadic basal cell carcinoma

Chapter 2.1

Superficial curettage followed by imiquimod 5% cream versus surgery for nodular basal cell carcinoma: a randomized, controlled trial with 5 years follow-up

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Submitted for publication

Chapter 2.2

Prognostic factors for treatment failure of imiquimod treatment in basal cell carcinoma – an observational study

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LETTER TO THE EDITOR

Imiquimod 5% cream is the most effective non-invasive treatment for superficial and nodular basal cell carcinoma (sBCC and nBCC). In two randomized controlled trials (RCTs), including patients with low risk sBCC and nBCC, treatment with imiquimod 5% cream for 6-12 weeks resulted in a probability of tumour-free survival around 80% after 5 years of follow-up.[1, 2] Little is known about factors that may influence the response to imiquimod treatment. Previous studies have shown that a less severe skin reaction and male sex are associated with treatment failure to imiquimod.[3-5] In the current study we aimed to confirm previous findings and to identify new histologic factors associated with risk of failure after imiquimod treatment.

Data were derived from 189 sBCC and 73 nBCC patients who participated in two RCTs on the efficacy of imiquimod.[6, 7] In both trials, imiquimod was applied once daily, five days a week, for 6 weeks. Treatment failure was evaluated by an investigator at 12-month post treatment and had to be histologically confirmed. Candidate prognostic factors were categorized into three groups: 1) patient and tumour characteristics, 2) factors related to treatment, and 3) histological characteristics. To evaluate the association between prognostic factors and 1-year treatment failure, odds ratios (OR) with 95% confidence intervals (95% CI) were calculated. Mixed-effects logistic regression analyses were used to account for the pooling of data from two studies. Multivariable models were used for mutual adjustment of factors in the factors in the first two groups, but for the large group of histologic factors only univariable models were used.

In total, 262 patients were included, 189 patients from the sBCC trial[7] and 73 patients from the nBCC trial.[6] Histologic characteristics were available in a subgroup of 136 patients (Table 1). Treatment failure ≤1 year after treatment occurred in 41/262 (15.6%) BCCs. The risk of treatment failure was significantly higher for males (OR 2.77, p=0.009) compared to females and for tumours on the lower extremities compared to tumours in the head and neck area (OR 3.02, p=0.044). Compared to patients with severe skin reaction, the OR with mild/moderate skin reaction was 4.75 (p=0.002) and increased to 8.28 (p=0.002) for patients without any skin reaction. The odds ratio for tumour thickness of 0.94 per 0.1mm increase in thickness was not significant (p=0.881) (Table 2). Four nBCCs invaded beyond the dermis and reached into the subcutis, all achieved treatment success (data not shown). Factors that may affect permeability of

the skin (hyperplastic epidermal aspect and parakeratosis) showed an OR of 2.23 (p=0.501) and 2.21 (p=0.160) for treatment failure, respectively. The odds ratio for presence of ulceration was 2.70 (p=0.078).

In this study, we confirmed that male sex, location on the lower extremities and a less severe/absent skin reaction were significantly associated with an increased risk of treatment failure following imiquimod cream in nBCC and sBCC. The results indicate that risk of treatment failure is not increased in thick tumours and tumours with a high amount of tumour infiltration. Presence of ulceration, parakeratosis and a hyperplastic epidermal aspect were associated with slightly increased odds ratios. These results might suggest that less permeability of the skin could play a role in the risk of treatment failure as well as the presence of ulceration, a well-known risk factor in melanoma. However, due to small numbers of patients with treatment failure the power of this study to detect small but relevant associations was small, and results need to be validated in larger datasets.

Table 1. Percentage with treatment failure according to level of prognostic factors.

	Number of patients	**Treatment failure, n (%)**
Clinical patient and tumour characteristics	**n=262**	
Age (years), median (IQR)	63.5 (55-70)	
<64 years	132	15 (11.4%)
≥64 years	130	26 (20.0%)
Female, n (%)	131	13 (9.9%)
Male, n (%)	131	28 (21.4%)
Location		
Head and neck, n (%)	45	8 (17.8%)
Lower extremities, n (%)	39	14 (35.9%)
Upper extremities, n (%)	35	3 (8.6%)
Trunk, n (%)	143	16 (11.2%)
Largest tumour diameter (mm), median (IQR)*	9.0 (7.0-13.0)	
≤9.0mm	139	25 (18.0%)
>9.0mm	122	16 (13.1%)
Treatment related characteristics>	**n=262**	
Skin reaction		
None	21	7 (33.3%)
Mild/moderate	135	27 (20.0%)
Severe	103	6 (5.8%)
Missing	3	1 (33.3%)

Table 1. Continued.

	Number of patients	Treatment failure, n (%)
Compliance		
30 days	183	32 (17.5%)
<30 days	65	7 (10.8%)
Missing	14	2 (14.3%)
Histologic tumour characteristics	**n=136**	
Tumour thickness (mm), median (IQR)*	0.50 (0.31-1.00)	
≤0.5mm	69	8 (11.6%)
>0.5mm	65	9 (13.8%)
Epidermal aspect		
Normal	100	13 (13.0%)
Atrophic	29	2 (6.9%)
Hyperplastic	4	1 (25.0%)
Missing†	3	1 (33.3%)
Ulceration		
Absent	110	11 (10.0%)
Present	26	6 (23.1%)
Parakeratosis		
Absent	62	5 (8.1%)
Present	74	12 (16.2%)
Erosion		
Absent	77	7 (9.1%)
Present	59	10 (16.9%)
Infiltrate		
None	14	2 (14.3%)
Mild	58	9 (15.5%)
Moderate	39	3 (7.7%)
Severe	25	3 (12.0%)
Amount of plasma cells		
Not pronounced	112	13 (11.6%)
Pronounced	10	2 (20.0%)
Missing^	14	2 (14.3%)
Amount of blood vessels		
Not pronounced	73	6 (8.2%)
Pronounced	63	11 (17.5%)

Table 1. Continued.

	Number of patients	Treatment failure, n (%)
Solar elastosis		
None	12	3 (25.0%)
Mild	53	6 (11.3%)
Severe	71	8 (11.3%)

BCC, basal cell carcinoma; SD, standard deviation; IQR, interquartile range.
*Information on tumour diameter was missing in one patient.
>Based on patient diaries.
+ Measured from the stratum granulosum, or base of overlying ulceration, to the deepest tumour nest with a 0.01-mm precise ocular micrometre.
‡Epidermal aspect could not be assessed in three BCCs due to coarse ulcerations (n=2) and poor quality of the biopsy (n=1).
^Plasma cells could only be assessed in biopsies where inflammation was present.

Table 2. Odds ratio with 95% confidence interval for treatment failure according to patient, tumour, treatment and histological characteristics. Mixed logistic effects models were used.

		OR	95% CI	P-value
Multivariable model of patient and tumour characteristics	**Patient and tumour characteristics**			
	Sex			
	Female	1.00		
	Male	2.77	1.29-5.94	*0.009*
	Age per year*	1.02	0.99-1.06	0.205
	Largest tumour diameter (mm)**	1.02	0.96-1.07	0.565
	Location			
	Head and neck	1.00		
	Upper extremities	0.49	0.12-2.09	0.337
	Trunk	0.61	0.23-1.64	0.327
	Lower extremities	3.02	1.03-8.82	*0.044*
Multivariable model of treatment characteristics	**Treatment characteristics**			
	Skin reaction			
	Severe	1.00		
	Mild/moderate	4.82	1.76-13.21	*0.002*
	None	9.10	2.38-34.82	*0.001*
	Compliance per day increase***	1.00	0.89-1.13	0.968
Separate univariable models of all histological factors	**Histologic characteristics**			
	Tumour thickness (mm)******	0.94	0.42-2.13	0.881
	Epidermal aspect			
	Normal	1.00		

Table 2. Continued.

		OR	95% CI	P-value
	Atrophic	0.50	0.11-2.34	0.375
	Hyperplastic	2.23	0.22-23.09	0.501
	Parakeratosis			
	Absent	1.00		
	Present	2.21	0.74-6.65	0.160
	Ulceration			
	Absent	1.00		
	Present	2.70	0.89-8.15	0.078
	Erosion			
	Absent	1.00		
	Present	2.04	0.73-5.73	0.176
	Infiltrate			
	None	1.00		
	Mild	1.10	0.21-5.78	0.909
	Moderate	0.50	0.07-3.36	0.476
	Severe	0.82	0.12-5.59	0.838
	Amount of plasma cells			
	Not pronounced	1.00		
	Pronounced	1.90	0.36-9.95	0.445
	Amount of blood vessels			
	Not pronounced	1.00		
	Pronounced	2.36	0.82-6.81	0.112
	Solar elastosis			
	None	1.00		
	Mild	0.38	0.08-1.82	0.227
	Severe	0.38	0.09-1.71	0.207

OR, odds ratio; 95% CI, 95% confidence interval. OR >1 and OR<1 indicate increased and decreased risk of treatment failure respectively, where categories with OR=1 were used as the reference category.

*The odds ratio for age represents increase in risk per year.

**The odds ratio for largest tumour diameter represents increase in risk per increase in mm.

***The odds ratio for compliance represents increase in risk per day increase of compliance.

****The odds ratio for tumour thickness represents increase in risk per 0.1mm increase.

P<0.05 is considered statistically significant. Italic values indicate statistically significant P-values (P<0.05).

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Chapter 3

Advanced basal cell carcinoma

Chapter 3.1

Eight years of experience with vismodegib for advanced and multiple basal cell carcinoma patients in the Netherlands: a retrospective cohort study

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ABSTRACT

Background: Vismodegib has been used for the treatment of locally advanced basal cell carcinoma (laBCC) and metastatic BCC (mBCC) since 2011. Most efficacy and safety data is provided by clinical trials. This study evaluates the effectiveness of vismodegib for the treatment of laBCC, mBCC and basal cell nevus syndrome (BCNS) patients, and the tumour characteristics associated with a higher probability of achieving a complete response in the Netherlands.

Methods: A retrospective cohort study that included all patients ≥18 years with histologically proven basal cell carcinoma that received ≥1 dose of vismodegib between July 2011 and September 2019 in the Netherlands.

Results: In total 48 laBCC, 11 mBCC, and 19 BCNS patients were included. Median progression-free survival was 10.3 months (95% confidence interval (CI), 7.5-22.6) for laBCC, 11.7 (95% CI, 5.2-17.5) for mBCC, and 19.1 (95% CI, 7.4-20.2) for BCNS. Larger laBCCs were associated with a lower probability of complete response (HR 0.77 per increase in cm, p=0.02). Of all BCNS patients, 63% received ≥2 treatment sequences with vismodegib; all achieved partial responses.

Conclusions: Half of the aBCC patients progress within 1 year after the start of vismodegib treatment. More research is needed to investigate other treatment strategies after vismodegib progression and to evaluate long term effects of repetitive vismodegib treatment.

INTRODUCTION

Basal cell carcinoma (BCC) is the most common skin cancer worldwide.[1] Therapeutic options vary from non-invasive therapies to local radiotherapy and surgery.[2] However, if a BCC stays untreated, it can develop into an advanced BCC (aBCC), comprising locally advanced BCC (laBCC) and metastatic BCC (mBCC). Surgery or radiotherapy is not always an option for the treatment of aBCCs.

In 2012, the Phase 2 ERIVANCE BCC trial investigated the efficacy and safety of vismodegib, the first-in-class molecule for targeted therapy for aBCCs that are not suitable for surgery and/or radiotherapy.[3] Vismodegib inhibits the oncogenic protein smoothened (SMO), a downstream signal of the hedgehog pathway that plays an important role in the pathogenesis of BCC. Mutations in the hedgehog pathway are found in the majority of BCCs.[4] An efficacy analysis of 96 patients in the ERIVANCE trial showed a median investigator-assessed progression-free survival (PFS) of 9.3 months (95% confidence interval (CI), 7.4–16.6) for those with mBCC and 12.9 months (95% CI, 10.2–28.0) for those with laBCC.[3] Based on the results of this trial and under priority review as a first-in-class molecule, targeted therapy with vismodegib was registered for the treatment of laBCC and mBCC in the Netherlands.[5] Another large Phase 2 trial assessed the safety of vismodegib (SafeTy Events in VIsmodEgib, STEVIE). The efficacy analysis of that trial included 1192 patients and showed a median investigator-assessed PFS of 13.1 months (95% CI, 12.0–17.7) for those with mBCC and 23.2 months (95% CI, 21.4–26.0) for those with laBCC.[6] Of all patients, 98% experienced at least one adverse event, with the most frequently observed adverse events being muscle spasms, alopecia, dysgeusia, decreased appetite, decreased weight, and asthenia.[6] In both the ERIVANCE BCC and STEVIE trials, only dose interruption of 4-8 weeks was accepted to recover from toxic effects and different treatment schedules were not allowed.[3, 7]

Some patients need long-term treatment with vismodegib and an intermittent treatment schedule could possibly optimise the balance between benefit and side effects. This seems especially relevant in patients with basal cell nevus syndrome (BCNS), as BCCs will keep on developing in these patients during their entire lives. Therefore, the multiple basal cell carcinomas (MIKIE) trial compared two different intermittent dosing regimens for vismodegib in patients with either BCNS or high-frequency BCC (HF-BCC) patients.[8] Both schedules showed similar response rates and adverse events rates; however, intermittent dosing was associated with fewer grade ≥3 treatment-emergent adverse events (TEAEs) compared to

the STEVIE trial.[8] The median durations of treatments in the MIKIE trial was 71.4 and 68.4 weeks depending on the dosing schedule, compared to 36.4 weeks for laBCC patients and 52.0 weeks for mBCC patients treated with the regular dosing schedule of 150mg daily in the STEVIE trial.[7, 8] Unfortunately, extensive information about the indication, use, safety and (predictors of) effectiveness of vismodegib is still sparse.[9]

This study presents effectiveness, safety, and the treatment course of all patients with aBCC or multiple BCCs who were treated with vismodegib in the Netherlands between July 2011 to September 2019.

METHODS

Study design and patients

This retrospective, multicentre, longitudinal cohort study included all patients treated with vismodegib for aBCC or multiple BCCs in the Netherlands from July 2011 till 9 September 2019. In the Netherlands, vismodegib is only prescribed in seven academic medical hospitals (verified by contacting insurance companies), and all patients were gathered from these centres. All patients were aged ≥18 years, had a histologically proven BCC and received at least one dose of vismodegib. All indications for vismodegib treatment in BCC were included; laBCC, mBCC, multiple BCCs in BCNS and in non-BCNS patients. Vismodegib was either started in a clinical trial setting (STEVIE, n=21 times, or MIKIE, n=8 times) or in daily practice (n=92 times).[7, 8] A new treatment sequence was defined as restarting vismodegib after a break of at least 8 weeks. Under supervision of a dermato-oncologist (K.M.), two investigators, B.J.A.V. and R.-J.A., extracted data from the electronic patient files and entered it into a standardized Castor database. This study was approved with waiver of informed consent by the Medical Ethics Committee of all participating centres.

Outcome measures

For the analysis on the effectiveness of vismodegib, the primary endpoint was the median PFS after the start of the first vismodegib prescription. Secondary endpoints were the difference in median PFS between the clinical trial and daily practice patients, probability of response (partial and complete) and PFS at 1, 3, 6, and 12 months, median duration of (complete) response, and median time to all response endpoints (the period after which 50% of patients had reached the endpoint of interest). Response and progression were measured according to investigator-assessed clinical response as noted in the patient file. For the indication of multiple BCCs in (non-)BCNS patients, progression was defined as the development of new or recurrent BCCs. An additional analysis was performed to evaluate which patient and tumour characteristics were associated with an increased probability for achieving a complete response in the first treatment sequence. For this purpose, data were recorded on the duration of tumour presence, tumour size, histologic subtype, bone invasion, and previous therapy. Tumour measurement information was gathered from patient files, clinical photographs of the tumour and/or computed tomography or magnetic resonance imaging.

Safety analysis included frequency, severity (measured according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0), and reversibility of TEAEs.

Data analysis and statistical method

Categorical variables were presented as percentages with absolute numbers and continuous variables as median with range, as appropriate.

Time-to-event (Kaplan-Meier) analyses were used to estimate the cumulative probability of an endpoint at pre-specified follow-up periods as well as median time to endpoints. The observation period of patients started at the date of first treatment with vismodegib and ended at the date of first documentation of response or progression or at the date of death, depending on the studied outcome. A log-rank test was used to calculate differences between clinical trial and daily practice patients. For the median duration of response, the observation period started at the date of first documentation of response and ended at the date of first documentation of progression. For the patients who had not experienced the event of interest, observations were censored at the date of the last tumour assessment. Effectiveness analyses were performed on first treatment sequence data.

To evaluate characteristics associated with increased probability for achieving complete response in the first sequence, univariable Cox regression analyses were performed and hazard ratios with 95% confidence intervals and *P* values were calculated. The variables with a significant or strong association (defined as at least halving of doubling of the hazard ratio) were entered into a multivariable Cox regression analysis to evaluate the independent effect of these variables. *P* values <0.05 were considered to indicate the statistical significance. Statistical analyses were performed with IBM SPSS Statistics version 25 and STATA version 13.0.

RESULTS

Between July 2011 and September 2019, 80 patients were treated with vismodegib in seven centres in the Netherlands. Patient, tumour, and treatment characteristics can be found in Table 1. Fifty-one patients were treated with only one sequence, 21 with only two, 3 with three, and 5 with four. Swimmer lane plots per treatment indication can be seen in Figure 1 and Kaplan-Meier curves from time-to-event analyses in Figure 2 and Table 2.

LaBCC

A total of 48 patients received vismodegib for a laBCC, five of them had BCNS. Tumours were located in the head and neck region in 83% (n=40), on the trunk in 15% (n=7), and on the extremities in 2% (n=1). Median self-reported tumour presence was 6 years (range, 0.3-20 years) and size was 5.0 cm (range, 1.0-30.0 cm), respectively. Thirty-seven tumours had an infiltrative component in the histologic sample (77%), nine were nodular (19%), and in two tumours (4%) this information was missing. Bone invasion was present in 16 of 48 tumours (33%). Of all 48 patients, 28 (58%) received at least one previous treatment for their tumour, mostly surgery or radiotherapy. At the start of vismodegib treatment, the median age of the patients was 75.5 years (range, 36-98 years).

Effectiveness

Four patients received vismodegib intentionally as neoadjuvant therapy and were therefore excluded leaving 44 patients for analysis. Median PFS was 10.3 months (95% CI, 7.5-22.6) for all 44 laBCC patients. There was no statistically significant difference in median PFS between daily practice and STEVIE trial patients (10.2 months (95% CI, 5.6-22.6) and 13.6 months (95% CI, 6.1-26.6), respectively (p=0.39)). At 3 months after the start of vismodegib, the probability of partial response was 94.6% (95% CI, 84.4-99.0) and probability of complete response after 6 months of treatment was 33.9% (95% CI, 20.6-52.5), with a median duration of complete response of 10.3 months (95% CI, 4.5-22.1). The HRs from the multivariable analysis showed a significantly decreased probability of achieving a complete response in larger tumours (HR 0.77 per increase in cm, p=0.02), whereas patients who participated in the STEVIE trial had a significantly increased probability of achieving a complete response compared to daily practice patients (HR 10.08, $p<0.01$) (Table 3). The main reasons for treatment discontinuation were toxicity (n=22) and tumour progression (n=15). Retreatment with vismodegib (n=12) led to a response in eight patients, six of them eventually developed progressive disease again. Six patients died due to the laBCC.

Table 1. Patient, tumour and treatment characteristics.

Sex
Men, n (%)
Women, n (%)
Age at start, median (range) years
<65 years
≥65 years
Caucasian, n (%)
Self-reported presence of BCC
Median (range), years
Unknown, n (%)
Basal cell nevus syndrome
Yes, n (%)
No, (n%)
Previous treatment*
None
Surgery
Radiotherapy
Cryotherapy
Curettage
Photodynamic therapy
5-fluorouracil cream
Imiquimod cream
Laser (type unknown)
Other
Site laBCC
Head and neck
Trunk
Extremities
Multiple sites
Size laBCC
Median (range), cm
Unknown, n (%)
Subtype laBCC
Infiltrative
Nodular
Unknown

	laBCC n=48	mBCC n=11	BCNS n=19	Multiple non-BCNS BCCs n=5
	24 (50%)	6 (55%)	12 (63%)	3 (60%)
	24 (50%)	5 (45%)	7 (37%)	2 (40%)
	75.5 (36-98)	70 (52-81)	46 (35-71)	77 (44-82)
	11 (23%)	4 (36%)	18 (95%)	1 (20%)
	37 (77%)	7 (64%)	1 (5%)	4 (80%)
	48 (100%)	11 (100%)	19 (100%)	5 (100%)
	6 (0.3-20)	5 (0.3-22)	-	-
	14 (29%)	3 (27%)	-	-
	5 (10%)	0 (0%)	19 (100%)	0 (0%)
	43 (90%)	11 (100%)	0 (0%)	5 (100%)
	20 (42%)	4 (36%)	0 (0%)	2 (40%)
	21 (44%)	6 (55%)	19 (100%)	5 (100%)
	7 (15%)	1 (9%)	1 (5%)	2 (40%)
	2 (4%)	0 (0%)	3 (16%)	2 (40%)
	1 (2%)	0 (0%)	3 (16%)	0 (0%)
	2 (4%)	0 (0%)	4 (21%)	2 (40%)
	2 (4%)	0 (0%)	3 (16%)	1 (20%)
	1 (2%)	0 (0%)	6 (32%)	0 (0%)
	0 (0%)	0 (0%)	2 (11%)	0 (0%)
	2 (4%)	1 (9%)	2 (11%)	0 (0%)
	40 (83%)	5 (46%)	-	-
	7 (15%)	4 (36%)	-	-
	1 (2%)	2 (18%)	-	-
	-	-	19 (100%)	5 (100%)
	5 (1-30)	14.5 (4-22)	-	-
	9 (19%)	5 (45%)	-	-
	37 (77%)	7 (64%)	-	-
	9 (19%)	0 (0%)	-	-
	2 (4%)	4 (36%)	-	-

Table 1. Continued.

Bone invasion laBCC
Present, n (%)
Absent n (%)
Site of metastasis
Regional lymph nodes, n (%)
Distant lymph nodes, n (%)
Lungs, n (%)
Bones
Median duration of first treatment sequence, months (range)
Start dosage
150 mg daily, n (%)
STEVIE, n (%)
MIKIE, n (%)
Short treatment interruptions
Yes, n (%)
No, n (%)
Dosage change
Yes, n (%)
No, n (%)
Sequences^
One, n (%)
Two, n (%)
Three, n (%)
Four, n (%)
Median duration between sequences, months (range)
Clinical review frequency in first sequence
Monthly, n (%)
2-monthly, n (%)
3-monthly, n (%)
Still on treatment
Yes, n (%)
No, n (%)
Stop reason
Tumour progression, n (%)
Toxicity, n (%)
Vismodegib as neoadjuvans, n (%)

	laBCC n=48	mBCC n=11	BCNS n=19	Multiple non-BCNS BCCs n=5
	16 (33%)	6 (55%)	-	-
	32 (67%)	5 (45%)	-	-
	-	3 (27%)	-	-
	-	1 (9%)	-	-
	-	6 (55%)	-	-
	-	2 (18%)	-	-
	6.4 (1.4-38.5)	7.5 (1.6-18.5)	6.6 (1.2-25.7)	14.4 (2.8-16.8)
	33 (69%)	11 (100%)	8 (42%)	2 (40%)
	15 (31%)	0 (0%)	6 (32%)	0 (0%)
	0 (0%)	0 (0%)	5 (26%)	3 (60%)
	6 (12%)	0 (0%)	1 (5%)	0 (0%)
	42 (88%)	11 (100%)	18 (95%)	5 (100%)
	3 (6%)	2 (18%)	1 (5%)	0 (0%)
	45 (94%)	9 (82%)	18 (95%)	5 (100%)
	37 (77%)	9 (82%)	7 (37%)	4 (80%)
	11 (23%)	2 (18%)	5 (26%)	1 (20%)
	0 (0%)	0 (0%)	4 (21%)	0 (0%)
	0 (0%)	0 (0%)	3 (16%)	0 (0%)
	6.0 (2.5-20.7)	6.9 (2.0-11.8)	11.2 (2.2-54.2)	3.0 (-)
	37 (77%)	8 (73%)	19 (100%)	5 (100%)
	9 (19%)	2 (18%)	0 (0%)	0 (0%)
	2 (4%)	1 (9%)	0 (0%)	0 (0%)
	2 (4%)	1 (9%)	2 (11%)	1 (20%)
	46 (96%)	10 (91%)	17 (89%)	4 (80%)
	15 (33%)	6 (60%)	1 (6%)	0 (0%)
	22 (48%)	2 (20%)	13 (76%)	2 (50%)
	4 (9%)	1 (10%)	0 (0%)	0 (0%)

Table 1. Continued.

Patient died, n (%)	
No therapy compliance, n (%)	
Physician fears development of resistance, n (%)	
End of trial, n (%)	
Median duration of follow-up from start vismodegib treatment, months (range)	

laBCC, locally advanced basal cell carcinoma; mBCC, metastatic basal cell carcinoma; BCNS, basal cell nevus syndrome; BCC, basal cell carcinoma.
*Percentages can add up to >100% because a patient can have had various previous treatments.

MBCC

Eleven patients received vismodegib for metastasised BCC; none of them had BCNS (Figure 1B). One patient had been treated for the primary laBCC with vismodegib and surgery 4.6 years before. Primary tumours were located in the head and neck region in 46% of patients (n=5), on the trunk in 36% (n=4) and on the extremities in 18% (n=2). The sites of metastases were: regional lymph nodes 27% (n=3), distant lymph nodes 9% (n=1), lungs 55% (n=6), and bones 18% (n=2). Median self-reported tumour presence was 5 years (range, 0.3-22 years) and size was 14.5 cm in diameter (range, 4.0-22.0 cm). All tumours with known subtype (n=7) were infiltrative. At the start of treatment, bone invasion was present in 55% of the patients (n=6). Of all mBCC patients, four did not receive any previous therapy and six had received previous surgery for the primary BCC (Figure 1b). The median age at the start of treatment was 70 years (range, 52-81 years).

Effectiveness

Of the 11 mBCC patients, one had previously been treated for the mBCC with vismodegib abroad, leaving ten patients for the effectiveness analysis. Median PFS was 11.7 months (95% CI, 5.2-17.5). At 3 months after the start of vismodegib, the probability of partial response was 52.0% (95% CI, 25.5-83.9). The main reason for treatment discontinuation was tumour progression (n=6). Only one patient achieved a complete response, which currently lasts for >2 years without treatment. This patient only had a regional lymph node metastasis and received previous surgical treatment of the primary BCC. After progressive disease, two patients were treated with radiotherapy, one with surgery, two with anti-programme death-1 inhibitors, two are not treated yet, and three patients died.

laBCC n=48	mBCC n=11	BCNS n=19	Multiple non-BCNS BCCs n=5
0 (0%)	1 (10%)	0 (0%)	0 (0%)
2 (4%)	0 (0%)	0 (0%)	0 (0%)
2 (4%)	0 (0%)	0 (0%)~	0 (0%)
1 (2%)	0 (0%)	3 (18%)	2 (50%)
24.6 (1.8-83.4)	15.2 (1.6-40.3)	54.7 (1.8-68.5)	32.4 (2.8-65.8)

^For the specific indication and which was started in the Netherlands.
~Six following sequences were ended because the physician feared development of resistance.

Multiple BCCs in BCNS

Nineteen BCNS patients received vismodegib for multiple BCCs. At the start of vismodegib treatment, median age was 46 years (range, 35-71 years). One patient had previously been treated with vismodegib for this indication abroad and two patients received vismodegib previously for a laBCC, leaving 16 patients for the effectiveness analysis. Median PFS was 19.1 months (95% CI, 7.4-20.2). Numbers were too small to compare effectiveness in clinical trial and daily practice patients.

In one patient, the time of response was unknown. In the remaining 15 patients, the probability of achieving partial response within 3 months after start of vismodegib was 93.3% (95% CI, 74.0-99.6) and probability of complete response after 6 months of treatment was 40.8% (95% CI, 19.3-72.2). The main reason for treatment discontinuation was toxicity (n=13).

Twelve patients (63%) received ≥2 treatment sequences, with a maximum of four sequences (Figure 1c). The median treatment break duration was 11.2 months (range, 2.2-54.2 months). All patients responded to vismodegib in all the following sequences.

Multiple BCCs in non-BCNS patients

Notably, five non-BCNS patients received vismodegib for multiple BCCs: three xeroderma pigmentosum (XP) patients and two HF-BCC patients (Figure 1C). Numbers were too small to perform effectiveness analyses. Reasons for termination of treatment were toxicity (n=2) and end of trial (n=2). One HF-BCC patient has been treated successfully alternating 3 months on and off vismodegib 150mg daily for >3 years.

Figure 1. Swimmer plots of all individual patients per treatment indication.

Time is shown on the horizontal axes in months and individual patients are shown on the vertical axes.

A. Swimmer plot of locally advanced basal cell carcinoma patients. Patients 14, 21, 22 and 23 received vismodegib as neoadjuvant therapy. Patients 8, 20, 28, 31 and 43 are basal cell nevus syndrome patients.

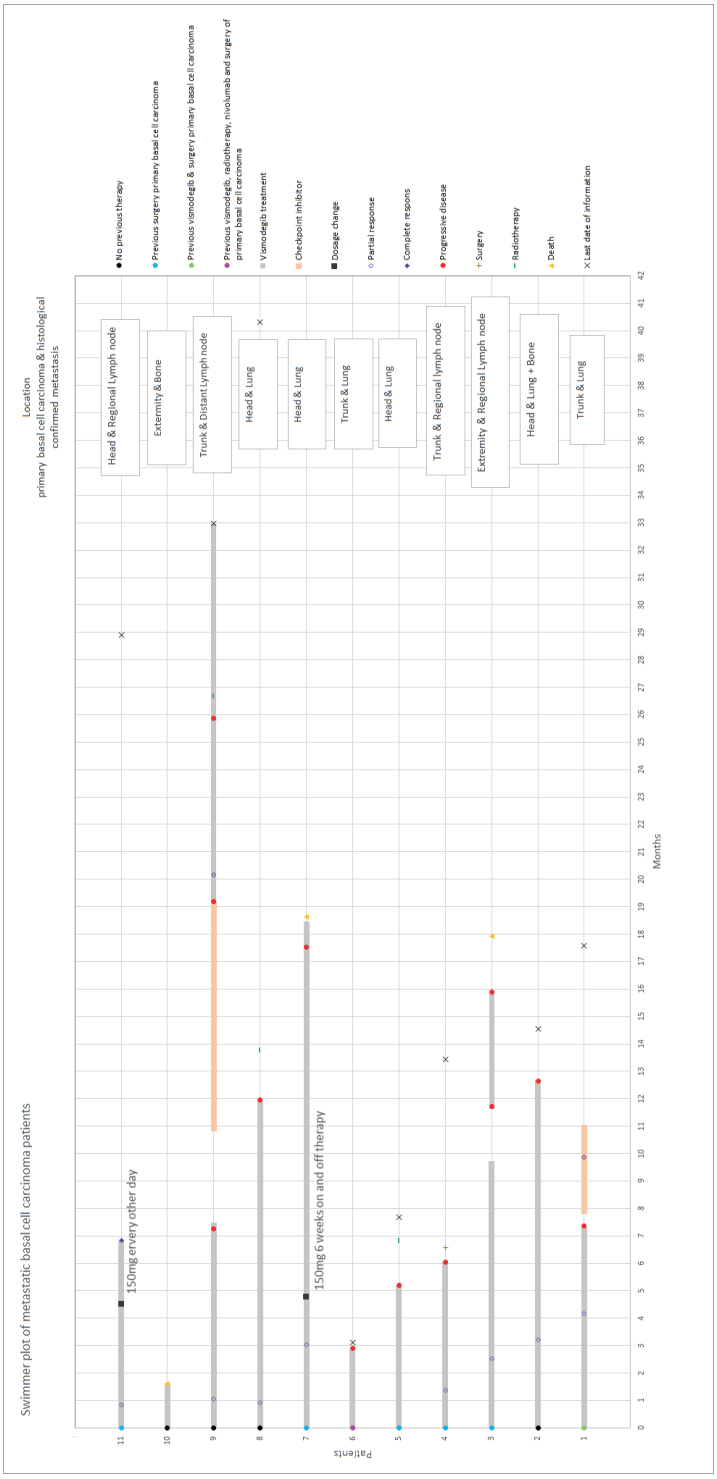

Figure 1. Swimmer plots of all individual patients per treatment indication.
B. Swimmer plot of metastatic basal cell carcinoma patients.

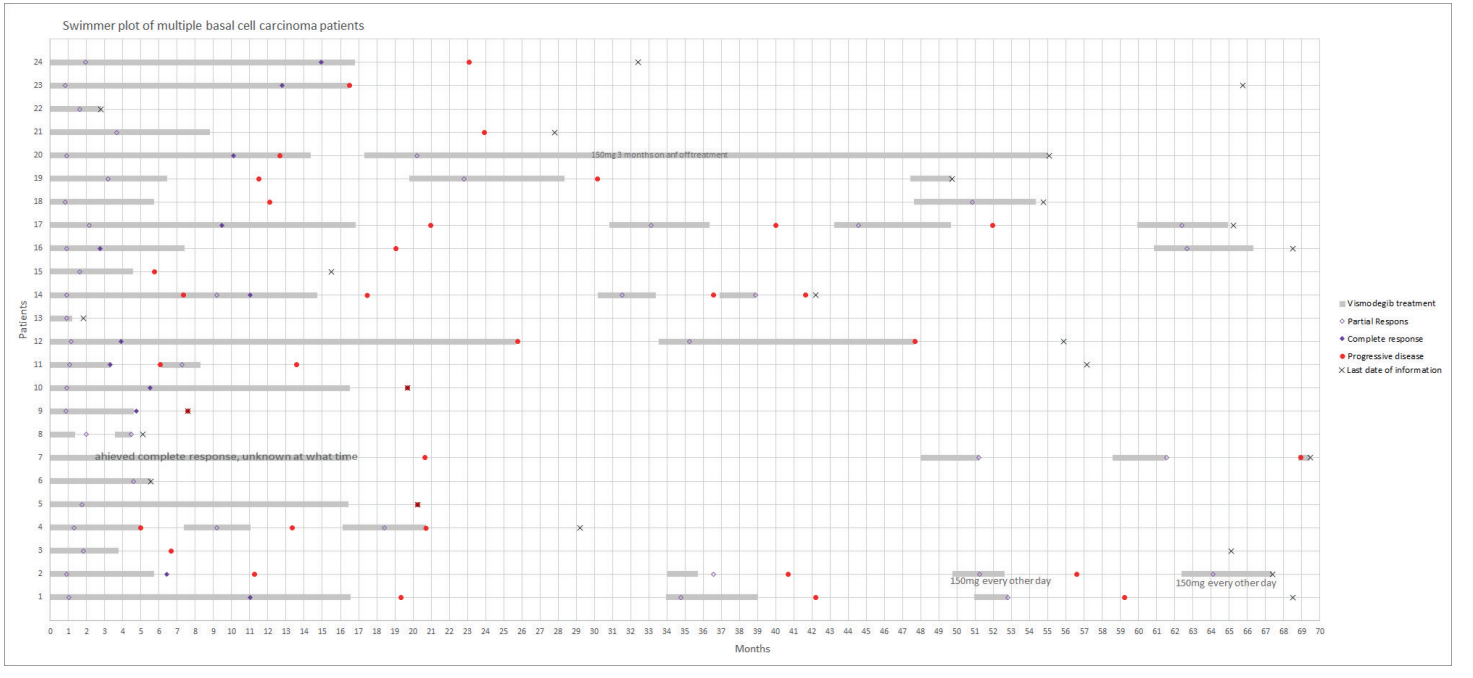

Figure 1. Continued.

C. Swimmer plot of patients with multiple basal cell carcinomas. Patients 20 and 21 have high-frequency basal cell carcinoma; patients 22, 23 and 24 are patients with xeroderma pigmentosum.

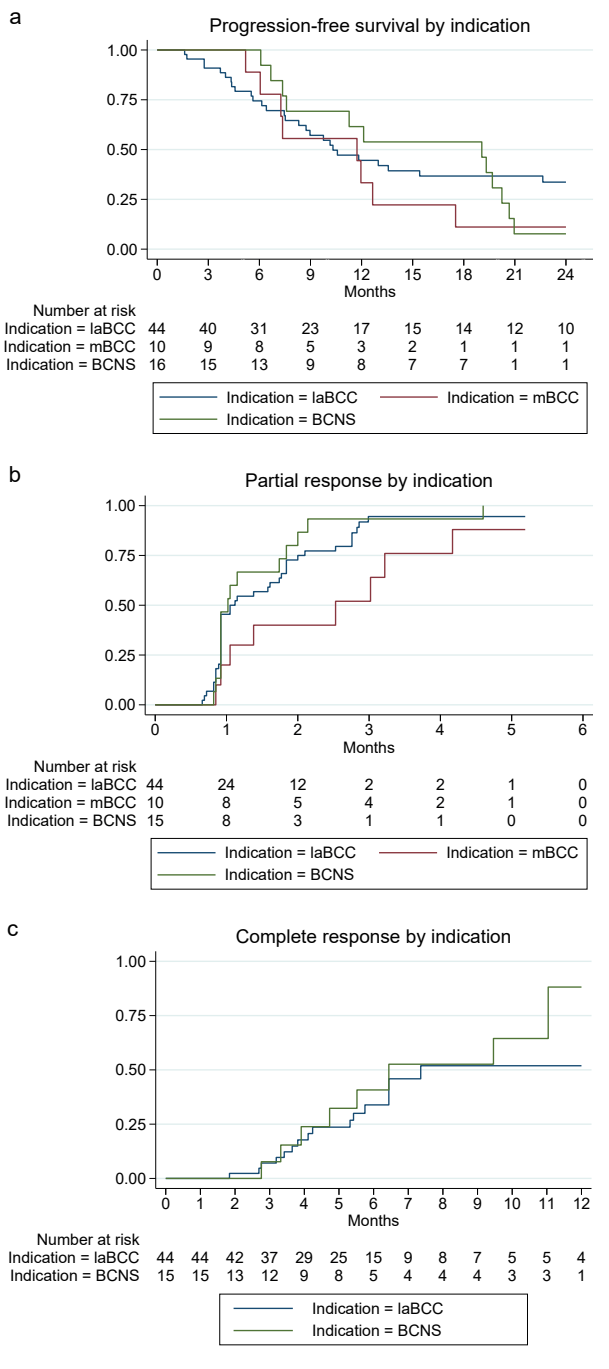

Figure 2. Kaplan-Meier curves of time-to-event analyses per treatment indication. laBCC, locally advanced basal cell carcinoma; mBCC, metastatic basal cell carcinoma; BCNS, basal cell nevus syndrome.

Table 2. Time to event analyses of progression and response endpoints.

Indication/Endpoint	1 month (95% CI)	3 months (95% CI)
laBCC		
PFS overall	100.0 (-)	90.9 (77.6-96.5)
PFS STEVIE	100.0 (-)	93.3 (61.3-99.0)
PFS daily practice	100.0 (-)	89.7 (71.3-96.5)
Partial response	45.5 (32.2-61.2)	94.6 (84.4-99.0)
Complete response	0.0 (-)	7.1 (2.3-20.4)
mBCC		
PFS	100.0 (-)	100.0 (-)
Partial response	20.0 (5.4-59.1)	52.0 (25.5-83.9)
Complete response	NA	NA
BCNS		
PFS	100.0 (-)	100.0 (-)
Partial response	46.7 (25.6-73.7)	93.3 (74.0-99.6)
Complete response	0.0 (-)	7.7 (1.1-43.4)

PFS, progression free survival; 95% CI, 95% confidence interval; NA, not applicable; NR, no more responders; NE, not estimable.
Cumulative probability of PFS, partial response and complete response with 95% CI, median time to endpoint with 95% CI and median duration of any and complete response with 95% CI.

	6 months (95% CI)	12 months (95% CI)	Median time to (95% CI)	Median duration of response (95% CI)*
	74.5 (58.6-85.0)	44.6 (29.1-58.9)	10.3 (7.5-22.6)	NA
	86.7 (56.4-96.5)	60.0 (31.8-79.7)	13.6 (6.1-26.6)	NA
	67.8 (47.1-81.8)	35.4 (17.8-53.6)	10.2 (5.6-22.6)	NA
	NR	NR	1.1 (0.9-1.8)	^9.7 (6.7-19.9)
	33.9 (20.6-52.5)	51.9 (33.2-73.5)	7.4 (5.8-NE)	10.3 (4.5-22.1)
	88.9 (43.3-98.4)	33.3 (7.8-62.3)	11.7 (5.2-17.5)	NA
	NR	NR	2.5 (0.9-4.2)	^9.2 (3.2-14.5)
	NA	NA	NA	NA
	100.0 (-)	61.5 (30.8-81.8)	19.1 (7.4-20.2)	NA
	NR	NR	1.0 (0.9-1.7)	^11.3 (5.0-18.8)
	40.8 (19.3-72.2)	88.2 (59.8-99.3)	6.4 (3.9-11.0)	8.3 (2.8-16.3)

*Analysis based on responders only.

^Median duration of any response.

Table 3. Hazard ratio with 95% confidence interval for complete response in locally advanced basal cell carcinoma associated with patient and tumour characteristics (n=44).

Characteristic	HR with 95% CI Univariable analysis	p-value	HR with 95% CI Multivariable analysis	p-value
Age (per year)*	0.99 (0.96-1.03)	0.85		
Sex				
Male	1.00			
Female	1.78 (0.63-5.07)	0.28		
Tumour size (per cm)**	0.91 (0.79-1.06)	0.24	0.77 (0.62-0.95)	0.02
Tumour location				
Not on the head	1.00	0.86		
On the head	0.90 (0.25-3.18)			
Tumour subtype				
Non-infiltrative	1.00	0.16	1.00	0.06
Infiltrative	0.46 (0.16-1.35)		0.21 (0.04-1.08)	
Bone invasion				
No	1.00	0.67		
Yes	0.78 (0.25-2.46)			
Previous therapy				
No	1.00	0.44	1.00	0.22
Yes	0.44 (0.24-1.86)		0.46 (0.13-1.58)	
Previous radiotherapy				
No	1.00	0.61		
Yes	1.40 (0.39-5.06)			
Participant in trial				
No	1.00	0.09	1.00	<0.01
Yes	2.38 (0.86-6.58)		10.08 (2.14-47.43)	

HR, hazard ratio; 95% CI, 95% confidence interval.
HR >1 and HR<1 indicate increased and decreased probability of response, respectively, where categories with HR=1 were used as the reference category. P<0.05 is considered statistically significant.
HRs, hazard ratios; 95% CI, 95% confidence interval.
*The hazard ratio for age represents increase in probability per year.
**The hazard ratio for tumour size represents increase in probability per cm.

Safety analysis

In total, 409 TEAEs were noted in all sequences (Table 4). Of those TEAEs, 77% were grade 1 or 2, 2.5% were grade 3 and only one patient experienced a grade 4 TEAE (liver toxicity); for the other TEAEs, the grade was not mentioned in the medical file. All patients experienced at least one TEAE, with a median number of four TEAEs per patient (range, 1-12 TEAEs) in the first treatment sequence. Patients who restarted treatment experienced the same TEAEs as in the previous sequence. Of all the side effects, 42% resolved, 19% was still present at the last control and for 39% this information was not noted in the patient file.

Table 4. Treatment-emergent adverse events per treatment sequence.

TEAE, n	Sequence 1 n=78*	Sequence 2 n=22*
Muscle spasms, 81	58 (74%)	14 (64%)
Dysgeusia, 76	56 (72%)	14 (64%)
Alopecia, 55	47 (60%)	6 (27%)
Weight loss, 29	22 (28%)	5 (23%)
Fatigue, 21	19 (24%)	1 (5%)
Decreased appetite, 17	12 (15%)	4 (18%)
Diarrhoea, 15	11 (14%)	2 (9%)
Nausea, 13	9 (12%)	3 (14%)
Headache, 9	9 (12%)	-
Myalgia, 8	7 (9%)	1 (5%)
Hepatotoxicity, 6	4 (5%)	2 (9%)
Dizziness, 6	5 (6%)	1 (5%)
Abdominal pain, 4	4 (5%)	-
Ageusia, 4	4 (5%)	-
Asthenia, 2	2 (3%)	-

TEAE, treatment-emergent adverse event.
*All individual patients who received the first or second treatment sequence in the Netherlands.

	Sequence 3 n=8	Sequence 4 n=5	Resolved	Not resolved	
	7 (88%)	2 (40%)	35 (43%)	12 (15%)	34 (42%)
	4 (50%)	2 (40%)	35 (46%)	13 (17%)	28 (37%)
	2 (25%)	-	24 (44%)	9 (16%)	22 (40%)
	2 (25%)	-	7 (24%)	5 (17%)	17 (59%)
	1 (13%)	-	4 (19%)	9 (43%)	8 (38%)
	1 (13%)	-	8 (47%)	6 (35%)	3 (18%)
	1 (13%)	1 (20%)	6 (40%)	1 (7%)	8 (53%)
	1 (13%)	-	6 (46%)	1 (8%)	6 (46%)
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	1 (25%)	-	3 (75%)
	-	-	-	-	2 (100%)

DISCUSSION

In this retrospective cohort study, data were provided about vismodegib use in the Netherlands. In the national guidelines, the indication for vismodegib treatment is "reserved only for patients with an aBCC where surgery and radiotherapy are ineffective or encounter major objections". In a population of ~17.2 million people and a suspected incidence of BCC of 3-10% per year, only 80 patients have been treated with vismodegib in a period of almost 8 years.[10,11] Over one-third of these 80 patients were initially included in a clinical trial, which indicates the reluctance to prescribe vismodegib in the Netherlands.

Unique for our study is the reflection of all data concerning the use and effectiveness of vismodegib and the course of treatment after vismodegib discontinuation. We found a median PFS of 10.3 months for the indicated laBCC, 11.7 months for mBCC, and 19.1 months for BCNS. Comparable results for the aBCC population were found in other studies. The ERIVANCE trial found a median PFS of 12.9 months for the laBCC group and 9.3 months for the mBCC group, and the STEVIE trial found 13.2 months for the mBCC group.[6,12] However, there was one exception, the STEVIE trial found a much longer PFS of 23.2 months in the laBCC group. The long duration of PFS in the laBCCs of the STEVIE trial is remarkable. An explanation might be a difference in included tumour types between our country and the STEVIE trial. Information on subtype and size of BCCs included in the STEVIE trial is not available. In our country, vismodegib was exclusively prescribed after evaluation of the tumour in a multidisciplinary tumour board, including a head and neck surgeon, a radiotherapist and an oncologist, which may result in defining a tumour "irresectable and not suitable for radiotherapy" at a more advanced stage. It can be speculated that tumours with a more advanced nature do worse and will show progression at an earlier stage. This hypothesis is confirmed by analyses of our own data in which we found that larger tumours have a lower probability of complete response versus smaller tumours. A second explanation for the difference in PFS between our study and the STEVIE trial can be the retrospective nature of our study in which effectiveness outcomes relied on the accuracy of record keeping and the frequency of patient visits. Less meticulous measurements in daily practice might affect the assessed PFS. Finally, the definition of tumour progression differed between the studies: in the STEVIE trial, it was defined as >20% increase in size, taking as reference the smallest tumour size measured during the study, whereas in our study, progression as noted by the physician was additionally defined as disease progression. In the latter definition of progression, the increase could be less than 20%, but with more other complaints, such as bleeding, pain or ulceration. This could have led to a shorter PFS in our study.

A few patients achieved a prolonged complete response, a phenomenon that has previously been described in a French population.[13] To determine what tumour types achieved a complete response, we compared several factors for probability of complete response in the multivariable Cox regression analysis (tumour size, histologic subtype, previous treatment and clinical trial participation). Irrespective of the other variables, patients with laBCCs that participated in the STEVIE trial had a very high probability of achieving a complete response compared to patients treated in daily practice. This higher effectiveness of treatments in patients participating in randomized controlled trials is known as the Hawthorne effect.[14]

According to the FDA (United States Food and Drug Administration) and EMA (European Medicines Agency) guidelines, vismodegib is only approved for the treatment of aBCC. Data on effectiveness for other indications are sparse and no such data are expected in the near future as there are currently no such clinical trials registered. In our cohort, 22 patients (26%) received vismodegib for a multiple BCC indication (20% BCNS, 4% XP, 2% HF-BCC patients). The large number of BCCs places a heavy burden on these patients and a therapy that can treat all lesions at once is very desirable.[15] In line with previous clinical trials, we found a high effectiveness of vismodegib in this patient population, but the majority of patients discontinued due to side effects. The frequency of most side effects was somewhat lower than in the STEVIE and ERIVANCE trials.[6, 16] A possible explanation is the retrospective nature of our study. Also, the shorter treatment duration could be causative, as it was found in the STEVIE trial that the frequency of most side effects increased with the treatment duration.[6] Two differences in side effects compared to previously published trials are notable: (1) a very low frequency of weight loss (28% vs. 41%) and (2) a higher frequency of dysgeusia (72% vs. 55%).[6] Weight measurement was obligatory in the STEVIE trial, but sometimes omitted in real life, which can explain the difference in the frequency of weight loss. We cannot explain the higher frequency of dysgeusia. However, we hypothesise that its inconvenience stresses patients more to mention this at their consultation, even if not specifically asked for, whereas in the STEVIE trial, all side effects had to be checked systematically.

To allow patients to recover from side effects, different intermittent dosing schedules were used. In the two intermittent vismodegib dosing regimens of the MIKIE trial (vismodegib daily alternate with 8 weeks of placebo), side effects still appeared substantial.[8] From our data, it becomes clear that in daily practice patients often have a much longer treatment break. Although our data show a lower frequency of side effects in the following sequences, it does not

mean patients will endure less side effects in the following sequence. As most patients stopped treatment due to side effects, selection of patients that have experienced less severe side effects could have occurred in the group that was treated with a second sequence. Moreover, the median treatment durations of following sequences were shorter compared to the fist sequence (6.4 months in the first, 5.3 months in the second, 3.3 in the third and 4.8 in the fourth sequence). From the STEVIE trial, it is known that median time to onset of alopecia is 5.6 and dysgeusia is 6.5 months, which might explain why those side effects were reported less in the second sequence.[7] Lastly, about ~20-30% of the patients in a following sequence received an alternate dose of vismodegib, specifically to lower side effects.

Seven BCNS patients have already been treated successfully for ≥3 times in eight years and one HF-BCC patient is treated successfully for years with 3 months on and off vismodegib treatment. Unfortunately, there is currently no information on the effects of lifelong intermittent treatment on the general health of patients and on the progression of BCC size and aggressiveness during treatment breaks. Although it is likely that intermittent vismodegib and multiple surgical procedures both affect the quality of life in this patient group, it is currently unknown which strategy has the least impact. Clustering data from different BCNS-centres worldwide can provide the best answers to these questions.

This study provides important information on vismodegib effectiveness and the course of treatment after vismodegib discontinuation. Median PFS was less than a year for aBCCs. Future research should focus on treatment combinations or options after vismodegib failure and defining which patients can achieve a prolonged complete response. In BCNS patients, PFS is longer than in aBCCs, but treatment is often discontinued due to side effects. Retreatment remains effective and can be applied in various schedules.

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Chapter 3.2

Molecular testing in metastatic basal cell carcinoma

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ABSTRACT

Background: Metastatic basal cell carcinoma (mBCC) is a very rare entity, and diagnosis can be challenging. Therapeutic options are limited, and response to targeted therapy is poor.

Objective: To demonstrate a clonal relationship between BCCs and their metastases and additionally, to explore which hedgehog pathway-related mutations are involved in mBCC.

Methods: Genetic analysis was conducted in ten primary BCCs and their metastases. Genes relevant for BCC development were analysed in tumour and metastasis material with small molecule molecular inversion probes (smMIPs) for *PTCH1*, *PTCH2*, *SMO*, *SUFU*, *GLI2* and *TP53* or with targeted next generation sequencing of the same genes and *CDKN2A*, *CDKN2B*, *CIC*, *DAXX*, *DDX3X*, *FUBP1*, *NF1*, *NF2*, *PTEN*, *SETD2*, *TRAF7*, and the *TERT* promoter.

Results: In eight of ten patients, identical gene mutations could be demonstrated in the primary tumours and their metastases. A broad spectrum of mutations was found. Four patients had *SMO* mutations in their tumour or metastasis, or both. All *SMO* mutations found were known to cause resistance to targeted therapy with vismodegib.

Limitations: In two patients there was insufficient qualitative DNA available for genetic analysis.

Conclusions: Molecular testing can help to identify the origin of a BCC metastasis and may be of prognostic and therapeutic value.

INTRODUCTION

Basal cell carcinoma (BCC) is the most common skin cancer among Caucasians and its incidence is still rising.[1] On the contrary, metastatic basal cell carcinoma (mBCC) is rare, with an estimated incidence varying from 0.0028 to 0.55% of all BCC cases.[2-4] The prognosis of mBCC is poor, with a median survival of 87 months in case of regional metastasis and 24 months in case of distant metastasis.[5] Surgery is the first choice of treatment, and if not feasible, radiotherapy should be considered. If both surgery and radiotherapy are contraindicated, targeted therapy with a hedgehog inhibitor is indicated. Vismodegib, currently the only registered systemic treatment for mBCC, inhibits the smoothened (SMO) protein in the hedgehog-signalling pathway.[6] Approximately 85% of sporadic BCCs harbour mutations in one or more genes of the hedgehog pathway. Of all sporadic BCCs, 79% have mutations in patched-1 (*PTCH1),* 22% in *SMO*, and 9% in suppressor of fused homolog (*SUFU*).[7]

In the STEVIE (SafeTy Events in VIsmodEgib) trial, clinical response of mBCC to vismodegib treatment was 36.9%, with only 4.8% being complete responses.[8] The observation that two-thirds of the patients with mBCC do not respond to vismodegib treatment could be explained by the fact that the metastases harbour vismodegib-resistant mutations. Mutations in *SMO*, either primarily present in the tumour or developed during treatment, have been proven to cause resistance to vismodegib in advanced BCC.[9-11] A second explanation for mBCC unresponsive to treatment could be misdiagnosis. Confirmation of the origin of the metastasis can sometimes be difficult with histology alone, especially in the presence of squamous or poor differentiation.[3,12] Generally, there can be difficulties distinguishing mBCC from primary non-small cell lung cancer or metastasis of unknown origin.[13]

This study used molecular testing to identify a clonal relationship between BCCs and their metastases. Furthermore, we explored which hedgehog pathway-related mutations are involved in mBCC.

METHODS

Between April 2016 and May 2019, genetic analysis was performed for eight patients with mBCC in the Maastricht University Medical Centre + (Maastricht UMC+) and the Erasmus MC Cancer Institute (Erasmus MC). Additionally, the Maastricht UMC+ received requests for genetic analysis of three patients with mBCC from two other centres. In the Maastricht UMC+, DNA was extracted and analysed using small molecule molecular inversion probes (smMIPs) and next-generation sequencing (NGS) on the NextSeq 500 (Illumina, Inc, San Diego, CA).[14] These smMIPs (826 probes, available on request) were limited to genes known to be involved in BCC development. This concerns *TP53* (NCBI RefSeq: NM_000546.5/NM_0011261132.2/NM_001126114.2) and the genes of the hedgehog pathway: *PTCH1, PTCH2, SMO, SUFU* and *GLI2* (respectively, NCBI RefSeq: NM_000264.3, NM_003738.4, NM_005631.4, NM_01619.3 and NM_005270.4).

In the Erasmus MC, targeted next generation sequencing (tNGS) was performed with a 20% detection limit and contained the following genes: *CDKN2A*, *CDKN2B, CIC, DAXX, DDX3X, FUBP1, NF1, NF2, PTCH1, PTCH2, PTEN, SETD2, SMO, SUFU, TRAF7, TP53* (respectively, NCBI RefSeq: NM_000077.4, NM_004936, NM_015125, NM_001141969, NM_001356, NM_003902, NM_000267, NM_000268, NM_000264, NM_003738, NM_000314, NM_014159, NM_005631, NM_016169, NM_032271, NM_000546) and additionally the *TERT* promoter region (NCBI RefSeq [Chr5, Hg19]: NC_000005.10:g.1295228G>A [C228T], g.1295242_1295243delinsAA [242_243delinsTT] and g.1295250G>A [C250T]).

Mutation detection was performed using the S5-XL system (Ion Torrent; Thermo Fisher Scientific, Rockford, IL) with the manufacturer's materials and protocols. Library preparations and sequencing was performed as described earlier.[15] Data analysis at Erasmus MC was performed using SeqPilot 4.2.2 software (JSI medical systems, Ettenheim, Germany). Copy number variation/loss of heterozygosity was evaluated using SNPitty, which visualizes B-allele frequencies from NGS sequencing data.[16] Variant filtering and interpretation was achieved with the Alamut 2.11 software tool (Interactive Biosoftware, Rouen, France) and included public databases such as the Genome Aggregation Database (gnomAD) and the Catalogue Of somatic Mutations in Cancer (COSMIC).

Clinical information was retrieved from the electronic patient files. Material from tumours and metastases were reviewed by academic dermatopathologists. According to Dutch guidelines, in cases when the histopathologic diagnosis is

uncertain, different immunohistochemical stainings are performed based on the localization and differentiation of a tumour.[17] All patients included gave written informed consent for genetic analysis except one. Only histologic analysis was performed on material from this patient.

RESULTS

The clinical characteristics of the 11 included patients are summarised in Table 1. The median age at diagnosis of the primary BCC was 63 years (range, 42-80 years), and seven patient (64%) were female. The primary BCC was located on the trunk in five patients, in the head and neck region in four, and on the lower extremity in two. The primary BCC of six patients was initially treated with surgery. The excision in two of those patients did not lead to tumour-free margins. One of these patients was treated with radiotherapy afterward, and the other patient did not receive adjuvant treatment. The primary locally advanced BCC of one patient was treated with vismodegib.

The metastases in four patients were already present at the time of the primary BCC diagnosis, and three of them were treated directly with vismodegib. One patient was treated with local surgery, underwent a cervical lymph node dissection, and received vismodegib as an adjuvant therapy.

The median time from primary BCC diagnosis to mBCC diagnosis was 3.4 years (range, 0-11 years). All patients had TNM stage IVA or IVB disease (Union for International Cancer Control TNM classification, eight edition). Four patients only had regional lymph node metastases. Distant metastases were present in seven patients: five in the lungs, one in the pleural cavity and one in the bones. Three patients with distant metastases also had proven regional lymph node metastases. Apart from one patient with basal cell nevus syndrome and one patient with HIV, there were no other patients with a genetic syndrome or immunosuppression.

The results of histologic characteristics can be found in Table 2. Of the 11 patients, seven had an infiltrative subtype of their primary BCC, three had a mixed nodular and infiltrative subtype and one patient had a primary nodular BCC (Table 2). Squamous differentiation was observed in four metastases and three primary tumours. In patients 4 and 9, a cytologic puncture was performed on the lymph node metastasis to obtain material.

Histologic samples were available for all other primary tumours and metastases. Histologic samples were available for both the primary tumour and metastasis in nine patients. Cell type and differentiation differed between the primary tumour and metastasis in seven of these nine patients. For example, the primary tumour in patient 1 showed a typical BCC histology, but

the lung metastasis showed more squamous differentiation (Figure 1). Owing to differences in histopathology, additional immunohistochemical staining was performed in the metastasis of all these patients (Table 2).

Genetic analysis of the primary tumours and metastases was performed in 10 of the 11 patients, as patient 11 died before informed consent for genetic analysis could be obtained. Genetic analysis was preferably performed on fresh material and obtained before systemic treatment was given. There were some exceptions, however. Two patients had received targeted therapy with vismodegib before material was obtained. Only formalin-fixed and paraffin-embedded (FFPE) material was available for three patients (Table 3). In one of those three patients, genetic analysis of the FFPE material of the primary tumour and metastasis failed with tNGS. In a different patient, genetic analysis of FFPE material with smMIP was successful in the primary tumour biopsy but failed in the cytological puncture of the metastasis.

In all eight patients in whom genetic analysis was successful for both samples, the mutations found in the metastases were identical to those found in the primary tumours. Four of those patients had distant metastases, three patients only had regional lymph node metastases and one had a parotid gland metastasis. All four patients with distant metastases had a known vismodegib-resistant *SMO* mutation, two of them received vismodegib therapy before material for genetic analysis was obtained. Specifications of the tumour mutation profiles and corresponding clinical courses are shown in Table 3. Nine patients were treated with vismodegib for their mBCC, of which two attained complete response. Progressive disease developed in the remaining seven patients within 1 year under this therapy, vismodegib treatment was discontinued. Of those, three died, two are currently in between treatments, and two are being treated with a checkpoint inhibitor in a clinical trial setting.[18]

Table 1. Clinical characteristics.

Patient	Age(y)/sex	Primary site	TNM/ Stage[a]	Size primary (cm)	Deep invasion[b]
1	68/F	Scapula	T3N0M1 IVB	15	Yes
2[c]	54/F	Head	T4N3bM0 IVA	20	Yes
3	63/F	Abdomen	T3N3M0 IVA	>10	Yes
4	49/F	Head	T3N1M1 IVB	10	Yes
5	52/F	Scapula	T3N2M1 IVB	5	No
6	57/M	Back	T2N3M0 IVA	3	No
7	70/F	Sternum	T4N0M1 IVB	7	Yes
8	80/M	Head	T3N2AM0 IVA	4	Yes
9[d]	72/M	Leg	T4N1M1 IVB	20	Yes
10	42/M	Head	T3NxM1 IVB	>5	Yes
11	76/F	Leg	T3NxM1 IVB	15	Yes

Y, years; F, female; M, male; RT, radiotherapy; NA, not applicable; LN, lymph node.

[a]Union for International Cancer Control TNM Classification, eight edition.

[b]Defined as invasion in structures beyond subcutaneous tissue.

	Treatment primary tumour	Recurrence	Time interval to metastasis (y)	Site of metastasis
	Vismodegib, excision	Yes	4.6	Lung
	Excision	NA	0	Cervical LN
	Vismodegib	NA	0	Axillary & inguinal LN
	Excision[e], RT	Yes	11	Pre-auricular LN & lung
	Excision	Yes	10	Axillary LN & lung
	Excision	Yes	7	Axillary LN
	Vismodegib	NA	0	Lung
	Excision	Yes	3.4	Parotid gland
	Excision[e]	No	1	Inguinal LN & lung
	Excision	Yes	10	Pleural cavity
	Vismodegib	NA	0	Bones

[c]Patient with basal cell nevus syndrome.
[d]Patient with HIV.
[e]No clear margins.

Table 2. Histologic characteristics.

Patient	Cell type/differentiation primary tumour	Growth pattern primary tumour	Metastasis material
1	Basaloid	Infiltrative	Histology
2[a]	Basaloid	Nodular & infiltrative	Histology Histology
3	Basaloid/ undifferentiated	Infiltrative	Histology
4	Basaloid	Nodular & infiltrative	Cytology Histology
5	Basaloid/squamous	Infiltrative	Histology Histology
6	Basaloid/squamous	Infiltrative	Histology
7[b]	Atypical epithelioid	Nodular	Histology
8	Basaloid	Nodular & infiltrative	Histology
9	Basaloid	Infiltrative	Cytology
10	Basaloid	Infiltrative	Histology
11	Basaloid/squamous	Infiltrative	Histology

ER, oestrogen receptor; LN, lymph node; PR, progesterone receptor, +, positive stain; **-**, negative stain.

	Cell type/differentiation metastasis	Stains in metastasis (+/-)
	Lung: basaloid/squamous	+ p40, BerEP4 - TTF1, CK7
	LN1: tumour cells	Not performed
	LN2: tumour cells	Not performed
	Axillary LN: basaloid	+ BerEP4 - EMA
	LN: tumour cells	Not performed
	Lung: basaloid	+ p40, GATA-3 - CEA, TTF1
	LN: basaloid	Not performed
	Lung: basaloid	+ p40, p63, GATA-3 - EMA, CD10, ER, PR
	LN: basaloid	+ BerEP4
	Lung: non-small cell carcinoma/squamous	+ BerEP4, p40, CD10 - TTF1, Napsin A
	Parotid gland: basaloid	+ BerEP4
	LN: tumour cells	Not performed
	Pleura: large cell carcinoma/squamous and adenoid	+ BerEP4, p40, p63 - TTF1, CD68, PD-L1, Vimentin
	Bone marrow: basaloid/ squamous	+ BerEP4, p63, CK7 - TTF1, CK20, ER, PR, PAX8, OCT3/4

[a]Patient with basal cell nevus syndrome.
[b]Material obtained during treatment with sonidegib.

Table 3. Genetic characteristics.

Patient	Origin of sample	Gene	Mutation	Frequency
1[a]	BCC	*PTCH1*	c.1728_1728+1delinsAA	64%
	Lung	*PTCH1*	c.1728_1728+1delinsAA	16%
		SMO	c.722C>T	11%
2[b]	BCC, *FFPE*	*PTCH1*	c.533A>C	56%
	Cervical LN1, *FFPE*	*PTCH1*	c.533A>C	91%
	Cervical LN2, *FFPE*	*PTCH1*	c.533A>C	82%
3	BCC	*PTCH1*	c.3053G>A	83%
		TP53	c.722C>T	44%
	Axillary LN	*PTCH1*	c.3053G>A	42%
		TP53	c.722C>T	13%
4	BCC	*SMO*	c.1234C>T	43%
		TP53	c.637C>T	74%
		PTCH1	c.2048C >T	46%
		TERT prom	C250T	38%
	Pre-auricular LN	*SMO*	c.1234C>T	45%
		TP53	c.637C>T	94%
		PTCH1	c.2048C >T	45%
		TERT prom	C250T	60%
	Lung	*SMO*	c.1234C>T	39%
		TP53	c.637C>T	58%
		PTCH1	c.2048C >T	42%
		TERT prom	C250T	43%
5	BCC	*SMO*	c.1234C>T	20%[c]
		TERT prom	C228T	63%
	Axillary LN	*SMO*	c.1234C>T	44%
		TERT prom	C228T	49%
	Lung	*SMO*	c.1234C>T	28%
		TERT prom	C228T	41%
6	BCC	*PTCH1*	c.466C>T	61%
		PTCH1	c.3261_3262insTGACC	27%
	Axillary LN	*PTCH1*	c.466C>T	43%
		PTCH1	c.3261_3262insTGACC	31%

	Protein change	Treatment of metastasis	Outcome	Currently under treatment
	r.spl? r.spl? p.(Thr241Met)*	Vismodegib, checkpoint inhibitor	SD	Yes
	p.(His178Pro) p.(His178Pro) p.(His178Pro)	CLND, vismodegib	CR	No
	p.(Trp1018*) p.(Ser241Phe) p.(Trp1018*) p.(Ser241Phe)	Vismodegib, checkpoint inhibitor	SD	Yes
	p.(Leu412Phe)* p.(Arg213*) p.(Ser638Phe) p.(Leu412Phe)* p.(Arg213*) p.(Ser638Phe) p.(Leu412Phe)* p.(Arg213*) p.(Ser638Phe)	CLND, local surgery	SD	No
	p.(Leu412Phe)* p.(Leu412Phe)* p.(Leu412Phe)*	ALND, RT axilla	SD	No
	p.(Gln156*) p.(Ala1099*) p.(Gln156*) p.(Ala1099*)	Vismodegib, ALND, RT axilla	SD	No, †[d]

Table 3. Continued.

Patient	Origin of sample	Gene	Mutation	Frequency
7[a]	BCC	*PTCH1*	c.2839G>T	90%
		TERT prom	C250T	59%
		SETD2	c.2002C>A	53%
	Lung	*SMO*	c.722C>T	54%
		PTCH1	c.2839G>T	72%
		TERT prom	C250T	46%
		SETD2	c.2002C>A	53%
8	BCC	*PTCH1*	c.767G>A	52%
	Parotid gland	*PTCH1*	c.767G>A	40%
9	BCC, *FFPE*	*TP53*	c.742C>T	24%
		SUFU	c.187G>A	46%
		SUFU	c.1165C>T	40%
	Inguinal LN, *FFPE*		Failed	
10	BCC, *FFPE*		Failed	
	Pleura, *FFPE*		Failed	

FFPE, formalin-fixed, paraffin-embedded material; CLND, cervical lymph node dissection; ALND, axillary lymph node dissection; RT, radiotherapy; CR, complete response; SD, stable disease; PD, progressive disease.
Materials from patients 1, 2, 3 8 and 9 were analysed with small molecule molecular inversion probes, and patients 4, 5, 6 and 7 and 10 were analysed with targeted next generation sequencing.

	Protein change	Treatment of metastasis	Outcome	Currently under treatment
	p.(Glu947*) p.(Pro668Thr) p.(Thr241Met)* p.(Glu947*) p.(Pro668Thr)	Vismodegib, surgery, RT, nivolumab, sonidegib	PD	Yes
	p.(Trp256*) p.(Trp256*)	Vismodegib	CR	No
	p.(Arg248Trp) p.(Gly63Ser) p.(Leu389Phe)	Vismodegib cyclic	PD	No, †
		Vismodegib	PD	Yes

[a]Material obtained after treatment with vismodegib.
[b]Patient with basal cell nevus syndrome.
[c]Confirmed with Sanger Sequencing Analysis.
[d]Unrelated to disease.
† = deceased.
**SMO* mutations p.(Leu412Phe) and p.(Thr241Met) are known to cause vismodegib resistance.

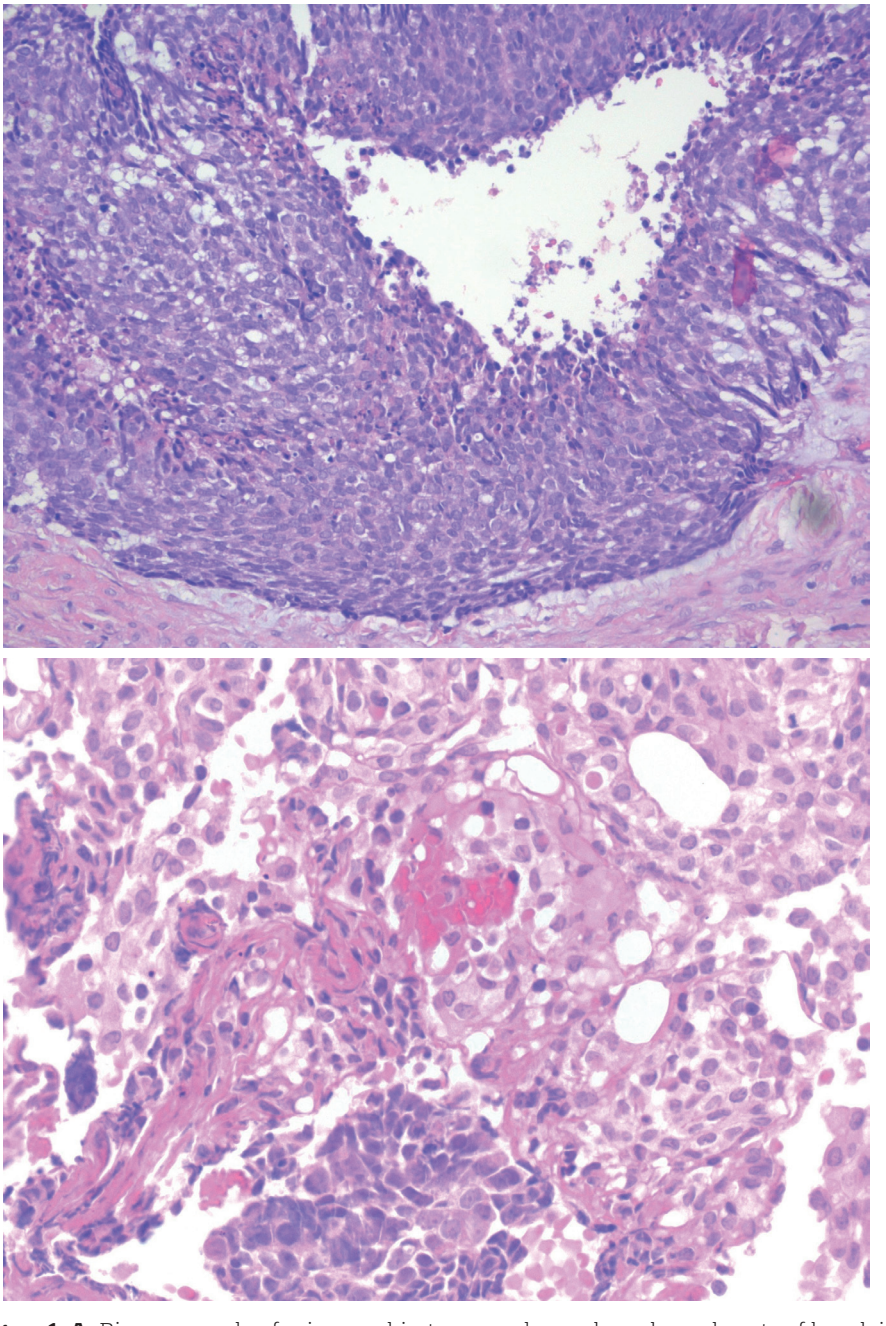

Figure 1. A. Biopsy sample of primary skin tumour shows deep dermal nests of basaloid epithelial cells with cystic degeneration, mucin deposits and central apoptosis. **B.** Biopsy sample of lung metastasis shows the tumour contains few basaloid cells but is particularly composed of nests of squamous cells with abundant cytoplasm and enlarged nuclei with prominent nucleoli. (**A** and **B**: hematoxylin and eosin stain; original magnification: x200).

DISCUSSION

In this case series we demonstrated the presence of identical gene mutations in eight primary BCCs and their metastases, providing strong evidence for clonal relationship. In most patients, there was a discrepancy in histologic features of the primary tumours and the metastases, resulting in some uncertainty about the origin of the metastases. The ability to confirm clonal relationship with genetic analysis can aid tumour staging. Knowledge of the mutation may be helpful in the decision to prescribe targeted therapies with hedgehog inhibitors or checkpoint inhibitors in case of mBCC.[19]

Notably, all patients with *SMO* mutations had distant metastases. This could indicate that *SMO* mutations are responsible for more aggressive behaviour in BCCs. The activating *SMO* mutation c.1234C>T was found twice in our case series, and was also previously found in a patient with an extraordinarily destructive BCC.[20] Because the number of patients is too small to draw firm conclusions, this finding should be confirmed in a larger cohort. The other *SMO* mutation that was found in two other patients should be interpreted with care, as material for molecular testing was obtained after previous treatment with vismodegib, which might have caused selection of a subpopulation in the tumour.

Among the nine patients in our cohort who were treated with vismodegib, progressive disease eventually developed under this treatment in seven of them within a year. This failure rate seems very high. In a different retrospective study with 28 patients with advanced BCC treated with vismodegib, vismodegib resistance developed within a year during treatment in only 21%.[11] This may be explained by the fact that our cohort only included patients with mBCC, who consequently have tumours with a more aggressive behaviour.

As we see in our case series, primary tumours and metastases sometimes differ histologically. Also, when a metastasis is diagnosed in a clinical setting, the primary tumour is not always present or known. If histologic confirmation is difficult, it is valuable to have fresh material for genetic analysis to confirm the diagnosis. Furthermore, the obtained genetic profile of the metastases could be useful to guide treatment choices, because the presence of mutations known to cause vismodegib resistance could predict the response to this treatment. This is especially relevant because the effect of vismodegib treatment only becomes visible after a median period of 3.7 months.[8] During these months, side effects can significantly impact the quality of life.[21] Also, the costs for 3.7 months of

treatment may be a 100-fold higher than the costs for genetic analysis.[14,22] We do have to keep in mind that a biopsy represents only a small part of the tumour and, consequently, that found vismodegib-resistant *SMO* mutations may be not representative for the entire tumour. A temporary tumour load reduction, improving a patient's quality of life, cannot be excluded. A different aspect of consideration is the fact that genes not involved in the hedgehog pathway may also be relevant in mBCC. Insight from other trials might lead to the discovery of other genes that could lead to new therapeutic options for patients with mBCC.[18]

Owing to the retrospective nature of this study, different methods were used to obtain material and detect mutations. In the Maastricht UMC+ and Erasmus MC, fresh material was available, but the material that was received from other centres was mostly FFPE, which probably caused the failure of analysis in two patients. Targeted NGS failed on FFPE biopsy tissue of the skin and pleural cavity, whereas smMIP analysis has been proven to be effective on FFPE material.[14] In our study, smMIP analysis was indeed successful on the FFPE material of one biopsy sample, but the quality of the FFPE material of a cytological puncture was too low to perform successful smMIP analysis. This was probably because the material was obtained with a fine-needle aspiration cytology, which contains a low amount of qualitative DNA.[23] For successful genetic analysis, we would advise obtaining a fresh biopsy sample of the primary tumour and metastasis.

One of the included patients had basal cell nevus syndrome caused by a germline mutation in *PTCH1*. In both the primary tumour (locally advanced BCC) and the metastases, only the germline *PTCH1* mutation was found in combination with loss of heterozygosity. Loss of heterozygosity is a frequently occurring event in sporadic tumour formation and therefore common loss of heterozygosity in both the primary and metastatic BCC may be a coincidental event.[24] Because no other variants were found in the genes tested, distinction between clonality or occurrence of independent events is not possible.

CONCLUSION

We demonstrated a clonal relationship between primary BCCs and their metastases. Molecular testing can be valuable if the diagnosis of this rare entity is difficult. Furthermore, genetic profiling of the metastases may become useful in tailoring the treatment of mBCC.

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Chapter 3.3

Hedgehog pathway and PD-1 inhibitors for advanced basal cell carcinoma

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Submitted for publication

Chapter 4

BASAL CELL NEVUS SYNDROME AND HIGH FREQUENCY BASAL CELL CARCINOMA

Chapter 4.1

A guideline for the clinical management of basal cell nevus syndrome (Gorlin–Goltz syndrome)

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1.0 PURPOSE AND SCOPE

The overall objective of this guideline is to provide up-to-date, evidence-based recommendations for the diagnosis and surveillance of all symptoms in children and adults with either basal cell nevus syndrome (BCNS), a clinical suspicion of BCNS, or a parent with BCNS. In the last two groups the guidelines should be followed until the diagnosis of BCNS can be rejected with certainty. The guideline aims to:

- Update and expand on the previous guidelines by an appraisal of all relevant literature from January 2011 up to January 2021
- Address important, practical, clinical questions relating to the primary guideline objective
- Provide guideline recommendations
- Discuss potential developments and future directions

The guideline is presented as a detailed review with highlighted recommendations for practical use in the clinic by dermatologist and other health professionals, including general practitioners, clinical geneticist, paediatricians, ophthalmologists, cranio-maxillofacial surgeons, neurologists, cardiologists and psychologists.

1.1 Exclusions

The guideline does not cover therapeutic recommendations for (nondermatological) symptoms, as the guideline mainly focuses on screening and follow-up of symptoms. Therapeutic recommendations for basal cell carcinomas (BCC) in general have been published in international BCC guidelines.[1,2]

1.2 Stakeholder involvement and peer review

The guideline was developed at the Maastricht University Medical Centre (MUMC+), the Dutch BCNS expert centre accredited by the Dutch Ministry of Health, Welfare and Sport.[3] The guideline development group (GDG) consisted of two dermatologist, a clinical geneticist, a molecular geneticist, an ophthalmologist, a paediatrician, a gynaecologist, a cranio-maxillofacial surgeon and a paediatric neurologist, all working at the MUMC+. Two residents in dermatology were also part of the GDG and acted as project managers. Furthermore, three patient/carer representatives commented on drafts of the guideline. The GDG developed clinical questions relevant for the management of patients with BCNS, all concerning the diagnosis and surveillance of symptoms. During the development of the guideline the GDG met twice face-to-face and if input from the complete GDG was requested for disagreements, it was solicited via e-mail.

2.0 METHODOLOGY

This guideline was developed using the Appraisal of Guidelines Research and Evaluation II and Grading of Recommendations Assessment, Development and Evaluation (GRADE) instruments.[4] A systematic literature search of the PubMed and Embase databases was conducted to identify key articles in English using the search terms 'basal cell nevus syndrome' and 'Gorlin syndrome' from January 2011 up to January 2021. A total of 2747 articles were found. All titles were screened and case reports on general features, image quizzes, and non-related articles were excluded (n=1112). The reference lists of all relevant articles were scanned for additional suitable articles (n=13). Titles and abstract of the remaining 1648 articles were screened and 1576 articles were excluded as they did not provide the highest level of evidence available or information pertinent to the scope of the guideline. In the final analysis, 72 articles were included (Table S1; see Supporting Information). Level of evidence was graded according to the GRADE system (high, moderate, low, very low) by two authors (B.J.A.V. and B.C.). Recommendations were based on evidence drawn from the systematic review of the literature and discussed with the GDG during the consensus meetings. For each recommendation, benefits, risks and side effects were systematically considered. Expert opinion of the GDG was used to generate recommendations if documented evidence-based data were not available.

3.0 LIMITATIONS OF THE GUIDELINE

This guideline was set up by a multidisciplinary team of physicians, which was restricted to a Dutch care centre. Diagnostic strategies and involved medical (sub) specialists may vary according to the healthcare system and local conditions. BCNS is a very rare disorder and the literature on specific symptoms is scarce. Randomized controlled trials are lacking and for most recommendations only indirect evidence was available. GRADE strength of recommendations (GSoR) are therefore often based on low or very low GRADE evidence certainty (GEC) levels.

4.0 UPDATING THE GUIDELINE

Results of future studies may require change of some of the recommendations. These guidelines will be re-evaluated after five years to determine whether an (modular) update will be necessary.

5.0 BACKGROUND

Basal cell nevus syndrome (BCNS, OMIM #109400), also known as Gorlin-Goltz syndrome, is a rare autosomal dominant disorder with an estimated prevalence varying from 1:31.000-1:256.000.[5,6] The most common genetic cause of BCNS is a heterozygous germline mutation in the patched-1 (*PTCH1)* gene.[7] This gene encodes the transmembrane glycoprotein PTCH1, a tumour suppressor of the Hedgehog (Hh) signalling pathway.[8] In addition to its important role in embryonic development, the Hh signalling pathway is involved in cell proliferation and differentiation. During the inactive state, PTCH1 has an inhibitory effect on smoothened (SMO) and downstream signalling is inhibited. Further downstream, the suppressor of fused (SUFU) also inhibits the pathway by directly binding to glioma-associated (GLI) transcription factors and preventing translocation to the nucleus. Mutations in *PTCH1* could relieve its inhibitory effect on *SMO* and subsequently this results in translocation of GLI transcription factors to the cell nucleus and upregulation of the Hh signalling pathway. Mutations in other genes of the Hh signalling pathway, either as germline mutation or postzygotic mosaicism, have been described but are less common.[9-12] The most common features of BCNS are BCCs and odontogenic keratocysts (OKCs) of the jaw, but a broad scale of other characteristic features has been described.[13] Because of the low prevalence and broad variety of symptoms, the management and follow-up of patients with BCNS is often challenging. In 2011, Bree et al. proposed a management protocol for surveillance of BCNS patients. However, at that time, genetic analysis played a less important role and the differences between patients with heterozygous mutations in *PTCH1* and *SUFU* were not evident yet.[14] Here we provide an up-to-date, multidisciplinary, practical, guideline for the clinical management of patients with BCNS (of suspicion of BCNS).

6.0 DIAGNOSIS

Diagnostic criteria for BCNS were first proposed by Evans et al. in 1993, modified by Kimonis et al. in 1997 and revised by Bree et al. in 2011.[14-16] According to the most recent publication[14], the diagnosis of BCNS can be established based on (i) one major criterion and genetic confirmation; (ii) two major criteria; or (iii) one major and two minor criteria (Table 1). In patients with suspected BCNS, it is important to obtain a complete medical (family) history during the first consultation and perform physical examination to search for dysmorphic features, skeletal abnormalities, and skin abnormalities. Possible features are listed in Table 2.

Table 1. Diagnostic criteria and clinical manifestations of basal cell nevus syndrome.

Basal cell nevus syndrome	
Diagnosis: The diagnosis of BCNS can be established based on:	
1) One major criterion and genetic confirmation; 2) Two major criteria; 3) Or one major and 2 minor criteria.	
Major criteria:	
1) BCCs prior to 20 years old or multiple BCCs;	4) Lamellar calcification of the falx cerebri;
2) OKCs prior to 20 years old;	5) Medulloblastoma (desmoplastic variant);
3) Palmar or plantar pitting;	6) First degree relative with BCNS.
Minor criteria:	
1) Rib anomalies;	5) Lymphomesenteric cysts;
2) Macrocephaly;	6) Ocular abnormalities (i.e. strabismus, hypertelorism, congenital cataracts, glaucoma, coloboma)
3) Cleft/lip palate;	7) Other specific skeletal malformations and radiologic changes (i.e., vertebral anomalies, kyphoscoliosis, short fourth metacarpals, postaxial polydactyly).
4) Ovarian/cardiac fibroma;	
Prevalence: 1 in 31.000-256.000	
Incidence: 1 in 18.976 births[5]	
Genetic test: In 40-64% of patients with a clinical diagnosis of BCNS, an underlying *PTCH1* mutation is found and in 6% an underlying *SUFU* mutation. In case of high clinical suspicion, postzygotic mosaicism can be ascertained by finding an identical mutation in at least 2 BCCs.	
Genetics: An autosomal dominant inheritance with 50% chance of passing on the mutated gene to the offspring. In 20-40% of the patients the disorder is due to a denovo mutation.	

BCNS, basal cell nevus syndrome; BCCs, basal cell carcinomas, OKCs, odontogenic keratocysts.

Table 2. Clinical manifestations of basal cell nevus syndrome.

Clinical manifestations	
Dysmorphic features	**Macrocephaly (> 95th 50%),** coarse face, bi-parietal/frontal bossing, broad nasal bridge, mandibular prognathism, facial asymmetry, congenital cleft lip/palate, malocclusion, hypertelorism, synophrys, coloboma, epicanthus
Development	Intellectual disability (5%)
Ocular system	**Hypertelorism (70%)**, **strabismus (10-20%), cysts on the eyelids (5-10%), congenital cataract (3-8%)**, nystagmus (1-5%), coloboma of the iris, choroid and / or n. opticus (1-5%), congenital glaucoma (1-5%), iris transillumination defects (1-5%), subconjunctival epidermoid cysts (1-5%), microphtalmia (1-2%) myelinated nerve fibers, epiretinal membranes, macular hole, retinal hamartomas
Stomatologic system	**Odontogenic keratocysts (44-92%)**, schisis (5%)
Skin	**BCCs (> 20 years 51.4%; > 40 years 71.7%), palmar (70%) and plantar (50%) pits (<10 years 30-65%; <15 years 80%; > 20 years 85%), facial milia (30%), epidermal cysts (50%), multiple naevi (<20 years 30-50%, > 20 years 70%)**
Skeletal system	**Macrocephaly (> 95th 50%), abnormal skull formation (frontal, biparietal / temporal bossing and large calvaria, 70%), scoliosis (40%), spina bifida occulta 40-60%, rib anomalies (bifid / fused / splayed) (30-60%), Sprengel deformity (10-40%), bone cysts (35% in metacarpalia),** kyphoscoliosis, increased mean height (women 174 cm, males 183 cm, 15% extremely long), pectus deformity, vertebral abnormalities, short fourth metacarpal, polydactyly, syndactyly, brachymetacarpalism
Gastro-enteric system	Lymphomesenteric cysts
Central nervous system	**Ectopic calcification of the: 1) falx cerebri (70-95% (13) / 65% (12), 2) tentory cerebelli (20-40%)**; 3) 'spotted' meningeal calcification (rare) (13); **4) complete or partial bridging of the sella turcica (25%)**. Medulloblastoma (1-4%), meningioma
Genito-urinary system	**Ovarian fibroma (6-60%)**, **ovarian cysts;** ovarian calcifications**; hypogonadotropic hypogonadism (5-10%)**, Horseshoe kidney, L-shaped kidney, unilateral renal agenesis, renal cysts, duplication of the renal pelvis and ureter (5%)
Cardio-vascular system	Cardiac fibroma (3-5%)

Bold manifestations occur in >5% of patients.

7.0 SUMMARY OF RECCOMENDATIONS

All recommendations are listed in Table 3. Table 4 provides a clear surveillance checklist for each age category.

Table 3. Recommendations and grades of evidence.

Recommendation concerning	Recommendation
Diagnosis	**Radiological examination for diagnostic criteria without therapeutic consequences should be avoided as much as possible.**
	If possible, we recommend performing genetic testing in all BCNS-suspected patients.
	We recommend a stepwise approach that first includes genetic testing of the *PTCH1* gene. If no mutation is found, but the clinical suspicion is high, we advise testing for mutations in *SUFU*. If again no variant is found in the presence of a high clinical suspicion, DNA from ≥2 different BCCs can be isolated and genetically tested for *PTCH1* and *SMO* with sensitive Next Generation Sequencing technologies to examine the possibility of postzygotic mosaicism. If a variation is found, the relevance of the mutation and its consequences for the protein function should be verified.
	There is insufficient evidence for genetic testing of *PTCH2*.
Dermatologists	**Adequate sun-protective measures are very important and should be discussed during every visit.**
	Total body inspection, including non-sun-exposed sites, is recommended annually until the development of the first BCC. From that moment on the follow-up frequency should be intensified to up to every three to six months, depending on the number and frequency of new BCCs.
	Treatment of BCCs should be done according to international guidelines.
	Radiotherapy is relatively contra-indicated.
	Treatment with oral HPIs can be considered for the treatment of multiple BCCs.
Non-dermatological symptoms of BCNS	
Development	**Physicians should be aware of the possible increased risk of developmental delay and monitor the development of children with BCNS.**
Bone deformities	**Physicians should identify bone deformities with physical examination at diagnosis to make early intervention possible when needed.**

	Grade evidence certainty*	Grade strength of recommendation*
	Very low	Strong
	Very low	Strong
	Very low	Weak
	Low	Strong
	Very low	Strong
	Very low	Strong
	Evidence varies per treatment and is summarized in these guidelines (1, 2)	Strong
	Very low	Strong
	Moderate	Strong
	Very low	Strong
	Very low	Strong

Table 3. Continued.

Recommendation concerning	Recommendation
Cardiac fibroma	**At diagnosis, all BCNS patients should be screened with a cardiac ultrasound. If cardiac symptoms occur in a BCNS patient, a cardiac ultrasound should be repeated to exclude a late onset cardiac tumour.**
Medulloblastoma	**In children with a *PTCH1* mutation MRI should be considered when clinical symptoms or abnormal psychomotor development are present. However, routine MRI is not indicated.** **In case of a clinical diagnosis without genetic testing or in children with a *SUFU* mutation a baseline MRI is recommended and should be repeated every 4 months until the age of 3 and twice per year until the age of 5.** **When BCNS is diagnosed in adulthood, a baseline brain MRI is not necessary.**
Ophthalmological symptoms	**In patients with BCNS a baseline ophthalmological examination, including an ocular pressure measurement if possible, is recommended.**
Odontogenic keratocysts	**From the age of 8 only heterozygous *PTCH1* patients should be screened for OKCs every two years with an orthopantomogram (OPG).** **After the first OKC, follow-up with an OPG is recommended annually.** **After the age of 22 years, follow-up can be continued by the dentist and additional OPG can be performed in case of pain/ unexplained positional change of the teeth.**
Ovarian fibroma	**Gynaecological ultrasound examination and surveillance in non-symptomatic patients is not strictly advised. In case of abdominal complaints such as pain or menstrual irregularities, female patients should undergo gynaecologic ultrasound examination to investigate the presence of an ovarian fibroma.**
(Lympho) mesenteric cysts	**Physicians should screen for (lympho)mesenteric cysts with ultrasound examination in patients with BCNS and inexplicable abdominal pain.**
Psychologic distress	**Psychological evaluation for support and counselling after the diagnosis is recommended for all patients (and their families). During follow-up, physicians should pay attention to psychological distress and address the possibility of a psychological consult.**
Patient care	**To provide optimal care for patients with BCNS we advocate a multidisciplinary approach.**

BCC, basal cell carcinoma; BCNS, basal cell nevus syndrome; MRI, magnetic resonance imaging; OKC, odontogenic keratocyst; OPG, orthopantomogram.

	Grade evidence certainty*	Grade strength of recommendation*
	Very low	Weak
	Low	Weak
	Low	Weak
	Low	Strong
	Low	Strong
	Very low	Weak
	Very low	Weak
	Very low	Weak
	Very low	Weak
	Very low	Strong
	Very low	Strong
	Very low	Strong

*According to Grading of Recommendations Assessment, Development and Evaluation (GRADE).

7.1 Establishing the diagnosis

7.1.1. Should radiological examination be avoided as diagnostic tool?

Patients with BCNS harbour a germline mutation in a tumour suppressor gene and only one additional mutation (second hit) is necessary for tumorigenesis.[17] It is known that children have a higher susceptibility to secondary malignancy after high-dose radiation due to the known age dependence of radiosensitivity.[18,19] However, the extent to which low-dose radiation (such as X-rays, <0.05 Gy) contributes to DNA damage is still unclear.[20]

Radiological examination for diagnostic criteria without therapeutic consequences should be avoided as much as possible. (GEC, very low; GSoR, strong)

7.1.2. Should genetic confirmation be preferred?

Genetic testing can be expensive, is not currently available in some centres/countries and presymptomatic testing can have social consequences (for example, when patients are taking out a life insurance policy). However, techniques have improved, which has made genetic testing more affordable in most countries. Knowing the causal familial mutation is helpful in providing (pre)symptomatic testing in family members. Furthermore, the specific gene involved (*PTCH1* or *SUFU*) warrants for a different follow-up regime.[21] Sites and countries that perform genetic testing are listed at Orphanet (https://www.orpha.net/consor/cgi-bin/index.php).

If possible, we recommend performing genetic testing in all BCNS-suspected patients. (GEC, very low; GSoR, strong)

7.1.3. Which steps should be followed in genetic confirmation of the diagnosis?

A mutation in the *PTCH1* gene can be detected in 50-70% of patients, depending on the clinical symptoms specified.[21-24] Standard genetic tests are not capable of detecting genomic rearrangments or deep intronic variants causing cryptic splicing in *PTCH1*.[25] Also, mutations could be located in other components of the Hh signalling pathway. The *SUFU* gene is responsible in approximately 4% of BCNS cases.[21] Mutation loads <5%, conceivably present in cases with postzygotic mosaicism (in *PTCH1* or *SMO*), are impossible to detect using Sanger sequencing.[11,12,26] Mutations in the *PTCH2* gene have also been reported,[10,27,28] but probably have an insignificant contribution to the cause of BCNS.[29] If a variation is found, the relevance of the mutation and its consequences for the protein function should be verified according to the standards and guidelines set forward by (inter) national organizations.[30] Only pathogenic variants, or likely pathogenic variants, may explain the patient's clinical symptoms and confirm the diagnosis.[30]

We recommend a stepwise approach that first includes genetic testing of the *PTCH1* gene.

If no mutation is found, but the clinical suspicion is high, we advise testing for mutations in *SUFU*. If again no variant is found in the presence of a high clinical suspicion, DNA from at least two different BCCs can be isolated and genetically tested for *PTCH1* and *SMO* with sensitive next-generation sequencing technologies to examine the possibility of postzygotic mosaicism. If a variation is found, the relevance of the mutation and its consequences for the protein function should be verified. (GEC, very low; GSoR, weak)

There is insufficient evidence for genetic testing of *PTCH2*. (GEC, low; GSoR, strong)

7.2 What should dermatologists be aware of?

The most common cutaneous manifestation in patients with BCNS are multiple BCCs, both nonpigmented and pigmented, involving all histological subtypes and occurring on both sun-exposed and nonsun-exposed parts of the body.[31] Some BCNS patients will develop >100 BCCs during their lifetime. The first BCC can develop during early childhood.[31] Sunscreen use must be discussed frequently, as it can prevent the development of BCCs in patients with BCNS.[32,33]

Other frequently found skin abnormalities (palmoplantar pits, basaloid follicular hamartomas, facial milia, and epidermoid cysts) are benign and do not need treatment, but may be helpful in establishing the diagnosis.[34-36]

Regarding the BCCs, treatment with surgical excision is the gold standard.[1,2] When localized in functionally/cosmetically sensitive areas such as the face, Mohs micrographic surgery is preferred.[1,2] As multiple excisions lead to many scars and can have a high psychological impact[37], non-invasive topical treatments can be useful alternatives.[1,2] Radiotherapy is relatively contraindicated in patients with BCNS, owing to the increased risk of BCCs in the irradiated area.[1,38] Hh pathway inhibitors (HPIs), such as vismodegib and sonidegib, may be indicated in advanced BCC[1,2] and are very effective in the treatment of multiple BCNS-associated BCCs (Tables S2 and S3; see Supporting Information).[39-43] These treatments are not a lifelong option because of side-effects, and BCCs will reoccur after treatment discontinuation (Table S4; see Supporting Information).[39-46] Recently, small phase II clinical trials on topical HPIs have been conducted.[47-49] Results from larger trials need to confirm whether topical HPIs could be a valuable addition to the treatment modalities.

Adequate sun-protective measures are very important and should be discussed during every visit. (GEC, very low; GSoR, strong)

Total body inspection, including nonsun-exposed sites, is recommended annually until the development of the first BCC. From that moment on the follow-up frequency should be intensified to up to every 3-6 months, depending on the number and frequency of new BCCs. (GEC, very low; GSoR, strong)

Treatment of BCCs should be done according to international guidelines. (GEC, evidence varies per treatment and is summarized in these guidelines[1,2]; GSoR, strong)

Radiotherapy is relatively contraindicated. (GEC, very low; GSoR, strong)

Treatment with oral HPIs can be considered for the treatment of multiple BCCs. (GEC, moderate; GSoR, strong)

7.3 When should surveillance for non-dermatologic symptoms be performed?
It is preferable that surveillance for specific symptoms and diseases is performed by the most experienced specialist and depends on the expertise of available (sub)specialists.

7.3.1. Overall development
The previous guideline of Bree et al. suggested routine developmental screening in all children with BCNS.[14] Children with BCNS may have an increased risk of developmental delay. Intellectual disability has been noted in 4-21% of the BCNS cohorts.[35,50] In most countries, routine developmental screening has been incorporated in the public health care system. Early recognition of developmental delay can ensure that adequate intervention and/or support is available when needed.

Physicians should be aware of the possible increased risk of developmental delay and monitor the development of children with BCNS (GEC, very low; GSoR, strong)

7.3.2. Bone deformities
Bone deformities are often described as a feature of BCNS and qualify as a minor criterion (Table 2).[14,35] Macrocephaly, frontal bossing, (kypho)scoliosis, Sprengel deformity, pectus deformity, short fourth metacarpal and poly- and syndactyly

can be found on direct physical examination. Features such as rib anomalies and frontal bossing do not have clinical consequences but can contribute to the diagnosis. Other bone deformities such as (kypho)scoliosis and Sprengel deformity might need treatment.

Physicians should identify bone deformities with physical examination at diagnosis to make early intervention possible when needed. (GEC, very low; GSoR, strong)

7.3.3. Cardiac fibromas

Approximately 3-5% of all patients with BCNS develop a cardiac fibroma, a benign and usually asymptomatic cardiac tumour with a mean age of onset of 0-1 month.[14,35] Although cardiac fibromas typically present in infancy, rare manifestations of a late-onset cardiac tumour have been described.[51] If a cardiac fibroma results in ventricular outflow obstructions or chamber abolition, it might lead to conduction delays, arrhythmia or heart failure. In such cases, excision of the cardiac fibroma is necessary.[52]

All children with BCNS, suspicion of BCNS or children at risk should be screened with a cardiac ultrasound. If cardiac symptoms occur in a patient with BCNS, a cardiac ultrasound should be repeated to exclude a late onset cardiac tumour. (GEC, very low; GSoR, weak)

7.3.4. Medulloblastomas

A medulloblastoma is a malignant tumour developing from the cerebellum. In BCNS patients, medulloblastomas are mainly of the desmoplastic subtype and usually develop in the first 3 years of life.[53] The risk for medulloblastoma differs between heterozygous mutations in *PTCH1* and *SUFU*. In the recent literature, the estimated risk for developing medulloblastoma in patients with a *PTCH1* mutation was 1.2-2.4%, whereas in patients with heterozygous *SUFU* mutations the risk was estimated to be 20 times higher.[21,54,55] Screening for medulloblastoma using magnetic resonance imaging (MRI) often requires general anaesthesia in young children. The risks related to general anaesthesia in global development in young children are still under debate.[56] Moreover, the MRI screening procedure with general anesthesia can be stressful for parents and children.

In children with a *PTCH1* mutation, MRI should be considered when clinical symptoms or abnormal psychomotor development are present. However, routine MRI is not indicated. (GEC, low; GSoR, weak)

In cases where there is a clinical diagnosis without genetic testing or in children with a *SUFU* mutation a baseline MRI is recommended and should be repeated every 4 months until the age of 3 and twice per year until the age of 5. (GEC, low; GSoR, weak)

When BCNS is diagnosed in adulthood, a baseline brain MRI is not necessary. (GEC, low; GSoR, strong)

7.3.5. Ophthalmologic symptoms

Several eye abnormalities have been described in patients with BCNS (Table 2).[57-59] To prevent a disturbed development of the visual system and visual loss, early recognition and intervention are important for more common ocular symptoms such as strabismus, microphthalmia, congenital cataract, coloboma of the iris/choroid/optic nerve, nystagmus, anterior segment dysgenesis and glaucoma.[59]

In patients with BCNS, suspicion of BCNS or patients at risk, a baseline ophthalmological examination, including an ocular pressure measurement if possible, is recommended. (GEC, low; GSoR, strong)

7.3.6. Odontogenic keratocysts of the jaw

OKCs are benign and initially asymptomatic, but the typically slow progression may result into major tooth dislocation and even fractures of the jaw. Early detection enables adequate treatment, which may be crucial for maintaining jaw function.[60] OKCs of the jaw are present in 44-92%[35] of BCNS patients and start to develop around the age of 8, when the deciduous teeth begin to change.[61] Around the age of 22 the teeth are permanent and do not grow or change anymore. From that moment onwards, change in position of teeth will be noticed by a patient. It is reported that the development of OKCs tend to decrease after the age of 30.[61,62] Patients with a *PTCH1* mutation often develop multiple OKCs with a recurrence rate ranging from 15.4-50.0% and a mean time to reoccurrence of 32 months.[63-66] To date, no OKCs have been described in patients with a heterozygous *SUFU* mutation.[21,67-71] Screening with a orthopantomogram (OPG) is recommended, as it is easily accessible, has low radiation levels (0.010 mSv) and low costs. Screening using MRI can be considered in order to avoid radiation, but accessibility and costs may be limiting factors. For preoperative planning of the OKC, a cone-beam CT(0.05 mSv) or CT (2.1 mSv) is preferred, because of a higher spatial resolution.[72,73]

From the age of 8 years, only patients with a heterozygous *PTCH1* mutation should be screened for OKCs every two years with an OPG/MRI. (GEC, very low; GSoR, weak)

After the first OKC, follow-up with an OPG/MRI is recommended annually. (GEC, very low; GSoR, weak)

After the age of 22 years, follow-up can be continued by the dentist and additional OPG/MRI can be performed in cases where there is pain or unexplained positional change of the teeth. (GEC, very low; GSoR, weak)

7.3.7. Ovarian fibromas

Ovarian fibromas are estimated to occur in 13-60% of women with BCNS usually between 16 and 45 years.[35,61] In patients with BCNS, the ovarian fibromas are often bilateral and calcified and have a multifocal/multinodular growth pattern.[74] The ovarian fibromas are usually asymptomatic, do not affect fertility and rarely cause ovarian torsion.[75] In the absence of gynaecologic symptoms, surgical treatment is not advised as it might result in decreased fertility or early menopause by reducing the amount of viable ovarian tissue.[76,77] When surgical treatment is indicated in patients with a fertility desire, they should be counselled about minimal invasive methods to maintain future reproductive options.[76,77]

Gynaecological ultrasound examination and surveillance in nonsymptomatic patients is not strictly advised. In cases of abdominal complaints such as pain or menstrual irregularities, female patients should undergo gynaecological ultrasound examination to investigate the presence of an ovarian fibroma. (GEC, very low; GSoR, weak)

7.3.8. (Lympho)mesenteric cysts

The presence of (lympho)mesenteric cysts is a minor diagnostic criterion with an unknown specific frequency.[14] Only a few case reports have been published, but these types of cysts are probably under-reported in patients with BCNS.[78,79] (Lympho)mesenteric cysts are benign, intra-abdominal tumours which are usually asymptomatic and often an occasional finding, although cases with abdominal pain have been reported.[80] The cysts can be seen on ultrasound examination, MRI, and CT, but for definitive diagnosis, histological examination is necessary.[80] Surgical excision of cysts has been performed, but it is unknown whether this leads to a decrease in abdominal complaints.[78,80]

Physicians should screen for (lympho)mesenteric cysts with ultrasound examination in patients with BCNS and inexplicable abdominal pain. (GEC, very low; GSoR, strong)

7.3.9. Psychological distress

The phenotype of BCNS varies to a great extend but in general patients will need many hospital visits and undergo multiple (mutilating) surgical procedures. All of this can have a substantial impact on quality of life.[37,81] Patients and patient carer representatives from our GDG expressed a strong need for psychological support in patients and patient carers. The underlying reasons for this need of psychological support were mainly the chronic aspect of BCNS, the multiple (mutilating) surgeries and the fear of developing new symptoms requiring treatment. Furthermore, patients often feel misunderstood by society, their employer and friends or family. A patient-reported outcome questionnaire specifically developed to monitor the impact of BCCs in BCNS patients can be used to monitor the health-related quality of life and gain insight in the patient's perspective.[82,83]

Psychological evaluation for support and counselling after the diagnosis is recommended for all patients (and their families). During follow-up, physicians should pay attention to psychological distress and address the possibility of a psychological consultation (GEC, very low; GSoR, strong)

8.0 HOW SHOULD PATIENT CARE BE ORGANISED?

In a national survey in the United Kingdom, the care of only 15% of patients with BCNS was managed by a multidisciplinary team.[84] In our expert centre, children and adult patients with (suspicion for) BCNS are seen together by the dermatologist and clinical geneticist at the first consultation. For children, it is preferable that consultations with the genetics and developmental paediatrician and neurologist are planned to take place on the same day. During follow-up the composition of the multidisciplinary team varies; in childhood a (paediatric) neurologist and dermatologist are involved. From the age of 8 years, the dermatologist and oral and maxillofacial surgeon play a key role in the management due to a high prevalence of BCCs and OKCs. Multidisciplinary care in the same (academic) centre decreases the burden of multiple visits to the hospital. Moreover, a case manager can play an important role in counselling patients and can ensure that patients receive all the necessary surveillance appointments. Providing all care in expert centres will probably increase the quality of care, avoiding delayed or incorrect diagnosis, treatment and follow-up of symptoms.

To provide optimal care for patients with BCNS we advocate a multidisciplinary approach. (GEC, very low; GSoR, strong)

9.0 RECOMMENDED AUDIT POINTS

Data collection should be coordinated between centres and include details of management used for each case of BCNS and patient outcomes. For specialist centres, the following questions should be answered for each patient with BCNS:

1. Is the family history known and documented?
2. Has diagnostic genetic testing been performed and is the outcome known?
3. Has the patient received sun-protective advice and an explanation of the importance of sunscreen application and is he/she aware of disadvantages of radiological examination?
4. Have surveillance appointments been planned for the patient involving all relevant specialties mentioned in this guideline?

Table 4. Surveillance checklist by age category.

Basal cell nevus syndrome checklist

Specialism	Screen for	Physical and additional examination
Clinical geneticist	Dysmorphic features Genetic counselling	Physical examination Mutation analysis *including prenatal testing*
Genetics and developmental paediatrician	General growth and development	General physical examination
Neurologist	Medulloblastoma Neurological development	Neurological examination MRI-cerebrum
Dermatologist	BCCs, palmoplantar pits, basaloid follicular hamartomas, milia, epidermoid cysts	Total body inspection, including non-sun-exposed sites
Oral and maxillofacial surgeon	Odontogenic keratocysts of the jaw	Orthopantomogram
Gynaecologist	Ovarian fibromas Prenatal screening	Pelvic ultrasound Depends on facilities per country
Cardiologist	Cardiac fibroma	Cardiac ultrasound
Ophthalmologist	Cataract, glaucoma, coloboma	Ophthalmologic examination including ocular pressure measurement
Psychologist	Psychologic distress	Psychological examination

BCC, basal cell carcinoma; MRI, magnetic resonance imaging.

*The difference between *PTCH1* and *SUFU* is based on currently, sparse,

	Surveillance recommendations 0-8 years	**Surveillance recommendations 8-16 years**	**Surveillance recommendations >16 years**
	At time of diagnosis *Repeat at time of family planning decisions*	At time of diagnosis *Repeat at time of family planning decisions*	At time of diagnosis *In case of family planning decisions or in transition phase from paediatric to adult care*
	At time of diagnosis *Referral to paediatric orthopaedic surgeon or psychologist if indicated*	At time of diagnosis *Referral to paediatric orthopaedic surgeon or psychologist if indicated*	Not applicable
	SUFU patients: MRI cerebrum 4-montly until the age of 3 and twice per year until the age of 5* *PTCH1 patients*: no standard MRI*	If indicated	If indicated
	Yearly, *and after first BCC every 3-6 months depending on frequency of new BCCs*	Yearly, *and after first BCC every 3-6 months depending on frequency of new BCCs*	Yearly, *and after first BCC every 3-6 months depending on frequency of new BCCs*
	Not applicable	*PTCH1 patients*: At time of diagnosis and once per two years in case of no abnormalities* *SUFU patients:* no standard screening*	*PTCH1 patients*: At time of diagnosis and once per two years in case of no abnormalities until the age of 22* *SUFU patients:* no standard screening*
	Not applicable	If indicated	If indicated
	At time of diagnosis *Repeat if indicated*	At time of diagnosis *Repeat if indicated*	If indicated
	At time of diagnosis *Repeat if indicated*	At time of diagnosis *Repeat if indicated*	At time of diagnosis *Repeat if indicated*
	At diagnosis, continue if indicated	At diagnosis, continue if indicated	At diagnosis, continue if indicated

available information in literature which is more elaborately discussed in the manuscript and Supplementary Table 1.

10. FUTURE DIRECTIONS

As these guidelines demonstrate, there is need for high-quality evidence to refine screening indications for different symptoms. Genotype-phenotype studies revealed that the occurrence of medulloblastomas is higher in patients with a *SUFU* heterozygous mutation, whereas OKCs do not occur in patients with this genotype. As BCNS is a rare disease, international collaboration between expert centres is important to be able to merge data on genetic substantiated cohorts. Furthermore, there should be more awareness for patients without a genetic mutation, as this lack of mutation can either be attributed to genetic mosaicism or an unknown genetic cause. In some patients, there will be a desire for treatment of multiple BCCs with oral HPIs. However, the associated adverse events make oral HPIs not suitable for lifelong use. Topical HPIs have been developed, but results of an international placebo-controlled trial have to be awaited to be able to make claims about efficacy and safety of this new medication.

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SUPPLEMENTARY INFORMATION

List of abbreviations

BCC = basal cell carcinoma
BCCNS = basal cell carcinoma nevus syndrome
BCNS = basal cell nevus syndrome
CI = confidence interval
GEC = grade evidence of certainty
GSoR = grade strength of recommendation
MLPA = multiple ligation-dependent probe amplification
NMSC = non melanoma skin cancer
OKC = odontogenic keratocyst
OR = odds ratio
PCR = polymerase chain reaction
RCT = randomized controlled trial
RR = relative risk
SCC = squamous cell carcinoma
smMIP-NGS = small molecule molecular inversion probes-next generation sequencing
USA = United Stated of America

Supplementary Table 1. Summary of findings.
Should radiological examination be avoided as diagnostic tool?
Radiological examination for diagnostic criteria without therapeutic consequences should be avoided as much as possible. (GEC: very low , GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Knudson 2001)	Review on the evolution of the concept that cancer occurs as a consequence of several somatic mutations. The two-hit-hypothesis is explained by the tumour suppressor gene *RB1* which causes retinoblastoma at a young age.	Not a systematic review
(Karagas, McDonald et al. 1996)	Cohort of 1690 patients from the U.S.A. with ≥1 BCC or SCC that participated in a RCT of beta-carotene for prevention of NMSC and filled out questionnaires on previous radiation therapy. The association between previous therapeutic radiotherapy and a new histopathological confirmed SCC/BCC (annual follow-up for a median period of 4 years) was examined with cox proportional hazard ratios. Time to new first BCC was associated with previous radiotherapy (RR=1.7, 95% CI 1.4-2.0). There was a trend towards a higher BCC risk after radiotherapy at a younger age.	Serious Flawed measurement of exposure: 1. Radiotherapy based on patients' recall 2. No information concerning the radiation doses.
(Ron, Preston et al. 1998)	Histopathological confirmed cases of a first melanoma, NMSC and Bowen's disease between 1958-1987 in 79.972 people from the Hiroshima and Nagasaki tumour registries. A linear excess relative risk model was used per radiation dose. A significant excess relative risk (ERR) for 1Sv for BCC was found (ERR_{1Sv} = 1.8 (90% CI 0.83-3.33)). ERR was strongly associated with age at exposure (ERR_{1Sv} at age 30= 1.9 (90% CI 0.6-4.3) with the risk decreasing with 11% (90% CI 6-16%) with each additional year of age at exposure.	Serious Failure to adequately control confounding and accurate measurement of all prognostic factors.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness The primary goal of the review was not sufficient to substantiate our recommendation.	Not applicable	Undected	Very low
	Not applicable	Very serious indirectness Differences in population → no BCNS patients Differences in exposure → Therapeutic radiotherapy. It was specifically mentioned in the questionnaires that diagnostic X-rays should not be counted as radiotherapy.	Not applicable	Strongly suspected Data collected for a previous RCT and therefore unknown whether the analyses in this paper represent all or a fraction of the analyses performed.	Very low
	Not applicable	Serious indirectness Differences in population → probably no BCNS patients as only first skin cancers were included.	Not applicable	Strongly suspected Data collected automatically and therefore unknown whether the studies and analyses conducted represent all or a fraction of those conducted.	Very low

Supplementary Table 1. Continued.

Article	Summary	Study limitation/ risk of bias;
(Piotrowski, Kulcenty et al. 2017)	Review on the biological consequences of low-dose radiation and possible induction of cancer. It concludes that 'even though many studies point toward a link between carcinogenesis and exposure to radiation, the exact mechanism is still not clear.'	Not a systematic review

Should genetic confirmation be preferred?
If possible, we recommend performing genetic testing in all BCNS-suspected patients. (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Evans, Oudit et al. 2017)	In total 232 individuals from 94 families seen since the early 1980s, with clinical diagnosed BCNS, were available in the Manchester Centre for Genomic Medicine. Syndromic features were entered into a database. In 72 families (182 individuals) DNA was available for screening for germline *PTCH1* pathogenic variants. All negative families with available DNA then underwent screening for *SUFU* mutations. Patients with *SUFU* pathogenic variants were significantly more likely compared to *PTCH1* to develop medulloblastoma (33% vs. 2.4%), meningioma (22.2% vs. 1.6%) and ovarian fibroma (42.9% vs. 5.9%), but less likely to develop a jaw cyst (0% vs. 62.7%).	Very serious Flawed measurement of exposure: Unknown when information concerning the presence of syndromic features were collected. Flawed measurement of outcome: No adjustment for multiple testing within the same family.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness The primary goal of the review was not sufficient to substantiate our recommendation.	Not applicable	Undetected	Low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Evidence
	Not applicable	Very serious The article focused on differences in phenotype between patients with *PTCH1* and *SUFU* pathogenic variants. There was no recommendation concerning genetic testing in BCNS-suspected patients.	Not applicable	Undetected	Very low

Which steps should be followed in genetic confirmation of the diagnosis?
We recommend a stepwise approach that first includes genetic testing of the *PTCH1* gene. If no mutation is found, but the clinical suspicion is high, we advise testing for mutations in *SUFU*. If again no variant is found in the presence of a high clinical suspicion, DNA from ≥2 different BCCs can be isolated and genetically tested for *PTCH1* and *SMO* with sensitive Next Generation Sequencing technologies to examine the possibility of postzygotic mosaicism. If a variation is found, the relevance of the mutation and its consequences for the protein function should be verified. (GEC: very low , GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(Klein, Dykas et al. 2005)	From February 1997-May 2005 blood samples from 143 individuals were sent for testing *PTCH* mutations. A request for clinical information was included as a part of the laboratory's sample requisition form and a one-page patient and family history questionnaire was mailed to the referring physician. DNA sequence abnormalities were identified by PCR and sequencing of the coding exons of the *PTCH* gene. All mutations were confirmed by bidirectional sequencing. Twenty-seven of 46 pedigrees (58.7%) with ≥2 typical BCNS features tested positive for *PTCH* mutations.	No serious limitations to answer the question on frequency of *PTCH1* pathogenic variants after DNA sequencing.
(Marsh, Wicking et al. 2005)	BCNS families were ascertained through dermatologists, plastic surgeons, oral surgeons, ophthalmologists and clinical geneticists in Australia and New Zealand. All patients were examined by a geneticist and met 2/4 major diagnostic criteria (oral keratocysts, palmar/plantar pits, multiple/early onset BCCs, calcification falx cerebri). Twenty eight patients were analysed for mutations in *PTCH* with denaturing high performance liquid chromatography (DHPLC). Twenty of them were previously evaluated by single stranded conformation polymorphism analysis but found to be negative. Protein truncating (n=10) and missense or indel (n=4) were found in 14/28 (50%) cases.	No serious limitations to answer the question on frequency of *PTCH1* pathogenic variants in a previously negative *PTCH1* mutation population after single stranded conformation polymorphism analysis.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness Answers the question on frequency of *PTCH1* pathogenic variants with Sanger sequencing.	Not applicable	Undetected	Very low
	Not applicable	Very serious indirectness Answers the question on frequency of *PTCH1* pathogenic variants in a previously negative *PTCH1* mutation population after single stranded conformation polymorphism analysis.	Not applicable	Undetected	Very low

Article	Summary	Study limitation/ risk of bias;
(Soufir, Gerard et al. 2006)	Between 2003 and 2005 17 index cases who displayed ≥2 major criteria (multiple BCCs, palmo/plantar pits, cerebral calcifications, odontogenic keratocysts) or one major and 2 minor criteria by Shanley et al. (1994) were included. The 23 exons of PTCH1 coding sequence were amplified and subsequently sequence analysis was performed. Furthermore, *PTCH* deletion and microsatellite analyses and multiplex ligation-dependent probe amplification were performed. In 12/17 patients (70%) a germline mutation in *PTCH* was identified.	No serious limitations to answer the question on frequency of *PTCH1* pathogenic variants by sequencing and MLPA.
(Evans, Oudit et al. 2017)	In total 232 individuals from 94 families seen since the early 1980s with clinical diagnosed BCNS were available in the Manchester Centre for Genomic Medicine. Syndromic features were entered into a database. In 72 families (182 individuals) DNA was available for screening for germline *PTCH1* pathogenic variants by Sanger sequencing and multiple ligation-dependent probe amplification (MLPA) and for deep intronic pathogenic variants using RNA. All negative families with available DNA then underwent Sanger sequencing and MLPA of *SUFU*. In 43/72 families (60%), *PTCH1* pathogenic variants were identified. In 3/72 families (4%) *SUFU* pathogenic variants were identified. No pathogenic variant in either *SUFU* or *PTCH1* was identified in 26/72 families (36%).	No serious limitations to answer the question on frequency of *PTCH1* and *SUFU* pathogenic variants.
(Bholah, Smith et al. 2014)	EBV transformed lymphoblastoid cell lines were obtained from 10 individuals fulfilling diagnostic criteria (clinical assessment performed by one of the authors) for BCNS but without molecular confirmation of a *PTCH1* mutation after Sanger sequencing of *PTCH1* exons 2-23 including a splice variant of exon 1 and MLPA. Possibility of mosaicism was avoided by selecting affected individuals with ≥1 prior generation with BCNS. RNA analysis was performed to detect altered *PTCH1* transcipts. In 2/10 (20%) cases RNA analysis detected novel pathogenic splice variants in *PTCH1*.	No serious limitations to demonstrate deep intronic variants are missed by Sanger sequencing and MLPA and identified with RNA analysis.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness Answers the question on frequency of *PTCH1* pathogenic variants with Sanger sequencing and MLPA.	Not applicable	Undetected	Very low
	Not applicable	Very serious indirectness Answers the question on frequency of *PTCH1* and *SUFU* pathogenic variant.	Not applicable	Undetected	Very low
	Not applicable	Very serious indirectness Demonstrates that deep intronic variants of *PTCH1* can cause BCNS phenotype.	Not applicable	Undetected	Very low

Article	Summary	Study limitation/ risk of bias;
(Khamaysi, Bochner et al. 2016)	A case report of a middle-aged Arab man with multiple BCCs, pits and comedones in a segmental distribution over the upper part of the body. He also had short first and third digits in his left hand, retinal detachment, hyperlipidaemia and perforation of the sigmoid colon due to constipation. CT demonstrated calcification of the falx cerebri. No family with similar symptoms. DNA from peripheral blood lymphocytes and uninvolved skin did not reveal pathogenic variants of *PTCH1, PTCH2, SUFU* and *SMO*. A heterozygous mutation in *SMO* (c.1234C>T, p.L412F), but not in *PTCH1, PTCH2* or *SUFU,* was detected in 3 BCCs. In conclusion, the patient was diagnosed with a type I mosaic form of BCNS caused by a mutation in *SMO*.	Very serious Selective outcome reporting
(Reinders, Cosgun et al. 2019)	Case report of 2 cases. The first case had several BCCs on the right side of her body. Sanger sequencing of the coding exons of *PTCH1* detected no mutation. Mutation analysis with small molecule molecular inversion probes-next generation sequencing (smMIP-NGS) was carried out of 4 different BCCs, which revealed a shared exon 14 *PTCH1* mutation (c.2197_2198del) in all BCCs. No mutations were found in unaffected skin. These results identified type 1 segmental mosaicism of *PTCH1*. The second case had the clinical diagnosis of BCNS (BCCs, thoracic scoliosis, palmoplantar pits) no *PTCH1* mutation was found with Sanger sequencing and smMIP-NGS in DNA extracted from blood. Mutation analysis on RNA from 2 different BCCs showed a shared mutation in PTCH1 (c.2460C>G, p.(Tyr820*)) with Sanger sequencing and smMIP-NGS. Reinterpretation of the smMIP-NGS on blood by visual inspection detected the mutation in 1% of the sequence reads. These results are indicative for type I postzygotic mosaicism.	Very serious Selective outcome reporting

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness Answers the question if somatic mosaicism in *SMO* is possible in a clinically diagnosed BCNS patient.	Not applicable	Suspected	Very low
	Not applicable	Very serious indirectness Case reports shows that somatic type I postzygotic and segmental mosaicism in *PTCH1* is possible in clinically diagnosed BCNS patients.	Not applicable	Suspected	Very low

Article	Summary	Study limitation/ risk of bias;
(Torrelo, Hernandez-Martin et al. 2013)	Case report of a 12-year old girl who had unilateral (right) segmentally arranged basaloid skin tumours present since birth, ipsilateral large palmoplantar pits distributed along Blaschko lines and ipsilateral odontogenic keratocysts and a family history of BCNS and several other BCNS symptoms. Complete sequencing of *PTCH1* was carried out on blood samples from the patient and her father and in skin samples from the patient's affected and unaffected skin. A P*TCH1* mutation was identified in exon 18 (p.Y1021C) in all samples studied. A second mutation in exon 3 of *PTCH1* (c.543_549delGGCACTC, (p.S181SfsX36)) was detected in 2 BCCs and 1 basaloid hamartoma on the right side of the skin but not in the unaffected skin and blood samples from both the patient and her father. In conclusion, the patient was diagnosed with type II segmental mosaicism.	Very serious Selective outcome reporting.
(Richards, Aziz et al. 2015)	Guideline from a workgroup formed in 2013 consisting of members from the American College of Medical Genetics (AMCG) and Genomics, the Association for Molecular Pathology (AMP) and the College of American Pathologists and surveys to 100 sequencing laboratories in the United States and Canada requesting input and sharing their protocols regarding variant interpretation. After developing the system it was again tested amongst the previous 100 laboratories and 2000 members of the AMP and a workshop with >50 attendees at an AMP meeting. Recommendations from other professional societies and workings groups were also evaluated. Guideline describes the interpretation process of found sequence variants.	Serious There is no comparison with other variant classifications.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness Case report shows that somatic type II segmental mosaicism in *PTCH1* is possible in a clinically diagnosed BCNS patient with a severe phenotype.	Not applicable	Suspected	Very low
	Not applicable	Serious indirectness Not specifically developed for BCNS patients.	Not applicable	Undetected	Very low

There is insufficient evidence for genetic testing of *PTCH2*. (GEC: low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Casano, Meddaugh et al. 2020) (Altaraihi, Wadt et al. 2019) (Fujii, Ohashi et al. 2013) (Fan, Li et al. 2008)	Two patients, a father and son were heterozygous for a *PTCH2* mutation, c.3347C>T, p.(Pro1116Leu) without fulfilling the diagnostic criteria for BCNS. Two patients, 39 year old mother and 18 year old daughter, were homozygous and heterozygous for a *PTCH2* mutation, c.269delG, p.(Gly90Alafs*4) in exon 3 leading to frameshift and premature stopcodon. Both did not present with features of BCNS. Furthermore, a review of all *PTCH2* reports in the literature (n=3) was provided. None of the found variants led to clinical diagnosis of BCNS defined by criteria of Evans et al. One *PTCH2* missense variant was found in a clinical BCNS patient, but it was present together with a *PTCH1* variant. The second *PTCH2* missense variant was found in 6 family members, but none of them were affected with BCNS. The third variant (frameshift) was found in a 13 year old female who developed jaw cysts and bifid ribs. This variant was also found in multiple alleles in the genome aggregation database, including 1 homozygous individual.	Serious Screening for all major criteria besides calcification of the falx cerebri was performed in the 39-year old mother. Nevertheless, no clinically relevant BCNS-symptoms were found. No details of the literature search have been given, although we suspect chances are very small that any article has been missed. Selective outcome reporting

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Suspected	Low

7.2 What should dermatologists be aware of?
Adequate sun-protective measures are very important and should be discussed during every visit. (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/risk of bias;
(Solis, Kwon et al. 2017)	Prospective registry (September 2014-March 2016) of 141 BCNS patients (94% from the U.S.A.), mean age 53 years; 93% of participants were white. In a multivariable analysis number of sunburns had an OR of 1.06 (95% CI 1.00-1.11) for a higher number of BCCs.	Very serious Biased patient population. Recall bias concerning the number of times a patient got sunburned. Sunburned was not objectified. Possible failure to adequately control confounding.
(Waldman and Grant-Kels 2019)	Retrospective 33-question survey study amongst BCNS patients, unknown how many were contacted, 47 patients responded. A trend towards a decrease in number of BCCs with an increase in childhood and current sunscreen use was seen.	Very serious Recall bias concerning sunscreen use in the past. The amount of sunscreen and SPF was not specified. Very small patient population, unknown how many patients were contacted, biased patient population. Patients who phenotypically develop more BCCs might be more likely to be more adherent to sunscreen use.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness	Not applicable	Strongly suspected Various possible risk factors that were investigated.	Very low
	Not applicable	Serious indirectness	Not applicable	Strongly suspected Various possible risk factors that were investigated.	Very low

Total body inspection, including non-sun-exposed sites, is recommended annually until the development of the first BCC. From that moment on the follow-up frequency should be intensified to up to every three to six months, depending on the number and frequency of new BCCs. (GEC: very low , GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Peris, Fargnoli et al. 2019)	European guidelines for diagnosis and treatment of BCC, based on the updated EDF guideline, German S2k guidelines, French guidelines, British association of Dermatologists' guidelines and de novo literature search by Medline. Methodology was based on the AGREE II instrument and levels of evidence were graded according to the Oxford classification. A structured consensus process was used to discuss and agree upon recommendations in 2018. Stakeholders were European Dermatology Forum, European Association of Dermato-Oncology, European Organization for Research and Treatment of Cancer and 24 experts from 11 countries, all of whom were delegates of national and/or international medical societies. Section 7 is dedicated to the diagnosis and management of patients with BCNS. The guidelines state: "Follow-up is recommended in patients with BCNS. However, skin examination should be scheduled on an individual basis." Skin examination should be carried out every 4-6 months.	Serious No literature that substantiates the recommendation.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low

Article	Summary	Study limitation/ risk of bias;
(Tom, Hurley et al. 2011)	A 65-question survey study sent to BCNS patients through the BCCNS Life Support Network. Confirmation of the diagnosis BCNS was made by the diagnostic criteria of Kimonis et al. (1997) and Ahn et al. (2004). A subset of the patients received a full skin examination during the Basal cell Nevus Syndrome Colloquium at which also patients participated. Sixty-one patients responded, 85% reported a positive history of BCC. Median age of first BCC was 16 years (range 2-34 years). Twenty-six patients reported >100 BCCs. Forty-one patients underwent full physical examination (Fitzpatrick skin type ranging from I-III) which revealed a small percentage of BCCs on the foot/groin/ buttocks.	Serious Flawed inclusion of patients: Likely only severe patients responded to the questionnaire Flawed measurement of outcome: Number and location of BCCs mainly based on patients recall Measurement of location of BCCs based on one point in time

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness. Only provides information concerning the amount, age of onset and location of BCCs in patients with BCNS. This study does not answer the question on how frequent follow-up should be performed.	Not applicable	Suspected Unknown if presented analyses represent all of a fraction of those conducted.	Very low

Radiotherapy is relatively contra-indicated (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Peris, Fargnoli et al. 2019)	European guidelines for diagnosis and treatment of BCC, based on the updated EDF guideline, German S2k guidelines, French guidelines, British association of Dermatologists' guidelines and de novo literature search by Medline. Methodology was based on the AGREE II instrument and levels of evidence were graded according to the Oxford classification. A structured consensus process was used to discuss and agree upon recommendations in 2018. Stakeholders were European Dermatology Forum, European Association of Dermato-Oncology, European Organization for Research and Treatment of Cancer and 24 experts from 11 countries, all of whom were delegates of national and/or international medical societies. Section 7 is dedicated to the diagnosis and management of patients with BCNS. The guidelines state: "Radiotherapy is not recommended because of the carcinogenic effect of x-rays resulting in the formation of new BCCs."	Very serious No literature that substantiates the recommendation.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low

Article	Summary	Study limitation/ risk of bias;
(Baker, Joseph et al. 2016)	A 65-year-old male BCNS patient was treated with 50 Gy in 20 fractions during 4 weeks. No secondary malignancy after 57 months follow-up. Furthermore, 15 cases have been reported in English literature which are listed in the article. During follow-up ranging between 14 months and >40 years, 6 cases developed new BCCs in the irradiated area and 9 did not.	Very serious Flawed measurement of exposure: 1. Not in all cases the dose of radiation therapy was known. Flawed measurement of outcome: 1. In several cases no information on the area of the new developed BCCs was provided. Failure to adequately control confounding: 1. No information in any case on the frequency of developing new BCCs within a patient. Enormous differences between the cases in the duration of follow-up.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness	Not applicable	Strongly suspected Cases who develop BCCs after radiation therapy are more likely not to be published (negative/ expected result)	Very low

Treatment with oral HPIs can be considered for the treatment of multiple BCCs (GEC: moderate, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Tang, Mackay-Wiggan et al. 2012, Tang, Ally et al. 2016)	Randomized, double-blind, placebo controlled trial to investigate the efficacy of vismodegib 150mg daily for the indication of multiple basal cell carcinomas in 41 BCNS patients.	No serious limitations for efficacy and adverse events
(Dreno, Kunstfeld et al. 2017)	Randomized, regimen-controlled, double-blind trial of two intermittent vismodegib dosing regiments in 229 multiple BCC patients (of which 85 patients had BCNS).	No serious limitations for efficacy and adverse events
(Lear, Hauschild et al. 2020)	Exploratory double-blind, randomized trial of sonidegib 400mg daily versus placebo in 9 patients with BCNS.	Serious limitations for efficacy and adverse events Short treatment period (16 weeks) Small sample size
(Verkouteren, Wakkee et al. 2021)	Retrospective cohort study of 80 patients that were treated with vismodegib for basal cell carcinoma in the Netherlands between 2011 and 2019. It includes a section on 19 BCNS patients treated for the indication multiple basal cell carcinoma with vismodegib 150mg/daily and various dosing schedules	Serious limitations for efficacy and adverse events No internal control Progression was defined as development of new/recurrent BCC, no tumour count has been performed. No information on adverse events available specifically for BCNS patients

Efficacy and adverse events are described in supplementary Tables III-IV.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Suspected Industry driven	See supplementary Tables III-IV.
	Not applicable	Serious indirectness Only 37% of the study population had BCNS and no additional analyses were performed for efficacy in patients with BCNS. It is expected that those patients have a higher efficacy.	Not applicable	Suspected Industry driven	See supplementary Tables III-IV.
	Not applicable	No indirectness	Not applicable	Suspected Industry driven, small RCT	See supplementary Tables III-IV.
	Not applicable	No indirectness	Not applicable	Suspected Small cohort study	See supplementary Tables III-IV.

Tumour reoccurrence after discontinuation of hedgehog pathway inhibitor in patients with BCNS.

Article	Summary	Study limitation/ risk of bias;
(Tang, Mackay-Wiggan et al. 2012, Tang, Ally et al. 2016)	Phase-2 double-blind RCT that compares vismodegib with placebo in 41 patients with BCNS and >2000 BCCs. During treatment breaks multiple BCCs reoccurred, but no exact number or percentage was provided in the primary and long-term result papers.	Very serious It is only mentioned in 1 sentence in the manuscript that multiple BCCs reoccurred without any measurements of this outcome.
(Sinx, Roemen et al. 2018)	Case report concerning a BCNS patient who received treatment with 150mg vismodegib for 3 years for the treatment of multiple BCCs. Two months after vismodegib discontinuation, >3 BCCs reoccurred at their pre-treatment locations.	Very serious Selective outcome reporting
(Valenzuela-Onate, Magdaleno-Tapial et al. 2020)	Case series about 3 BCNS patients in whom at least 19 BCCs developed within 2 years after discontinuing vismodegib treatment (unknown treatment duration).	Very serious Selective outcome reporting
(Wolfe, Green et al. 2012)	Case report concerning a BCNS patients who received 7 months of treatment with vismodegib. Two years after discontinuing treatment, 10/19 BCCs on the head and neck reoccurred.	Very serious Selective outcome reporting

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Strongly suspected Industry driven, small RCT	Very low
	Not applicable	No indirectness	Not applicable	Strongly suspected	Very low
	Not applicable	No indirectness	Not applicable	Strongly suspected	Very low
	Not applicable	No indirectness	Not applicable	Strongly suspected	Very low

Overall development

Physicians should be aware of the possible increased risk of developmental delay and monitor the development of children with BCNS (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Bree, Shah et al. 2011)	International BCNS colloquium on May 2005 with 55 patients and their families and medical, dental, and research experts from various countries. On the first day of the conference subspecialty literature reviews were presented to the group by faculty members. A survey was conducted among patients. The second day included a panel discussion to develop updated protocols for diagnosis and surveillance after specific questions. One specific questions was; what changes should be considered to the surveillance protocol for paediatric patients with BCNS? "Routine developmental screening with well-child visits should be conducted. If a patient fails screening or not meets milestones or in case of school age and difficulty with learning in school, further cognitive evaluation and/developmental assessment and testing is warranted."	Serious. No details regarding the literature review on which the recommendation is based are provided.
(MacDonald 2015)	A systematic review of the literature was performed for clinical and radiologically apparent features of BCNS patients. The systematic review included 14 case series (7 from Asia and 7 from Europe/Canada/United States/Australia). Prevalence of mental retardation ranged between 4-21% and was mentioned in 6 case series. In the remaining 8 case series no information on mental retardation was given. No predilection was found for the origin of the patients.	Serious Flawed measurement of outcome 1. No definition of mental retardation is given 2. Unknown how mental retardation was measured in the individual case series.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low
	Not applicable	Very serious The primary goal of the systematic review was not sufficient to substantiate our recommendation. Screening and surveillance were not mentioned in this review.	Not applicable	Suspected	Very low

Article	Summary	Study limitation/ risk of bias;
(Shiohama, Fujii et al. 2017)	Retrospective case-control study concerning 9 children with BCNS, diagnosed according to Kimonis' clinical criteria and 15 healthy control children. Morphological brain analysis was performed using MRI. In 8/9 patients a *PTCH1* mutation was confirmed, 1 refused mutational analysis. BCNS patients had a larger cerebrum, with more wide frontal horns of the lateral ventricles, a thicker corpus callosum, a larger brain stem and larger cerebellum compared to healthy controls. These results suggest that sonic hedgehog signalling affects human brain morphology.	Very serious Flawed measurement of outcome 1. Unknown if measurements were performed by an investigator that was blinded.

Bone deformities

Physicians should identify bone deformities with physical examination at diagnosis to make early intervention possible when needed. (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Bree, Shah et al. 2011)	International BCNS colloquium on May 2005 with 55 patients and their families and medical, dental, and research experts from the U.S.A. and internationally. On the first day of the conference subspecialty literature reviews were presented to the group by faculty members. A survey was conducted among patients. The second day included a panel discussion to develop updated protocols for diagnosis and surveillance after specific questions. One specific questions was; what changes should be considered to the surveillance protocol for paediatric patients with BCNS? "A baseline spine film at age 1 or at time of diagnosis is recommended."	Very serious No details regarding the literature review were provided. No further consideration for the recommendation was given in the guideline.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious The primary goal was to examine the brain morphological characteristics of children with BCNS.	Not applicable	Strongly suspected Various measurements were performed and tested.	Very low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low

Article	Summary	Study limitation/ risk of bias;
(Shiohama, Fujii et al. 2017)	A systematic review of the literature was performed for clinical and radiologically apparent features of BCNS patients. The systematic review included 14 case series (7 from Asia and 7 from Europe/Canada/United States/Australia). Prevalence of several bone deformities was: rib anomalies (18-91%), scoliosis/vertebral anomalies (4-91%), syndactyly/polydactyly (0-33%), shorth 4th metacarpal (4-29%), sprengel deformity (3-12%), pectus deformity (6-23%). Not all bone deformities were described in all 14 case series.	Serious Flawed measurement of outcome 1. Unknown if all patients were examined for bone deformities in the individual case series.

Cardiac fibromas

All children with BCNS, suspicion of BCNS or children at risk should be screened with a cardiac ultrasound. If cardiac symptoms occur in a BCNS adult a cardiac ultrasound should be repeated to exclude a late onset cardiac tumour. (GEC: very low, GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(MacDonald 2015)	A systematic review of the literature was performed for clinical and radiologically apparent features of BCNS patients. The systematic review included 14 case series (7 from Asia and 7 from Europe/Canada/United States/Australia). Prevalence of cardiac fibroma ranged between 2-4% and was mentioned in 3 case series. In the remaining 11 case series no information on cardiac fibroma was given.	Serious Flawed measurement of outcome 1. Unknown if and how all patients were examined for cardiac fibromas in the individual case series.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious The primary goal of the systematic review was not sufficient to substantiate our recommendation. Screening and surveillance were not mentioned in this review.	Not applicable	Suspected	Very low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious The primary goal of the systematic review was not sufficient to substantiate our recommendation. Screening and surveillance were not mentioned in this review.	Not applicable	Suspected	Very Low

Article	Summary	Study limitation/ risk of bias;
(Bree, Shah et al. 2011)	International BCNS colloquium on May 2005 with 55 patients and their families and medical, dental, and research experts from the U.S.A. and internationally. On the first day of the conference subspecialty literature reviews were presented to the group by faculty members. A survey was conducted among patients. The second day included a panel discussion to develop updated protocols for diagnosis and surveillance after specific questions. Two specific questions were; what changes should be considered to the surveillance protocol for paediatric and for adult patients with BCNS? "A baseline cardiac ultrasound was recommended for children as it was considered to be a reasonable, non-invasive test to rule out a potentially life-threatening cardiac fibroma; although it was felt to be of potentially low yield."	Serious No details regarding the literature reviews were provided.
(Miyake, Del Nido et al. 2011)	A single-centre retrospective review of 173 patients ≤21 years diagnosed with a primary cardiac tumour between 1968 and 2010. The diagnosis had to be made based on an echocardiogram and/or MRI or angiography in the years before the abovementioned non-invasive imaging methods were available. A total of 25 patients were diagnosed with a cardiac fibroma, with presenting symptoms being arrhythmia (32%), murmur (20%), abnormal chest x-ray (20%). Sixteen patients had documented ventricular tachycardia (VT). In 13 patients with VT the fibroma was excised. Of the 3 patients with VT who did not undergo resection, 2 continue to have nonsustained VT while receiving antiarrhythmic medication but are asymptomatic and the third improved over a 35-year period to a pattern of low-grade ectopy off medication.	Very serious Flawed measurement of the outcome 1. Patients without any symptoms are more likely to be excluded from this review and therefore the frequency of patients with arrhythmias is overestimated.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low
	Not applicable	Very serious The primary goal of the review was not sufficient to substantiate our recommendation. Screening and surveillance were not mentioned in this review.	Not applicable	Undetected	Very low

Article	Summary	Study limitation/ risk of bias;
(Kluger, Marco-Baertich et al. 2007)	A case report of a woman with BCNS since the age of 15 years (frameshift *PTCH1* mutation, c942delC on exon 6) and presented at the age of 59 with several episodes of syncope. Cardiac examination and electrocardiography showed no abnormalities. MRI revealed a mass compatible with a cardiac fibroma. This was confirmed with a cardiac ultrasound.	Very serious Unknown since when the cardiac fibroma was present. Selective outcome reporting.

Medulloblastomas

In children with a *PTCH1* mutation MRI should be considered when clinical symptoms or abnormal psychomotor development are present. However, routine MRI is not indicated. (GEC: low, GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(Evans, Oudit et al. 2017)	In total 232 individuals from 94 families seen since the early 1980s with clinical diagnosed BCNS were available in the Manchester Centre for Genomic Medicine. Syndromic features were entered into a database. In 72 families (182 individuals) DNA was available for screening for germline *PTCH1* pathogenic variants by Sanger sequencing and multiple ligation-dependent probe amplification (MLPA) and for deep intronic pathogenic variants using RNA. Of all 126 *PTCH1* patients, 3 (2.4%) developed a medulloblastoma.	Serious Flawed measurement of outcome: 1. Information concerning the presence of syndromic features were collected at a single point in time.
(Verkouteren, Cosgun et al. 2021)	A retrospective cohort study in two centres in the Netherlands who conducted *PTCH1* analysis for all clinical requests from the Netherlands between April 1999 and December 2015. Analysis was done by Sanger sequencing and multiple ligation-dependent probe amplification (MLPA). Patients with a pathogenic *PTCH1* mutation were selected and information of the presence of medulloblastoma was retrieved from the medical records. Clinical data were available for 81 patients, of whom 1 (1.2%) developed a medulloblastoma at the age of 11 months.	Minimal Flawed measurement of exposure: 1. No RNA analysis for deep intronic pathogenic *PTCH1* variants was performed.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious It is unknown whether the cardiac fibroma was already present during childhood.	Not applicable	Strongly suspected	Very low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness Provides information concerning the frequency of medulloblastoma in patients with *PTCH1* pathogenic variants.	Not applicable	Undetected	Low
	Not applicable	Serious indirectness Provides information concerning the frequency of medulloblastoma in patients with *PTCH1* pathogenic variants.	Not applicable	Undetected	Low

Article	Summary	Study limitation/ risk of bias;
(Foulkes, Kamihara et al. 2017)	Expert consensus recommendation based upon literature review and a discussion in the AACR Childhood Cancer Predisposition Workshop held in Boston, Massachusetts, in 2016. Based upon the risk of <2% for medulloblastoma in *PTCH1* individuals from Smith et al. (2014), the following is recommended: no radiographic screening unless concerning neurologic exam, head circumference change, or other unusual signs or symptoms.	Minimal No details on literature review were given.
(Vutskits and Davidson 2017)	Review on the long-term impact of general anesthesia on the developing brain. Both experimental and human observations/studies have been performed with mixed evidence. The review concludes there is experimental evidence suggesting that administration of general anesthetics during early postnatal life can induce a variety of lasting morphological and functional changes in the developing central nervous system. The underlying mechanisms have been partially revealed. However, population-based human epidemiological studies provide mixed evidence. A prospective trial and ambidirectional cohort study using psychometric outcome measures did not show an association between short anesthesia exposures and neuromorbidity.	Minimal No details on literature review are given.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low
	Not applicable	Very serious Indirectness in population. Non-human studies and studies on children without BCNS. Provides information on long-term effect of general anaesthesia necessary to perform MRI in young children.	Not applicable	Undetected	Low

In case of a clinical diagnosis without genetic testing or in children with a *SUFU* mutation a baseline MRI is recommended and should be repeated every 4 months until the age of 3 and twice per year until the age of 5. (GEC: low, GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(Smith, Beetz et al. 2014)	A study that aimed to find other mutations causative for clinical BCNS diagnosis in patients without a PTCH1 mutation from an English cohort. Through Sanger sequencing and MLPA of *SUFU* a mutation was found in 9 individuals from 3 families. Three patients developed a medulloblastoma, all before the age of 3.	Very serious Flawed measurement of exposure: 1. Information concerning the presence of syndromic features were collected at a single point in time.
(Foulkes, Kamihara et al. 2017)	Expert consensus recommendation based upon literature review and a discussion in the AACR Childhood Cancer Predisposition Workshop held in Boston, Massachusetts, in 2016. The following is recommended for patients with a *SUFU* mutation: consider every-4-month brain MRI through age 3 and then every-6-month brain MRI until the age of 5. It is noted that there are currently no data available to support optimal frequency and timing of imaging.	Minimal No details on literature review are given.
(Kool, Jones et al. 2014)	An international cohort of sonic-hedgehog-driven medulloblastomas was sequenced. In 116/133 cases a known sonic hedgehog mutation was detected. Six germline *SUFU* mutations were found. All six patients developed a medulloblastoma under the age of 3 years.	Minimal Germline information was available in 45 patients.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness Provides information concerning the frequency and age of onset of medulloblastoma in patients with a *SUFU* pathogenic variant.	Not applicable	Undetected	Low
	Not applicable	No indirectness	Not applicable	Undetected	Low
	Not applicable	Serious indirectness Provides information concerning the frequency and age of onset of medulloblastoma in patients with a *SUFU* pathogenic variant.	Not applicable	Undetected	Low

(Vutskits and Davidson 2017)	Review on the long-term impact of general anesthesia on the developing brain. Both experimental and human observations/studies have been performed with mixed evidence. The review concludes there is experimental evidence suggesting that administration of general anesthetics during early postnatal life can induce a variety of lasting morphological and functional changes in the developing central nervous system. The underlying mechanisms have been partially revealed. However, population-based human epidemiological studies provide mixed evidence. A prospective trial and ambidirectional cohort study using psychometric outcome measures did not show an association between short anesthesia exposures and neuromorbidity.	Minimal No details on literature review are given.

When BCNS is diagnosed in adulthood, a baseline brain MRI is not necessary. (GEC: low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Kool, Jones et al. 2014)	An international cohort of sonic-hedgehog-driven medulloblastomas was sequenced. In 116/133 cases a known sonic hedgehog mutation was detected. Six germline *SUFU* mutations were found. All six patients developed a medulloblastoma under the age of 3 years. Two patients harboured a *PTCH1* germline mutation and developed a medulloblastoma (1 <3 years and 1 between 4-17 years).	Minimal Specific age of onset of medullobastoma was unknown.
(Smith, Beetz et al. 2014)	A study that aimed to find other mutations causative for clinical BCNS diagnosis in patients without a PTCH1 mutation from an English cohort and to reassess the risk for medulloblastoma. Through Sanger sequencing and MLPA of *SUFU*, a mutation was found in 9 individuals from 3 families. Three *SUFU* patients and two *PTCH1* patients developed a medulloblastoma, all before the age of 3. An additional patient that did not get tested but had a clinical diagnosis also developed a medulloblastoma before the age of 3 years.	None

	Not applicable	Very serious Indirectness in population. Non-human studies and studies on children without BCNS. Provides information on long-term effect of general anaesthesia necessary to perform MRI in young children.	Not applicable	Undetected	Low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness Provides information regarding the age of onset of medulloblastoma in patients with a germline *PTCH1* and *SUFU* pathogenic variants.	Not applicable	Undetected	Low
	Not applicable	Serious indirectness Provides information regarding the outcome age of onset of medulloblastoma in patients with a *SUFU* pathogenic variant.	Not applicable	Undetected	Low

Ophthalmologic symptoms

In patients with BCNS, suspicion of BCNS or patients at risk, a baseline ophthalmological examination, including an ocular pressure measurement if possible, is recommended. (GEC: low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias;
(Jen and Nallasamy 2016)	A review on ocular findings in genetic skin disorders. In BCNS the following ocular manifestations can be found: periocular basal cell carcinoma, hypertelorism, exophthalmos, iris/ uveal/optic nerve colobomas, microphthalmia, nystagmus, strabismus, anterior segment dysgenesis, congenital cataracts, glaucoma, eyelid epidermal or dermal cysts, orbital cysts, myelinated nerve fibers, epiretinal membranes, macular hole, retinal hamartomas and milia. It is recommended to refer all BCNS patients to an ophthalmologist at diagnosis for a screening and follow-up according to the significance of the found manifestations.	Serious No literature review is provided.
(Chen, Sartori et al. 2015)	A case report and review of literature on ocular findings in patients with BCNS. A literature search was performed in PubMed between 1984-2014, 33 articles were found of which 31 were included. The following manifestations are described: periocular BCC, hypertelorism, strabismus (which can cause irreversible visual loss), myelinated nerve fibers, retinal abnormalities, eyelid cysts, microphthalmia, congenital cataracts, anerior segment dysgenesis, nystagmus, colobmas of the optic nerve/ iris/choroid, congenital glaucoma and iris transillumination defects.	Minimal

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness This study summarizes possible ocular findings and based on these findings recommends screening for all BCNS patients.	Not applicable	Undetected	Low
	Not applicable	Very serious This study summarizes possible ocular findings and their frequency.	Not applicable	Undetected	Low

Article	Summary	Study limitation/ risk of bias;
(Moramarco, Himmelblau et al. 2019)	An observational cross-sectional study on 11 BCNS patients (all *PTCH1* heterozygotes). All patients went through a complete ophthalmological examination including history, best-corrected visual acuity, intraocular pressure measurement using Goldmann applanation tonometry after topical anesthetic drops application, slit-lamp biomicroscopy, mydriatic indirect fundus biomicroscopy and Spectral domain optical coherence tomography. Mean age was 38.5 years, 82% had myopia, 63% strabismus, 45.5% hypertelorism, 18% congenital cataract, 9% glaucoma. Fundus examination showed myelinated nerve fiber in 36% and coloboma of the optic nerve in 9%.	Serious Unknown how cases were selected

From the age of 8 only heterozygous *PTCH1* patients should be screened for OKCs every two years with an orthopantomogram (OPG)/MRI. (GEC: very low, GSoR: weak)
Evidence supporting that screening only applies to *PTCH1* and not *SUFU* heterozygous patients:

Article	Summary	Study limitation/ risk of bias;
(Evans, Oudit et al. 2017)	In total 232 individuals from 94 families seen since the early 1980s with clinical diagnosed BCNS were available in the Manchester Centre for Genomic Medicine. Syndromic features were entered into a database. In 72 families (182 individuals) DNA was available for screening for germline *PTCH1* pathogenic variants by Sanger sequencing and multiple ligation-dependent probe amplification (MLPA) and for deep intronic pathogenic variants using RNA. All negative families with available DNA then underwent Sanger sequencing and MLPA of *SUFU*. In total 79/126 (62.7%) patients with a *PTCH1* variant developed odontogenic jaw keratocysts. None of the 9 individuals with a *SUFU* variant developed odontogenic jaw keratocysts. Jaw cysts were ascertained by orthopantogram screening.	Very serious Flawed measurement of outcome: Unknown when information concerning the presence of syndromic features were collected.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious This study summarizes possible ocular findings and their frequency.	Not applicable	Undetected	Low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness Provides evidence for the outcome frequency of odontogenic jaw keratocysts in patients with a *PTCH1* and *SUFU* pathogenic variant.	Not applicable	Undetected	Low

Article	Summary	Study limitation/ risk of bias;
(Cosgun, Reinders et al. 2020)	Multicentre retrospective cohort study of individuals with a *PTCH1* variant between 1999 and 2015. Data was shown for 78 patients with 2 or more symptoms of BCNS. In total 71/78 (91%) patients with a *PTCH1* variant had odontogenic keratocysts of the jaw.	Very serious Flawed measurement of outcome: Unknown when information concerning the presence of syndromic features were collected and how this was done.
(Huq, Walsh et al. 2018)	A case report concerning a Caucasian family with multiple BCCs, meningiomas and a medulloblastoma. In two sisters (58 and 62-years-old) a heterozygous splice site variant of *SUFU* (c.1365+2T?A) was detected. None of the family members developed odontogenic keratocysts of the jaw.	Very serious Flawed measurement of outcome: No screening for odontogenic keratocysts of the jaw was performed.
(Kijima, Miyashita et al. 2012)	A case report concerning two patients with BCNS who developed a meningioma. In one of the cases a germline nonsense mutation in *SUFU* (c.550C>T) was found. This patient (35-year-old male) had not developed odontogenic jaw keratocysts.	Serious Flawed measurement of outcome: A head CT was performed to identify intracerebral tumours, but nothing was mentioned regarding the presence or absence of odontogenic keratocysts of the jaw.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness Provides evidence for the outcome frequency of odontogenic jaw keratocysts in patients with a *PTCH1* pathogenic variant.	Not applicable	Undetected	Low
	Not applicable	Very serious indirectness Absence of odontogenic jaw keratocysts in patients with a *SUFU* pathogenic variant.	Not applicable	Strongly suspected	Very low
	Not applicable	Very serious indirectness Absence of odontogenic jaw keratocysts in a patient with a *SUFU* pathogenic variant.	Not applicable	Strongly suspected	Very low

Article	Summary	Study limitation/ risk of bias;
(Mann, Magee et al. 2015)	Case report concerning a 55-year-old woman with macrocephaly, hypertelorism and facial papules and with 2 children who developed medulloblastoma. Sequencing revealed a splice-site mutation in *SUFU* (c.756+1G>A). A skull radiograph did not show evidence of calcification of the falx cerebri. Nothing was mentioned about odontogenic keratocysts of the jaw.	Very serious Flawed measurement of outcome: No screening for odontogenic keratocysts of the jaw was performed.
(Pastorino, Ghiorzo et al. 2009)	A case report concerning a Caucasian family with a child that developed medulloblastoma at 8 months old. The 37-year-old father had pitting of the soles and calcification of the falx cerebri, no other radiological screening was performed. The mother did not show any clinical manifestations of BCNS. In DNA of both the father and son a germline splice site mutation in *SUFU* (c.1022+1G>A) was detected.	Very serious Flawed measurement of outcome: No screening for odontogenic keratocysts of the jaw was performed.
(Schulman, Oh et al. 2016)	Case report concerning a 60-year-old woman with multiple infundibulocystic basal cell carcinomas. Radiographic study did not reveal any jaw cysts. A splice acceptor site mutation in *SUFU* was detected (c.757-2A>G) in the infundibulocystic BCCs and in the normal tissue sample.	Very serious Flawed measurement of outcome: No screening for odontogenic keratocysts of the jaw was performed.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness. Absence of odontogenic jaw keratocysts in patients with a *SUFU* pathogenic variant. No specific information regarding odontogenic keratocysts of the jaw was provided.	Not applicable	Strongly suspected	Very low
	Not applicable	Very serious indirectness Absence of odontogenic jaw keratocysts in patients with a *SUFU* pathogenic variant.	Not applicable	Strongly suspected	Very low
	Not applicable	Very serious indirectness Absence of odontogenic jaw keratocysts in a patient with a *SUFU* pathogenic variant.	Not applicable	Strongly suspected	Very low

Evidence that supports screening for OKCs and start age and frequency;

Article	Summary	Study limitation/ risk of bias;
(MacDonald 2015)	A systematic review of the literature was performed for clinical and radiologically apparent features of BCNS patients in North European and East Asian communities based on case series. The prevalence of OKCs was between 44-92% and 88-100% in 7 North European and 7 Asian case series respectively.	Serious Flawed measurement of outcome Unknown if and how all patients were examined for odontogenic keratocysts of the jaw in all case series.
(Lo Muzio 2008)	A review of the clinical features in BCNS patients with diagnostic protocols, management and prognosis. (Recurrent) OKCs are being present in 90% of patients. They may start to develop as early as 7 or 8 years of age. A panoramic radiograph of the jaws once a year from the age of 8 years is suggested.	Very serious No information is given about the search in methods and included/ excluded articles for this review.
(Carlson, Oreadi et al. 2015)	Prospective case series of 16 patients with BCNS and 32 previously untreated OKCs. Postoperative screening was performed with panoramic radiographs at 3, 6 and 12 months post treatment and annually thereafter and a CT scan 1 year postoperatively and annually thereafter. One patient presented in the first decade of life, 9 in the second, 4 in the fourth, 1 in the fifth and 1 in the sixth. During a mean follow-up time of 7 years, 49 OKCs were managed with 61 procedures. The authors recommend annual dental radiographs including panoramic radiograph beginning at 8 years of age.	Very serious The case series does not provide evidence for the screening frequency and start age.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious The primary goal of the systematic review was not sufficient to substantiate our recommendation. Screening and surveillance were not mentioned in this review.	Not applicable	Suspected	Very low
	Not applicable	No indirectness	Not applicable	Strongly suspected	Very low
	Not applicable	No indirectness	Not applicable	Undetected	Very low

Evidence that supports screening method;

Article	Summary	Study limitation/ risk of bias;
(Avril, Lombardi et al. 2014)	A review based on retrospective evaluation of 11725 panoramic radiographs seen in one institution during 6 years. OKCs are defined as radiolucent lesions with well-defined borders. OPG can reveal radiolucent lesions and no additional imaging is required for diagnosis. Limitations are 2D projections of 3D structures and therefore limited value of lesion size, margins and extension.	Very serious No information is given on the retrospective evaluation of 11725 panoramic radiographs. No clear recommendations regarding OKCs in BCNS patients.
(Borghesi, Nardi et al. 2018)	A review presenting the image appearance of OKCs underlining the specific findings of different imaging modalities. Panoramic radiography is helpful in preliminary assessment of the OKC. However, for preoperative planning 3D imaging modality is required. MRI is mainly performed as complementary technique in selected cases to provide a better demonstration of internal features and soft tissue involvement. CT and cone beam CT have a higher spatial resolution compared to MRI and are therefore optimal imaging modalities for preoperative planning.	Serious No information is given about the search in methods and included/ excluded articles for this review.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness Review concerns all radiolucent lesions of the mandible in all patients. Nothing is stated about screening.	Not applicable	Suspected	Very low
	Not applicable	Very serious indirectness Review concerns OKCs in all patients, not specifically BCNS patients. Nothing is stated about screening.	Not applicable	Undetected	Very low

After the first OKC, follow-up with an OPG/MRI is recommended annually. (GEC: very low, GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(Lo Muzio 2008)	A review of the clinical features in BCNS patients with diagnostic protocols, management and prognosis. Recurrent OKCs are being present in 90% of patients. They may start to develop as early as 7 or 8 years of age. A panoramic radiograph of the jaws once a year from the age of 8 years is suggested.	Very serious No information is given about the search in methods and included/ excluded articles for this review.
(Noy, Rachmiel et al. 2017)	A retrospective cohort study of 118 OKCs in patients with and without BCNS between 1995-2015. Thirty-two OKCs were diagnosed in 8 BCNS patients. Of these 32 OKCS, 13% presented with pain and 38% with swelling. Recurrence was seen in 47% and median time to recurrence was 32 months (IQR 12-48 months). They also employed a multivariable model of prediction which showed that the most relevant period for occurring recurrences is approximately within 3 years after the surgery for the OKC. This provides evidence to follow patients after a OKC very closely.	Very serious No detailed information is provided about the multivariable prediction model. Small BCNS population.
(Ribeiro-Junior, Borba et al. 2017)	Retrospective cohort study of 40 OKCs from 31 patients (between 2003-2009) which aimed to investigate treatment of OKCs and its relation to BCNS. Four BCNS patients that developed 13 OKCS were identified, of which 15.4% recurred.	Very serious Small BCNS population.
(Titinchi and Nortje 2012)	Retrospective cohort of 145 OKCs from 106 patient between 1971-2011, of which 15 patients had BCNS. Prominent presenting symptoms were swelling (50.9%) and purulent discharge (17.0%). The overall recurrence rate was 50.0% after a mean follow-up period of 22.2 months.	Very serious Small BCNS population.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness No distinction is made in the frequency of screening by a panoramic radiograph after the development of a first OKC.	Not applicable	Strongly suspected	Very low
	Not applicable	Serious indirectness No specific follow-up frequency is suggested.	Not applicable	Suspected	Very low
	Not applicable	Very serious indirectness Only shows recurrence percentages. No specific follow-up frequency is suggested.	Not applicable	Suspected	Very low
	Not applicable	Very serious indirectness Only shows recurrence percentages. No specific follow-up frequency is suggested.	Not applicable	Suspected	Very low

After the age of 22 years, follow-up can be continued by the dentist and additional OPG can be performed in case of pain/unexplained positional change of the teeth. (GEC: very low, GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(Lo Muzio 2008)	Review article. A review of the clinical features in BCNS patients with diagnostic protocols, management and prognosis. Recurrent OKCs are being present in 90% of patients. They may start to develop as early as 7 or 8 years of age. A panoramic radiograph of the jaws once a year from the age of 8 years is suggested. New and recurring cysts develop until about age 30, afterwards the rate of development tends to decrease.	Very serious limitation No information is given about the search in methods and included/ excluded articles for this review.
(Mustaciuolo, Brahney et al. 1989)	Case report. Case about a 13-year-old girl with OKCs confirmed on radiographic examination. The patient was followed routinely. In the introduction was mentioned that the trend for continued development of new and recurring cysts at increasing frequency is until about age 30 and afterwards the rate of development tends to decrease.	Very serious limitation Selective outcome reporting

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness No statement is made about screening after a certain age, only that the rate of development tends to decrease after the age of 30.	Not applicable	Strongly suspected	Very low
	Not applicable	Very serious indirectness No screening recommendation about the frequency of follow-up and age limit to decrease the follow-up is made.	Not applicable	Strongly suspected	Very low

Ovarian fibromas

Gynaecological ultrasound examination and surveillance in non-symptomatic patients is not strictly advised. In case of abdominal complaints such as pain or menstrual irregularities, female patients should undergo gynaecologic ultrasound examination to investigate the presence of an ovarian fibroma. (GEC: very low, GSoR: weak)

Article	Summary	Study limitation/ risk of bias;
(MacDonald 2015)	A systematic review of the literature was performed for clinically and radiologically apparent features of BCNS patients in North European and East Asian communities based on case series. The prevalence of ovarian fibroma's in 7 North European case series (13-60%) and 7 Asian case series (6-17%) where shown.	Serious limitation Results based on 14 North European and Asian case series with small populations. Unknown how and when screening of ovarian fibromas was performed in individual case series.
(Lo Muzio 2008)	A review of the clinical features in BCNS patients with diagnostic protocols, management and prognosis. Ovarian cysts and fibromas are present in 25-50% of affected women. A diagnostic protocol has been set up for BCNS patients and their relatives with a suspicion on BCNS, in which they advise an ovarian ultrasound in female patients to exclude ovarian fibroma.	Very serious limitation No information is given about the search and included/excluded articles.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness The primary goal of the systematic review is different and not sufficient to substantiate our recommendation. Screening and surveillance were not mentioned in this review. Moreover, the Asian population is also included in this study and is not directly comparable due to differences in prevalence of ovarian fibromas.	Not applicable	Suspected	Very low
	Not applicable	Serious indirectness Ultrasound is advised to exclude ovarian fibromas in BCNS patients and relatives. No recommendation about surveillance is given. No distinction is made in screening between symptomatic and non-symptomatic patients.	Not applicable	Suspected	Very low

Article	Summary	Study limitation/ risk of bias;
(DeLair and Soslow 2016)	Review about gynaecologic manifestations in rare hereditary syndromes, among which BCNS. Ovarian fibromas occur in approximately 17-24% of women with BCNS. In rare cases, the fibroma may be the first manifestation of the disease. Usually, patients are asymptomatic, but symptoms of a mass effect or acute abdomen could occur. The review states: "Conservative treatment with preservation of normal ovarian tissue is recommended to prevent premature menopause and to preserve fertility."	Very serious limitation No information is given about the search and included/excluded articles.
(Scalia, Farulla et al. 2018)	Case report about a 25-year old Caucasian woman with irregular menses and BCNS. The presence of several uterine and ovarian hypoechoic masses were detected with transrectal ultrasonography. A MRI and CT were performed for further research. The diagnoses of ovarian and uterine fibromas were confirmed with laparascopic surgery. In the article is appointed that ultrasound is generally the first-line imaging technique for the evaluation of ovarian abnormalities, but since it is non-specific a MRI is advised to screen.	Very serious Selective outcome reporting

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness. The review states a clear conservative treatment recommendation concerning treatment for ovarian fibromas in BCNS patients.	Not applicable	Suspected	Very low
	Not applicable	Serious indirectness The article is mainly focused on the imaging features of ovarian fibromas on different imaging modalities. Although an ultrasound is generally the first line imaging technique, a MRI is recommended in the article because it is more specific. No statement is made about a screening recommendation in asymptomatic patients.	Not applicable	Strongly suspected	Very low

Article	Summary	Study limitation/ risk of bias;
(Khodaverdi, Nazari et al. 2018)	A 25 year old female patient with BCNS underwent a trans-abdominal ultrasonography and pelvic MRI due to abdominal pain. The ovarian fibromas were removed by laparoscopic surgery.	Very serious Selective outcome reporting
(Morse, McLaren et al. 2011)	Case about a 15-year-old girl with irregular menses. Transvaginal ultrasonography and MRI was performed followed by surgery. A MRI is recommended for workup and follow-up due to the sensitivity and specificity, also in young patients who may not tolerate transvaginal ultrasound.	Very serious limitation Selective outcome reporting

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness This report focused on preservation of ovarian function and fertility after surgical management of ovarian fibromas detected with trans-abdominal ultrasonography and pelvic MRI. No statement is made about screening recommendations in non-symptomatic patients.	Not applicable	Strongly suspected	Very low
	Not applicable	Serious indirectness A MRI is recommended for screening and follow-up instead of ultrasound due to the sensitivity and specificity, also in young patients a MRI was advised if they can't tolerate an ultrasound. No statement is made about screening recommendations in non-symptomatic patients. The case report mainly focussed on fertility preservation and surgical excision of ovarian fibromas.	Not applicable	Strongly suspected	Very low

Lymphomesenteric cysts

Physicians should screen for (lympho)mesenteric cysts with ultrasound examination in patients with BCNS and inexplicable abdominal pain. (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias
(Bree, Shah et al. 2011)	Consensus group participants reviewed the literature and defined surveillance recommendations for BCNS patients. Moreover, they analysed 55 BCNS patients and their family members. No statement is made about the screening for (lympho)mesenteric cysts. It is only appointed as a minor criterion.	Very serious limitation No medical information was given about the included 55 BCNS patients. Unknown to what extent the recommendations are based on the findings of the included BCNS patients, expert opinion and literature.
(Haenen, Hubens et al. 2013)	Case report of a 30-year-old male with Gorlin-Goltz syndrome and increasingly severe abdominal pain. Physical examination showed obvious palpable abdominal masses in the umbilical region. A MRI from 2 years earlier had shown a cyst located around the umbilical region and in the mesoderm around the left hemi-abdomen. A conservative stance was taken and a follow-up CT 1 year later showed an increase in size of the cysts. Because of symptoms and growing nature the cysts were surgically removed. Based on histology the diagnosis of epidermal inclusion cysts was made.	Very serious limitation Selective outcome reporting bias. (Probably overestimation of effect).

	Inconsistency of the pooled results	Indirectness of the evidence	Imprecision of the pooled results	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness No statement is made about the screening for (lympho)mesenteric cysts. This is not one of the outcomes in this article.	Not applicable	Undetected	Very low
	Not applicable	Very serious indirectness The case report is focused on the occurrence of an epidermal inclusion cysts in a BCNS patient. No recommendation or statements were made about screening for (lympho)mesenteric cysts.	Not applicable	Strongly suspected	Very low

Article	Summary	Study limitation/ risk of bias
(Rajan, Brown et al. 2016)	Case report of a 61-year-old woman that presented with multiple mesenteric lesions seen on a CT scan. CT scan was performed per trial protocol for vismodegib treatment. The mesenteric cysts were smaller on CT imaging after 2 months of vismodegib treatment. Mesenteric cysts are often identified after imaging of the abdomen as they are usually asymptomatic.	Very serious limitation Selective outcome reporting bias. (Probably overestimation of effect).
(Kayser, Kayser et al. 2007)	Case report of a 41-year-old female who suffered from recurrent and severe abdominal pain. On abdominal sonography three abdominal masses were detected. Additional CT scan was performed and followed by laparotomy to confirm the diagnosis mesenteric cystic lymphangiomas. Excellent results can be found by ultrasound examination. Moreover it is very cheap, fast and absolutely harmless for the patients and therefore should become a standard procedure in regular follow-up examinations of BCNS patients.	Very serious limitation Selective outcome reporting bias. (Probably overestimation of effect).

	Inconsistency of the pooled results	Indirectness of the evidence	Imprecision of the pooled results	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Very serious indirectness The case report is focused on the fact that (lympho) mesenteric cysts are mostly accidentally discovered and that they can mimic metastatic disease. No recommendation or statements were made about screening for (lympho)mesenteric cysts.	Not applicable	Strongly suspected	Very low
	Not applicable	Very serious indirectness	Not applicable	Strongly suspected	Very low

Psychological distress

Psychological evaluation for support and counselling after the diagnosis is recommended for all patients (and their families). During follow-up, physicians should pay attention to psychological distress and address the possibility of a psychological consultation. (GEC: very low, GSoR: strong)

Article	Summary	Study limitation/ risk of bias
(Huq, Bogwitz et al. 2017)	A cohort study was carried out to collect standardised phenotypic information and health-related quality of life (QoL) information in 19 BCNS patients with a clinical diagnosis of BCNS. Medical information was obtained by file reviews and participants were also surveyed over the phone for more details. QoL was ascertained using the AQoL-6D questionnaire. The outcomes of the QoL scores were compared with population norms. Within the cohort the only variable that reached statistical significance was the presence of ≥100 BCCs when compared with individuals with <100 BCCs.	Serious limitation Small group of patients included (n=19)
(Shah, Mavers et al. 2011)	81 patients attending a national BCNS support group meeting were included in a survey-based assessments of quality of life and depressive symptoms. In- and exclusion criteria were clearly described. Skin-related quality of life (Skindex-29) was completed by 32 participants to evaluate the specific impact of BCCs. The Skindex-29 showed a wide range of scores in each sub item (emotions, symptoms and functioning). Depressive symptomatology (CES-D) was completed by 18 participants and suggested that 50% of participants had significant depressive symptomatology and indicated a need for clinical evaluation. Because BCNS is a chronic genetic disease and many of its symptoms and treatments can cause significant cosmetic effects, physicians may need to evaluate patients for psychological impact.	Serious limitation Small sample size. Only 32 of the 82 patients were included to fill out the Skindex-29. Of these 32, only 18 also completed the CES-D questionnaire. Disproportionate representation of females among the participants.

	Inconsistency of the pooled results	Indirectness of the evidence	Imprecision of the pooled results	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness In this cohort the quality of life has been researched in BCNS patients and it was found that ≥100 BCCs has significant impact on the QoL. However, no recommendations or advice about a psychological consult was given based on the results. Psychological distress was not directly measured.	Not applicable	Strongly suspected Unknown whether the analyses represent all or a fraction of those conducted.	Very low
	Not applicable	Serious indirectness The goal of this study was to investigate the psychological effects in patients with BCNS.	Not applicable	Strongly suspected Individuals with a more severe phenotype may be more likely to be involved in the BCNS support group and be included. The lifetime number of BCCs in the population is probably over-representative of severe disease. Over 60% of the population had more than 100 BCCs in their lifetime and none reported fewer than 10.	Very low

Article	Summary	Study limitation/ risk of bias
(Mathias, Chren et al. 2014)	A questionnaire was developed to measure patient-reported outcomes (PROs) in patients with advanced basal cell carcinoma (aBCC) and BCNS. Interviews were conducted by telephone with aBCC patients, BCNS patients and physicians who treat aBCC and BCNS individuals. Patients were recruited from 5 US clinical sites and BCCNS Life Support Network. After that, 2 separate questionnaires for aBCC and BCNS patients were drafted. 40% of BCNS patients reported that their condition was currently affecting their quality of life. Furthermore, BCNS patients reported that the disease had an impact on their social life, work or job opportunities, and ability to enjoy outdoor activities. BCNS patients reported distress about passing the condition to their children, their appearance, jaw cysts, the inconvenience of frequent physician visits and surgical procedures, the toll taken on their bodies and fear of the future. 80% of BCNS patients reported impact on emotional functioning.	Serious limitation Small sample size. Only 30 patients completed concept elicitation interviews, whereof 16 patients had BCNS.
(Ali, Collier et al. 2016)	National survey based on a questionnaire completed by 73 patients with Gorlin syndrome (GS). One of the outcomes was about the most bothersome aspects of Gorlin syndrome that patients were worried about their skin condition, appearance of the skin, frustrated and embarrassed. About 32% of the patients needed improved support from healthcare professionals.	Serious limitation The questionnaire was sent to 243 patients and only 73 were returned. Not a validated questionnaire.

	Inconsistency of the pooled results	Indirectness of the evidence	Imprecision of the pooled results	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness This article included both aBCC and BCNS individuals. A aBCC- and BCNS-specific questionnaire was developed to measure the disease effect on the quality of life of patients.	Not applicable	Strongly suspected Participants were recruited from clinical sites that participated previously in vismodegib clinical trials or the BCCNS Life support Network. The results likely reflect a treatment bias with vismodegib.	Very low
	Not applicable	Serious indirectness The psychological aspects of the disease were mentioned, but psychological evaluation after the diagnosis for all patients and their families were not explicitly advised.	Not applicable	Strongly suspected The questionnaire was only returned by a small group of patients (n=73), whereof 67 were completed by patients and 6 by family and friends on behalf of patients.	Very low

Article	Summary	Study limitation/ risk of bias
(Peris, Fargnoli et al. 2019)	European guideline for the diagnosis and treatment of basal cell carcinoma. The psychological impact of disfiguring surgery and need to discuss surgery-associated morbidity in basal cell nevus syndrome was appointed. Moreover long-term follow-up is advised because of anxiety about having multiple skin cancers.	Serious limitation Despite the psychological aspect of BCNS is a well-known issue, it is not appointed on which studies these statements and recommendations were based on.

How should patient care be organized?
To provide optimal care for patients with BCNS we advocate a multidisciplinary approach. (GEC: very low, GSoR : strong)

Article	Summary	Study limitation/ risk of bias;
(Ali, Collier et al. 2016)	National survey based on a questionnaire completed by 73 patients with Gorlin syndrome (GS). Fifteen percent of patients were seen by a multidisciplinary team.	Serious limitation The questionnaire was sent to 243 patients and only 73 were returned. Not a validated questionnaire.

	Inconsistency of the pooled results	Indirectness of the evidence	Imprecision of the pooled results	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness The psychological aspects of the disease were mentioned, but psychological evaluation after the diagnosis for all patients and their families were not explicitly advised.	Not applicable	Undetected	Very low

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	Serious indirectness It was only discussed that 15% of the patients was seen in a multidisciplinary setting without any recommendations.	Not applicable	Strongly suspected The questionnaire was only returned by a small group of patients (n=73). Of those, 67 were completed by patients and 6 by family and friends on behalf of patients.	Very low

Article	Summary	Study limitation/ risk of bias;
(Peris, Fargnoli et al. 2019)	European guidelines for diagnosis and treatment of BCC, based on the updated EDF guideline, German S2k guidelines, French guidelines, British association of Dermatologists' guidelines and de novo literature search by Medline. Methodology was based on the AGREE II instrument and levels of evidence were graded according to the Oxford classification. A structured consensus process was used to discuss and agree upon recommendations in 2018. Stakeholders were European Dermatology Forum, European Association of Dermato-Oncology, European Organization for Research and Treatment of Cancer and 24 experts from 11 countries, all of whom were delegates of national and/or international medical societies. Section 7 is dedicated to the diagnosis and management of patients with BCNS. The guidelines state: "A multidisciplinary approach is required to manage patients with NBCCS."	Minimal No literature on which the recommendation is based.

	Inconsistency of the pooled results;	Indirectness of the evidence;	Imprecision of the pooled results;	Reporting/ publication bias.	Quality of Body of Evidence
	Not applicable	No indirectness	Not applicable	Undetected	Very low

Supplementary Table 2. Overview of all papers discussing oral hedgehog pathway inhibitor monotherapy for multiple BCCs in BCNS patients.

(Tang, Mackay-Wiggan et al. 2012)
(Tang, Ally et al. 2016)
(Dreno, Kunstfeld et al. 2017)
(Lear, Hauschild et al. 2020)
(Verkouteren, Wakkee et al. 2021)
(Valenzuela-Onate, Magdaleno-Tapial et al. 2020)
(Ozgur, Yin et al. 2015)
(Wong, Poblete-Lopez et al. 2020)
(Ojevwe, Ojevwe et al. 2015)
(Yang and Dinehart 2016)
(Kesireddy, Mendiola et al. 2019)
(Canha, Bajiric et al. 2019)
(González-González, Ferreras et al. 2018)

Only studies which reported overall outcome were included in Supplementary Tables 3 & 4 on efficacy and safety.

	RCT of vismodegib 150mg daily versus placebo in 41 patients with BCNS.
	RCT of vismodegib 150mg daily versus placebo in 41 patients with BCNS, final results.
	RCT of two intermittent vismodegib dosing regiments in 229 multiple BCC patients (85 BCNS patients).
	RCT of sonidegib 400mg daily versus placebo in 9 patients with BCNS.
	Retrospective cohort study of patients treated with vismodegib 150mg daily and various dosing schedules for basal cell carcinoma, including 19 patients with BCNS treated for the indication multiple basal cell carcinomas.
	Case series of 3 patients with BCNS who were treated with vismodegib 150mg daily for multiple BCCs in an intermittent dosing schedule.
	Retrospective cohort study of patients treated with vismodegib 150mg daily for basal cell carcinoma, including 2 patients with BCNS treated for the indication multiple basal cell carcinomas.
	Retrospective case series that compares 150mg daily dosing and Monday through Friday dosing of vismodegib for BCC, including 3 patients with BCNS treated for the indication multiple basal cell carcinomas.
	Case report concerning a 31-year-old male patient with BCNS and multiple BCC treated with vismodegib 150mg daily.
	Case report of 2 patients with BCNS treated with vismodegib 150mg daily in specific schedules (1 or 2 months on treatment and 2 months off treatment) for multiple basal cell carcinomas.
	Case report of a patient with BCNS treated with vismodegib 150mg daily for multiple basal cell carcinomas for 2.5 years. After vismodegib was discontinued, the basal cell carcinomas reoccurred.
	Case report of a 52-year-old patient with BCNS treated with sonidegib 200mg daily for multiple basal cell carcinomas. A near complete response was seen over the course of 6 monhts.
	Case report of a 62-year-old patient with BCNS treated with vismodegib 150mg daily for multiple basal cell carcinomas. After 10 months treatment was discontinued due to adverse events.

Supplementary Table 3. Efficacy of oral hedgehog pathway inhibitors in the treatment of multiple BCCs in patients with BCNS.

Study	Hedgehog pathway inhibitor	Response criteria	Total – BCNS patients	Randomization: n
Tang et al.[11,12]	Vismodegib 150mg	Reduction in rate of new surgically eligible BCCs (nSEBs) after 3 months compared to placebo	41 – 41	Vismodegib: 26 Placebo: 15
Lear et al.[14]	Sonidegib 400mg	Clinical clearance rate of main target BCC using a 6-point scale (worsening, no change, 1-25%, 26-75%, 76-99% or 100% improvement)	10 – 10	LDE225: 8, of which 7 were included in the analysis Placebo: 2
Dreno et al.[15]	Vismodegib 150mg alternated with placebo	Percentage reduction in number of clinically evident BCCs at week 73	229 – 85	Group A: 116 Group B: 113

	Baseline tumours	Primary outcome	Secondary outcome	Completion of treatment until primary outcome	GEC
	Mean number nSEBs at baseline:	Mean rate nSEBs/ year at month 3:	Change in size of existing SEBs:	38 patients completed.	Moderate
	44	2	-60mm		
	37	34	+55mm		
		p<0.001	p<0.001		
	Total BCCs at baseline:	Clinical clearance rate at week 16:	BCC tumour count at week 16:	All patients completed. Only 7 LDE225 allocated patients included in efficacy analysis due to receipt of placebo in 5 of 13 doses in 1 patient.	Low
	566	100%: 3	309		
		76-99%: 3			
		26-75%: 1			
	510	1-25%: 1	619		
		Worsening: 1			
	Mean number of BCCs at baseline:	Mean reduction at week 73:	Mean reduction 3 target BCCs at week 73:	55% in group A and 50% in group B completed 73 weeks of treatment. Median treatment duration was 71.4 weeks.	Moderate
	9.8	55.2%	82.9%		
	9.1	56.6%	68.8%		
		p=0.21	p=0.02		

Supplementary Table 3. Efficacy of oral hedgehog pathway inhibitors in the treatment of multiple BCCs in patients with BCNS.

Study	Hedgehog pathway inhibitor	Response criteria	Total – BCNS patients	Randomization: n
Verkouteren et al.[13]	Vismodegib 150mg	Progression-free survival	80 – 19	Not applicable

HPI = hedgehog pathway inhibitor, RCT = randomized controlled trial, BCCs = basal cell carcinomas, SEBs = new surgically eligible basal cell carcinomas, nSEBs = new surgically eligible basal cell carcinomas (SEBs were defined as clinically diagnosed basal cell carcinoma (BCC) 5 millimeters (mm) or greater in diameter on the face, excluding the nose and periorbital skin, and 9 mm or greater at sites other than the face), CI = confidence interval.* Consisted of two groups; group A: 12 weeks vismodegib 150mg/day – 8 weeks placebo alternately, group B: 24 weeks vismodegib 150mg/day followed by 8 weeks

	Baseline tumours	Primary outcome	Secondary outcome	Completion of treatment until primary outcome	GEC
	Not measured	Median progression free survival: 19.1 months (95% CI 7.4-20.2)	Probability of partial response within 3 months after start of vismodegib: 93.3% (95% CI, 74.0–99.6) Probability of complete response after 6 months of treatment: 40.8% (95% CI, 19.3–72.2)	Not appiclable.	Very low

placebo – 8 weeks vismodegib 150mg alternately. ^Twelve patients (63%) received ≥2 treatment sequences, with a maximum of four sequences. The median treatment break duration was 11.2 months (range 2.2–54.2 months). All patients responded to vismodegib in all the following sequences. A new sequence was defined as restarting vismodegib treatment after a break of >8 weeks. Results could not be pooled due to enormous differences in outcome measurements.

Supplementary Table 4. Side effects of oral hedgehog pathway inhibitors in the treatment of multiple BCCs in patients with BCNS.

	(Tang, Mackay-Wiggan et al. 2012, Tang, Ally et al. 2016)	**(Lear, Hauschild et al. 2020)**
Hedgehog pathway inhibitor	Vismodegib	Sonidegib
Dosage	150mg daily	400mg daily
Treatment duration	Unknown. Ten patients were treated for >15 months continuously	16 weeks
Patients available for safety results	40	8
Alopecia	100% (40)	25% (2)
Muscle spasms	100% (40)	38% (3)
Dysgeusia	93% (37)	13% (1)
Weight decreased	78% (31)	NM
Gastrointestinal upset/ diarrhea	65% (26)	13% (1)
Fatigue	48% (19)	25% (2)
Nausea	10% (4)	25% (2)
Runny nose/ nasopharyngitis	18% (7)	25% (2)
Common cold/asthenia	20% (8)	NM
Headache	NM	25% (2)
Treatment discontinuation/interruption	21/40 within 18 months	2/8 within 16 weeks
Reason for treatment discontinuation		
AE / lab abnormalities	30% (12)	25% (2)
Patients decision/refused treatment	NM	NM
Patient satisfaction	3% (1)	NM
Site method	15% (6)	NM
Withdrew consent	NM	NM
Investigators decision	NM	NM
Disease progression	NM	NM
Died	5% (2)	NM
End of trial	-	-
GEC	Moderate	Low

AE = adverse event, NM = not mentioned, *Group A: 12 weeks vismodegib 150mg/day – 8 weeks placebo alternately, group B: 24 weeks vismodegib 150mg/day followed by 8 weeks placebo – 8 weeks vismodegib 150mg alternately. ^Two patients were still on treatment.

	(Dreno, Kunstfeld et al. 2017) Group A*	**(Dreno, Kunstfeld t al. 2017) group B***	**(Verkouteren, Wakkee et al. 2021)**
	Vismodegib	Vismodegib	Vismodegib
	150mg daily alternated with placebo	150mg daily alternated with placebo	Various dosing schedules
	71.6 weeks	68.4 weeks	Median 14.4 months (2.8-16.8 months)
	114	113	No specific adverse event data available for the BCNS cohort.
	63% (72)	65% (73)	NM
	73% (83)	83% (93)	NM
	66% (75)	67% (75)	NM
	21% (24)	19% (21)	NM
	18% (20)	16% (18)	NM
	21% (24)	23% (26)	NM
	20% (23)	13% (15)	NM
	NM	NM	NM
	13% (15)	18% (20)	NM
	10% (11)	11% (12)	NM
	50/116 within 73 weeks	57/113 within 73 weeks	Median treatment duration 14.4 months (2.8-16.8 months)
			^
	20% (23)	27% (30)	76% (13)
	6% (7)	3% (3)	0% (0)
	NM	NM	0% (0)
	NM	NM	0% (0)
	10% (12)	12% (13)	0% (0)
	2% (2)	5% (6)	0% (0)
	3% (3)	3% (3)	6% (1)
	NM	NM	0% (0)
	-	-	18% (3)
	Moderate	Moderate	Very low

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Chapter 4.2

Update on hedgehog pathway inhibitor therapy for patients with basal cell nevus syndrome or high-frequency basal cell carcinoma – a systematic review

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ABSTRACT

Some patients with basal cell carcinoma develop a large number of basal cell carcinomas during their lives. The most common underlying genetic disease that causes multiple BCCs is basal cell nevus syndrome. Basal cell nevus syndrome is caused by a germline mutation in patched-1 (*PTCH1*), a tumour suppressor gene of the hedgehog signalling pathway. However, in a significant portion of the patients with multiple basal cell carcinomas an underlying genetic cause is not found. Nevertheless, these patients can experience a treatment burden comparable to that of patients with basal cell nevus syndrome. They are referred to as high-frequency basal cell carcinoma patients. Hedgehog pathway inhibitors were the first group of targeted therapy for basal cell carcinomas. This study reviews the literature on hedgehog pathway inhibitor therapy for patients with basal cell nevus syndrome or high-frequency basal cell carcinoma, to provide an overview on efficacy, safety, dosing regimens, tumour resistance and reoccurrence, and health-related quality of life.

INTRODUCTION

A subset of patients with basal cell carcinoma (BCC) will develop a large number of BCCs during their lives. The most common genetic disease that causes multiple BCCs is basal cell nevus syndrome (BCNS), which has an estimated incidence in the range 1:31,000-256,000.[1,2] In up to 85% of all patients with BCNS, a germline mutation in the tumour suppressor gene patched-1 (*PTCH1*), part of the hedgehog signalling pathway, is responsible.[3] In a smaller proportion of patients with BCNS, a postyzygotic mutation in *PTCH1* or germline mutation in another hedgehog pathway gene, such as suppressor of fused (*SUFU*), can be found.[3] In addition to BCNS, xeroderma pigmentosum, Bazex-Dupré-Christol and Rombo syndrome are also diseases with a susceptibility for developing multiple BCCs. In a subset of patients with multiple BCCs the underlying cause is unknown. These patients are referred to as high-frequency BCC (HF-BCC) patients, although there is no clear definition for the number and frequency of BCCs in patients with HF-BCC.

In general, BCCs in patients with BCNS and HF-BCC can be treated with local surgery.[4,5] However, there is an unmet need for new treatment options for patients with BCNS and HF-BCC. Some patients develop so many BCCs during their lives that surgical treatment can become physically challenging due to the large number of scars, but treatment also has a high emotional impact because of the burden of multiple hospital visits.[6] The impact of multiple BCCs on the health-related quality of life (HRQoL) can be substantial, as was found in a small cohort study of BCNS patients.[6] A treatment that could cure all lesions at the same time, with limited scarring and without major side-effects, is therefore highly desirable.

In 2012 the US Food and Drug Administration (FDA) approved the first oral hedgehog pathway inhibitor (HPI), vismodegib, for the treatment of advanced BCC.[7] Its mechanism of action consists of inhibition of smoothened (SMO) and consequently inactivation of the hedgehog pathway. Unfortunately, tumour resistance, predominantly caused by *SMO* mutations, is a common problem in the treatment of advanced BCC with vismodegib.[8,9]

Vismodegib was the first HPI investigated in patients with HF-BCC and BCNS, but other types of oral HPIs have been investigated since. In general, side-effects such as muscle spasms, alopecia and dysgeusia eventually lead to treatment discontinuation in the BCNS and HF-BCC population.[10] However, patients have a lifelong indication for treatment and in order to maintain long-term treatment, different dosing schedules are applied in clinical practice. Furthermore, topical

HPIs have been developed for the treatment of multiple non-advanced BCCs. Although the mechanism of topical HPIs is the same, i.e. inhibition of SMO, the typical side-effects of oral HPIs are expected to be absent because of the local application and therefore minimal systemic effect.

The aim of this review is to outline the available clinical data for patients with BCNS and HF-BCC treated with any type or dosage of oral and topical HPIs.

MATERIALS AND METHODS

This systematic review, conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, was performed in the following 5 areas of interest: efficacy, safety, dosing regimen, tumour resistance and reoccurrence, and HRQoL in patients with BCNS and HF-BCC that were treated with HPI. Systematic reviews are exempted for institutional board review at Maastricht University Medical Centre+.

First, a broad search was performed in clinicaltrials.gov, ISRCTN.org and clinicaltrialsregister.eu to determine which HPIs have been used for the treatment of BCCs. The following HPIs were identified: (*i*) oral; vismodegib/GDC-0449, sonidegib/LDE225, saridegib/IPI-926, itraconazole, BMS-833923, LEQ506 and TAK-441, and (*ii*) topical; patidegib/IPI-926, sonidegib/LDE225 and itraconazole. Multiple searches were performed using either "basal cell nevus syndrome/Gorlin syndrome," "high-frequency basal cell carcinoma," "multiple basal cell carcinoma," or "basal cell carcinoma" in combination with one of the HPIs to identify suitable articles in clinicaltrials.gov, PubMed, Embase from database inception to 17 September 2021.

One author (BV) performed the searches and independent review of the titles and abstracts. Studies describing treatment of patients with BCNS or HF-BCC with HPI monotherapy, which were relevant for the areas of interest, were selected for full article review. To assess efficacy and safety, all studies that reported outcomes on a group level were included, regardless of the used outcome and safety measurements used. Furthermore, all case reports and series that described treatment of patients with BCNS or HF-BCC with HPI monotherapy were evaluated for any information regarding dosing schedules, tumour resistance and reoccurrence and HR-QoL.

The following information was extracted: type and dosage of HPI, study design, level of evidence, treatment indication, number of participants, duration of treatment and follow-up, response criteria, efficacy, industry driven. Quality of evidence was assessed by using Oxford Centre for Evidence-Based Medicine levels. A list of common adverse events (AEs) and reasons for treatment discontinuation were also collected. Additional information on mutation analysis, resistance criteria, time to reoccurrence, and a brief summary was collected from tumour resistance and reoccurrence studies.

Additional information on type of questionnaire and time points of its measurements were collected for HRQoL studies.

RESULTS

A total of 879 individual records were identified, of which 723 were removed after screening the titles and abstracts, and another 120 were removed after full-text review (Figure 1 and Appendix S1. A final total of 24 individual studies (36 different reports) were included, which discussed results on either efficacy (n=8), safety (n=7), dosing regimens (n=8) tumour resistance and reoccurrence (n=15), and/or HRQoL (n=2) in patients with BCNS and HF-BCC.

Efficacy results of all HPIs in all dosing schedules are shown in Table 1.

Oral hedgehog pathway inhibitors

Continuous vismodegib. One randomized controlled trial (RCT), and 1 retrospective cohort reported outcomes for continuous vismodegib treatment.[11-13]

In the RCT, treatment with vismodegib 150mg/day (n=26) compared to placebo (n=15) resulted in a mean rate of 2 new surgically eligible BCCs (SEBs) per year, compared with 34 in the placebo group. Furthermore, the vismodegib group showed a 65% reduction in mean size of existing SEBs.[11,12] A SEB was defined as clinically diagnosed BCC, regardless of subtype, of ≥5mm in diameter on the face or ≥9mm on other body parts (no upper limit).

The retrospective cohort study determined the progression-free survival in 16 BCNS patients treated with vismodegib 150mg daily and found a progression-free survival of 19.1 months (95% confidence interval (CI) 7.4-20.2).[13] Probability of partial response within 3 months after treatment was 93.3% (95% CI 74.0-99.6).

Continuous sonidegib. Only one randomized placebo-controlled trial reported on continuous sonidegib treatment in 9 patients with BCNS.[14] Treatment with sonidegib 400mg daily (n=7) resulted in a 100% target BCC clinical clearance rate in 3 patients, 76-99% in 3 others, and 26-75% in 1 patient. In the placebo group (n=2), 1 patient had a 1-25% clinical clearance rate and the target BCC of 1 patient showed worsening.[14] The total number of BCCs decreased from 566 at baseline to 309 at week 16 in the sonidegib and increased from 510 at baseline to 619 at week 16 in the placebo group. Numbers were too small for statistical analysis.

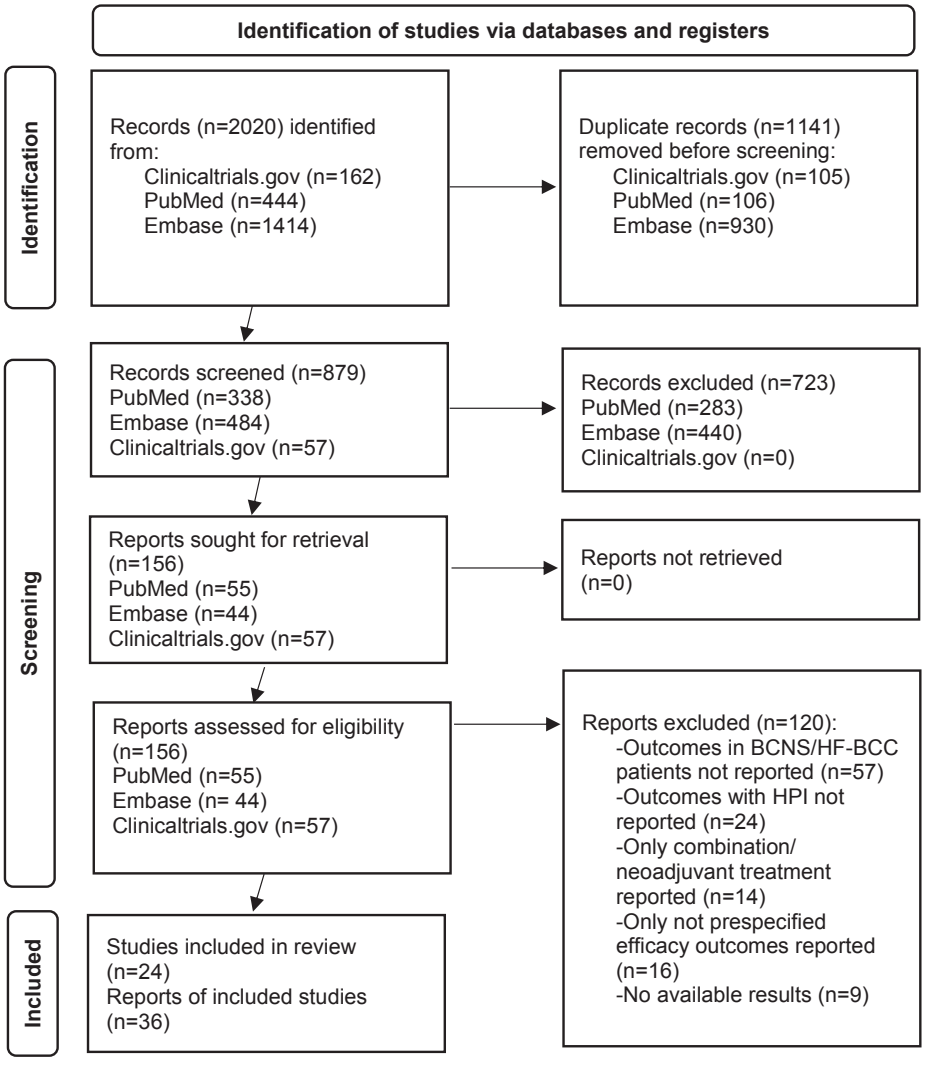

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow-diagram.

BCNS, basal cell nevus syndrome; HF-BCC, high-frequency basal cell carcinoma; HPI, hedgehog pathway inhibitor.

Dosing regimens for oral HPIs. One RCT determined the efficacy of 2 vismodegib regimens in 85 BCNS and 144 HF-BCC patients.[15] Group A received 12 weeks of vismodegib 150mg/day alternated with 8 weeks of placebo and group B received 24 weeks of vismodegib 150mg/day followed by 8 weeks of vismodegib 150mg/day alternated with 8 weeks of placebo. At week 73, the mean relative reduction

of the number of clinical BCCs was 62.7% in group A compared to 54.0% in group B (p=0.21). Furthermore, of all 34 case reports/series/cohorts about HPIs in HF-BCC/BCNS patients that were found in the literature search, 9 reported on dosing regimens.[13,15-21] All but one of these reports concerned different dosing for vismodegib. Most schedules were based on several weeks/months on and off treatment (n=25 patients), but also every other day (n=4 patients) and Monday-Friday dosing (n=2 patients) schedules have been used. Overall, outcomes were badly reported and too heterogeneous for effective comparison between different schedules. Results are summarized in Table 2.

Topical hedgehog pathway inhibitor

Three randomized-vehicle-controlled phase-2 trials investigating twice daily application of topical HPIs were registered at clinicalrials.gov.[22-24]

The first study compared itraconazole 0.7% gel for 47 BCCs with vehicle for 25 BCCs within the same 9 patients (6 patients with BCNS and 3 patients with HF-BCC).[22] Four target lesions were identified at baseline and at least one was treated with placebo according to the study protocol. The change in tumour area was +0.04% in the itraconazole-treated BCCs compared to -10.9% in the vehicle treated BCCs after 4 weeks compared to baseline. After 12 weeks the change in tumour area was +8.9% in the itraconazole and +26.5% in the vehicle BCCs.

The second trial compared patidegib 2%, 4% and vehicle gel in BCCs >5mm at baseline in 16 patients with BCNS, randomized in a 1:1:1 ratio.[23] After 26 weeks of application, the tumour size decreased by 51.3% in 21 BCCs the patidegib 2% group (n=6 patients), 26.6% in 24 BCCs in the patidegib 4% group (n=6 patients) and 21.8% in 16 BCCs in the vehicle group (n=4 patients).

In the third trial, LDE225 0.75% cream on 13 BCCs was compared with vehicle on 14 BCCs within the same 8 patients with BCNS.[24] The mean decrease in 2D tumour size, was 38.4% after 4 weeks of treatment in the LDE225 0.75% group compared to an increase of 9.6% in the placebo group. In part two of the trial, LDE225 0.75% cream in 7 patients was compared with LDE225 0.25% cream in 3 patients and showed a mean decrease in 2D tumour size of 28.5% and 36.3% respectively after 6 weeks of treatment.[24]

Safety

The most commonly reported AEs and reasons for treatment discontinuation of oral HPIs are shown in Table 3.

Oral hedgehog pathway inhibitors. In the trial of Tang et al., 40 patients were eventually treated with vismodegib. Thirty-one patients (77.5%) needed a temporary or permanent treatment discontinuation due to AEs during a 36 month study period.[11,12]

Dreno et al. found that intermittent dosing of vismodegib lead to treatment discontinuation because of AEs in 23/116 (19.8%) in group A and 30/113 (26.5%) patients in group B.[15] The regiment in group A was associated with fewer severe treatment-related AEs compared to group B. The median duration of treatment was 71.6 weeks and 68.4 weeks in group A and B respectively.

Treatment with continuous sonidegib lead to treatment discontinuation in 2 (25%) out of 8 patients due to AEs during 16 weeks of treatment.[14]

Topical hedgehog pathway inhibitors. All three topical HPIs were applied twice daily on several BCCs within a single patient. Itraconazole 0.7% gel for 4 weeks caused application site reaction and pruritus in 4/9, lesion pain in 3/9, and xerosis and dysgeusia in 1/9 patients (Table 4).[22]

Patidegib 4% gel lead to application site alopecia, dermatitis, pain and rash in 1/6 patients during 26 weeks of treatment.[23] None of these AEs occurred in the 6 patients treated with patidegib 2% gel.

In part I of the topical LDE225 trial, 4/8 patients reported local skin irritation and 1/8 reported skin fissures, it is unknown if this happened following application with placebo or LDE225 0.75%.[24] Urticaria and increased hepatic enzyme activity in blood investigations were seen in 1/8 patients. In part II, 1/7 patients treated with LDE225 0.75% cream reported local skin irritation and urticaria. None of these AEs occurred in the 3 patients treated with LDE225 0.25% cream.[24] It is unknown if any of the adverse events led to treatment discontinuation in the three trials describing topical HPI treatment.

Table 1. Studies on hedgehog pathway inhibitors for basal cell nevus syndrome and high-frequency basal cell carcinoma patients.

Study	HPI - dosing	Study type	Quality of evidence	Patient inclusion criteria	Response criteria
Tang et al.[11,12]	Vismodegib 150mg/d	Phase-2 double-blind RCT	1	Clinical diagnosis of BCNS and ≥10 SEBs within last 2 years	Reduction in rate of nSEBs
Lear et al.[14]	Sonidegib 400mg/d	Phase-2 double-blind RCT	1	Clinical diagnosis of BCNS and ≥2 BCCs	Clearance rate of main target BCC
Dreno et al.[15]	Vismodegib 150mg alternated with placebo	Phase-2 double-blind RCT*	2	Patients with ≥6 BCCs	Mean relative reduction in number of BCCs
Verkouteren et al.[13]	Vismodegib 150mg/d	Retrospective cohort study	4	Clinical diagnosis of BCNS	Progression-free survival and response rate
Sohn et al.[22]	Itraconazole 0.7% gel twice daily	Phase-2 open-label intrapatient	3	Patients with >3 BCCs annually	Change in BCC tumour area

Total – BCNS patients	Randomization: n	Baseline tumours	Primary outcome	Secondary outcome
41 – 41		Mean number nSEBs at baseline:	Mean rate nSEBs/ year at month 3:	Change in size of existing SEBs:
	Vismodegib: 26	44	2	-60mm
	Placebo: 15	37	34	+55mm
			p<0.001	p<0.001
10 – 10		Total BCCs at baseline:	Clinical clearance rate at week 16:	BCC tumour count at week 16:
	LDE225: 8, of which 7 were included in the analysis	566	100%: 3	309
			76-99%: 3	
			26-75%: 1	
	Placebo: 2	510	1-25%: 1	619
			Worsening: 1	
229 – 85		Mean number of BCCs at baseline:	Mean reduction at week 73:	Mean reduction 3 target BCCs at week 73:
	Group A: 116	9.8	55.2%	82.9%
	Group B: 113	9.1	56.6%	68.8%
			p=0.21	p=0.02
24 – 19	Not applicable	Unknown	Median progression free survival: 19.1 months (95% CI 7.4-20.2)	Probability of PR within 3 months: 93.3% (95% CI 74.0-99.6)
9 – 6		Total BCCs treated:	Change at week 4:	Change at week 12:
	Itraconazole: 9	65	0.04%	8.9%
	Vehicle: 9	42	-10.9%	26.5%
			p=0.40	p=0.40

Table 1. Continued.

Study	HPI - dosing	Study type	Quality of evidence	Patient inclusion criteria	Response criteria
Epstein et al.[23]	Patidegib 2% and 4% gel twice daily	Phase-2 double-blind parallel assignment	1	Clinical diagnosis of BCNS and ≥10 BCCs within last 2 years	Change in BCC size
Skvara et al.[24]	Sonidegib 0.25% and 0.75% cream twice daily	Phase-2 double-blind, 2 parts, parallel assignment^	1	Clinical diagnosis of BCNS with BCCs on 2 different body parts	Percentage of BCCs with clearance

HPI, hedgehog pathway inhibitor; BCNS, basal cell nevus syndrome; RCT, randomized controlled trial; BCCs, basal cell carcinomas; SEBs, surgically eligible basal cell carcinomas; nSEBs, new surgically eligible basal cell carcinomas (SEBs were defined as clinically diagnosed basal cell carcinoma (BCC) 5 millimetres (mm) or greater in diameter on the face, excluding the nose and periorbital skin, and 9 mm or greater at sites other than the face); RR, relative reduction; PR, partial response; CI, confidence interval.

Total – BCNS patients	Randomization: n	Baseline tumours	Primary outcome	Secondary outcome
17 – 17		Total number of SEBs at baseline:	Decrease in SEB size at week 26:	Mean number nSEBs at week 26:
	Patidegib 2%: 6	21	51.3% (p=0.03)	0.4
	Patidegib 4%: 6	24	26.6% (p=0.76)	0.4
	Vehicle: 5	16	21.8%	1.4
				p=0.048
18 - 18		Total number of BCCs at baseline:	BCCs with partial/complete clearance at week 4:	Mean change in 3D tumour size at week 4:
	Part 1 – 4 weeks			
	Vehicle: 8	14	7% / 0%	7.0
	LDE225 0.75%: 8	13	92% / 23%	-35.5
	Part 2 – 6 weeks			
	LDE225 0.25%: 3	12	83% / 0%	-19.3
	LDE225 0.75%: 7	22	77% / 0%	-43.4

*Consisted of two groups; group A: 12 weeks vismodegib 150mg/day – 8 weeks placebo alternately, group B: 24 weeks vismodegib 150mg/day followed by 8 weeks placebo – 8 weeks vismodegib 150mg alternately. ^Part 1: participants were exposed to both topically applied 0.75% LDE225 cream and LDE225 vehicle cream twice daily for 28 days where each treatment was randomized to two different test areas on each participant, part 2: participants were exposed to topically applied 0.25% or 0.75% LDE225 cream twice daily for 6 weeks or 0.75% LDE225 cream twice daily for 9 weeks. +Using a 6-point scale (worsening, no change, 1-25%, 26-75%, 76-99% or 100% improvement).

Table 2. Overview of studies reporting on dosing schedules and outcomes.

	Patients/schedule	Outcome
Verkouteren et al.[13]	12/19 BCNS patients received ≥2 treatment sequences (restart after break >8 weeks) with a maximum of 4 sequences in 6 years	All patients responded to vismodegib in all following sequences
	1 HF-BCC patient: 3 months on and off treatment	'Successfully' >3 years
Yang et al.[16]	2 BCNS patients	Biopsy-detected BCC in years (4-3-2-1) before / after (1-2) treatment:
	Patient 1: 1 month on and 2 months off treatment	12-11-15-9 / 2-1
	Patient 2: 2 months on and off treatment	4-1-4-5 / 1
Valenzuela-Onate et al.[17]	3 BCNS patients	
	Patient 1: 3 months on and off treatment	No new BCCs after 6 months
	Patient 2: Monday-Friday dosing	No new BCCs after 9 months, 11/14 BCC: CR, 3/14: PR
	Patient 3: Monday-Friday dosing	Size reduction >30% after 3 months
Mendes et al.[18]	BCNS patient on and off treatment for >3 years (reintroduction after recurrence and discontinuation after complete response)	'Well controlled'
Hoffmann et al.[19]	HF-BCC patient with >100 BCCs and sonidegib 200mg once every day	Clinical remission of all but 1 BCC after 9 months
Tronconi et al.[20]	4/8 multiple BCC/Gorlin patients changed from daily to 4 weeks on and 2 weeks off treatment	All patients had complete response after a total treatment duration of 27.3 months (95% CI 11.7-38.8)

Table 2. Continued.

	Patients/schedule	Outcome
Villani et al.[21]	7 HF-BCC patients 1: 20 weeks on, 12 weeks off and on treatment 2: 16 weeks on, 8 weeks off and 12 weeks on treatment 3: 12 weeks on and off treatment 4: 8 weeks on and off treatment 5: once every second day for 16 months 6: once every second day for 22 months 7: once every second day for 16 months	Not reported

BCCs, basal cell carcinomas; BCNS, basal cell nevus syndrome; HF-BCC, high-frequency basal cell carcinoma; CR, complete response; PR, partial response; 95% CI, 95% confidence interval.
All patients were treated with vismodegib unless stated otherwise.

Table 3. Prevalence of side effects in oral hedgehog pathway inhibitors.

	Tang et al.(11, 12)
HPI	Vismodegib
Dosage	150mg daily
Treatment duration	Unknown, 10 patients were treated for more than 15 months continuously
Patients available for safety results	40
Alopecia	100% (40)
Muscle spasms	100% (40)
Dysgeusia	93% (37)
Weight decreased	78% (31)
Gastrointestinal upset/ diarrhoea	65% (26)
Fatigue	48% (19)
Nausea	10% (4)
Runny nose/ nasopharyngitis	18% (7)
Common cold/asthenia	20% (8)
Headache	NM
Treatment discontinuation/interruption	21/40 within 18 months
Reason for treatment discontinuation	
AE / lab abnormalities	30% (12)
Patients decision/refused treatment	NM
Patient satisfaction	3% (1)
Site method	15% (6)
Withdrew consent	NM
Investigators decision	NM
Disease progression	NM
Died	5% (2)

HPI, hedgehog pathway inhibitor; AE, adverse event; NM, not mentioned.
*Group A: 12 weeks vismodegib 150mg/day – 8 weeks placebo alternately,

	Lear et al.(14)	**Dreno et al.(15) Group A***	**Dreno et al.(15) group B***
	Sonidegib	Vismodegib	Vismodegib
	400mg daily	150mg daily alternated with placebo	150mg daily alternated with placebo
	16 weeks	71.6 weeks	68.4 weeks
	8	114	113
	25% (2)	63% (72)	65% (73)
	38% (3)	73% (83)	83% (93)
	13% (1)	66% (75)	67% (75)
	NM	21% (24)	19% (21)
	13% (1)	18% (20)	16% (18)
	25% (2)	21% (24)	23% (26)
	25% (2)	20% (23)	13% (15)
	25% (2)	NM	NM
	NM	13% (15)	18% (20)
	25% (2)	10% (11)	11% (12)
	2/8 within 16 weeks	50/116 within 73 weeks	57/113 within 73 weeks
	25% (2)	20% (23)	27% (30)
	NM	6% (7)	3% (3)
	NM	NM	NM
	NM	NM	NM
	NM	10% (12)	12% (13)
	NM	2% (2)	5% (6)
	NM	3% (3)	3% (3)
	NM	NM	NM

group B: 24 weeks vismodegib 150mg/day followed by 8 weeks placebo – 8 weeks vismodegib 150mg alternately.

Table 4. Prevalence of side effects in topical hedgehog pathway inhibitors.

	Skvara et al. [34]		
	Part I LDE225 0.75% & vehicle n=8	Part II LDE225 0.25% n=3	Part II LDE225 0.75% n=7
SAE	0 (0%)	0 (0%)	1 (14%) Hepatic enzyme increased
Skin fissures	1 (13%)	0 (0%)	0 (0%)
Skin irritation	4 (50%)	0 (0%)	1 (14%)
Urticaria	0 (0%)	0 (0%)	1 (14%)
Application site alopecia	-	-	-
Application site dermatitis	-	-	-
Application site pain	-	-	-
Application site rash	-	-	-
Application site reaction	-	-	-
Alopecia	-	-	-
Abnormal hair growth	-	-	-
Pruritus	-	-	-
Rash	-	-	-
Lesion pain	-	-	-

SAE, serious adverse event.
*Adverse effect in itraconazole 0.7% gel patients resolved after the end of the trial except in 2 patients who had persistent mild lesion pain, pruritus and xerosis cutis.

Tumour resistance and reoccurrence

After eligibility assessment of the previously described RCTs, cohort studies and 34 case reports/series, we found information on resistance/reoccurrence in 15 different studies.[11-13,17-19,25-34] Development of resistance during treatment with an oral HPI was reported in 9 articles and tumour reoccurrence after treatment discontinuation also in 9 articles (Table 5). Only one article reported reoccurrence in a patient treated with sonidegib, all other concerned resistance/reoccurrence in vismodegib. No information on tumour reoccurrence after topical HPIs was found, but, as was described in the efficacy section, not all BCCs responded to topical HPI treatment which might be caused by primary resistance.

	Epstein et al.[23]			Sohn et al.[37]
	Patidegib 2% n=6	Patidegib 4% n=6	Vehicle n=5	Itraconazole 0.7% & vehicle* n=9
	0 (0%)	0 (0%)	1 (20%) Pneumonia	0 (0%)
	-	-	-	-
	-	-	-	-
	-	-	-	-
	0 (0%)	1 (17%)	0 (0%)	-
	0 (0%)	1 (17%)	0 (0%)	-
	0 (0%)	1 (17%)	0 (0%)	-
	0 (0%)	1 (17%)	0 (0%)	-
	0 (0%)	0 (0%)	1 (20%)	4 (44%)
	0 (0%)	0 (0%)	1 (20%)	-
	1 (17%)	0 (0%)	0 (0%)	-
	0 (0%)	0 (0%)	1 (20%)	4 (44%)
	0 (0%)	1 (17%)	0 (0%)	-
	-	-	-	1 (11%)

Health-related quality of life

Only Dreno et al. measured HRQoL of 229 patients with a validated questionnaire.[15] The Skindex-16, which comprises 3 domains (symptoms, emotions and function) was measured 8 times between baseline and end-of-treatment (week 73), and at 12, 24 and 52 weeks follow-up.[35] Outcomes ranged from 0 (never bothered) to 100 (always bothered). Both alternating treatment regimens with vismodegib showed a decrease of ≥10 points from baseline to week 9 and every point post-baseline in all domains, which was considered to be a clinical meaningful improvement.[36] A decrease of HRQoL was seen in all domains after treatment discontinuation, but HRQoL scores had not returned to baseline scores yet after 52 weeks discontinuation of treatment.

Table 5. Resistance and reoccurrence.

Study	Study type – Quality of evidence	Patients, n (BCNS/HF-BCC)	BCCs described, n
Tang et al.[11,12]	Phase-2 double-blind RCT (placebo) – 1	41 – BCNS	>2000
Chang and Oro[25]	Retrospective cohort – 2	3	133
Sinx et al.[26]	Case report – 5	1 - BCNS	>3
Banvolgyi et al.[27]	Retrospective cohort – 5	4	Unknown
Valenzuela-Onate et al.[17]	Case series – 5	3	5
Wolfe et al.[28]	Case report – 5	1	19
Verkouteren et al.[13]	Retrospective cohort – 5	24 – 19 BCNS and 5 HF-BCC	Unknown
Tauber et al.[29]	Cohort – 5	8 – HF-BCC (4 or more BCCs)	53
Kirkpatrick et al.[30]	Case report – 5	1 – BCNS	Unknown

	***Resistance* during vismodegib treatment, primary or secondary**	***(Re)occurrence* after discontinuing vismodegib treatment**
	During vismodegib treatment two pre-existing BCCs did not respond Mutational profile: one had a vismodegib-resistant *SMO* mutation (Val231Met) No information on secondary resistance described	During treatment breaks BCC reoccurred, no exact number or percentage was provided
	After a mean period of 55.3 weeks of vismodegib, during treatment 6 out of 133 BCCs regrew	Not described
	During 3 years vismodegib treatment 2 BCCs regrew after initial complete response Mutational profile: both had vismodegib-resistant *SMO* mutations (Ser241Phe and Asp473Asn)	Two months after discontinuing 3 years vismodegib treatment, BCCs reoccurred at their pre-treatment locations
	Not described	Three months after discontinuing 4 years vismodegib treatment, BCCs reappeared in 1 patient
	Not described	In 3 cases, at least 19 BCCs developed within 2 years after discontinuing vismodegib of unknown treatment duration
	Not described	Two years after discontinuing 7 months vismodegib treatment, 10 out of 19 BCCs on the head and neck reoccurred
	Five out of 24 patients had progressive disease during vismodegib 150mg/daily treatment	In 17 out of 24 patients progressive disease was seen after vismodegib discontinuation
	In one patient with 7 BCCs, new BCCs developed after reduction of vismodegib 150mg daily to an unknown reduced dose BCCs regressed after increasing the dose to 150mg daily	Not described
	After 36 months of vismodegib 150mg/daily 1 new BCC had developed	Not described

Table 5. Continued.

Study	Study type – Quality of evidence	Patients, n (BCNS/HF-BCC)	BCCs described, n
Soura et al.[31]	Case report – 5	1 – BCNS	79
Piccerillo et al.[32]	Case report – 5	1 – BCNS	>50
Van Eecke et al.[33]	Case report – 5	1 – BCNS	Multiple
Kesireddy et al.[34]	Case report – 5	1 – BCNS	Multiple
Hoffmann et al.[19]	Case report – 5	1 – HF-BCC	>100
Mendes et al.[18]	Case report – 5	1 - BCNS	High count

BCCs, basal cell carcinomas; BCNS, basal cell nevus syndrome; HF-BCC, high-frequency basal cell carcinoma; RCT, randomized controlled trial.

Furthermore, Tang et al. reported that 23/41 included patients with BCNS responded to a telephone questionnaire evaluating vismodegib treatment at some time-point after the end of the trial. Of those 23 patients, 18 stated they preferred treatment with vismodegib over surgery.[11,12]

	***Resistance* during vismodegib treatment, primary or secondary**	***(Re)occurrence* after discontinuing vismodegib treatment**
	After 12 months of vismodegib 150mg/daily: 1/79 BCCs partially responded, 78/79 completely responded After 30 months of vismodegib the remaining BCCs increased in size and 2 BCCs reoccurred	Not described
	Not described	Thirty-six months after discontinuing 6 months vismodegib 150mg daily treatment, relapse of all previously treated BCCs was seen
	Not described	Two months after discontinuing 24 months of vismodegib treatment (dose not mentioned), regrowth of BCCs was seen
	After 11 months of vismodegib 150mg daily, pre-existing BCCs increased and new BCCs developed Patient continued for another 18 months of vismodegib during which 22 BCCs developed	Not described
	After sonidegib 200mg every second day for 9 months only 1 BCC remained for which no therapy was initiated (patient desire)	Not described
	Not described	Patient received vismodegib, (dose not mentioned) in on-off regimen for >3 years, vismodegib is reintroduced after recurrence of BCCs

DISCUSSION

After reviewing all literature on oral and topical HPI therapy in patients with BCNS and HF-BCC, we can conclude that high-quality evidence for HPI treatment in this population is scarce. Both continuous vismodegib and sonidegib and alternating vismodegib have been proven effective in patients with BCNS. No head-to-head trial comparing vismodegib and sonidegib treatment have been performed and the reviewed trials are too heterogeneous to compare.

During continuous oral HPI treatment, AEs are very common, and are often the reason for treatment discontinuation. The reported percentage of patients that interrupt or cease treatment due to AEs is 25-77.5%.[12,14] This range is broad, and variation may partly be caused by various other reasons reported for treatment cessation, such as "patient's decision," "withdrawal of consent," or "refusing of treatment". Furthermore, it is not clear from the studies what AEs at what grades caused treatment discontinuation. After treatment discontinuation, at least a part of the BCCs will reoccur but there appears to be a broad range of time to tumour reoccurrence.

In the continuous vismodegib trial, 77.5% interrupted treatment for ≥2 months. Intermittent dosing alternating several weeks of oral HPI with no treatment has been proposed as a strategy for better toleration of the AEs. In the 1 RCT investigating the efficacy of intermittent vismodegib by Dreno et al.[15], alternating 12 weeks of treatment with 8 weeks of placebo appeared to be more effective compared to 8 weeks on and off treatment, and was associated with fewer severe treatment-related AEs. Only a few other articles report on alternating dosing schedules and most of them investigated similar dosing strategies to those reported by Dreno et al.[15] However, in 4 patients a daily alternating schedule was reported and in 2 patients investigators opted for a Monday-Friday dosing. These dosing schedules also appear to be effective, but the level of evidence is low.

Topical HPIs have been developed to avoid AEs in patients requiring long-term treatment for multiple BCCs. From the 3 reported phase-2 trials on 3 different HPIs, it can be concluded that the effectiveness varies per active pharmaceutical ingredient. Although the trials could not be compared due to heterogeneity in population and outcome measurements, topical itraconazole 0.7% gel application for 4 weeks appeared not to be effective in 9 patients and topical patidegib 2% and LDE225 0.75% investigated in 17 and 8 patients, respectively, showing more promising results. A follow-up phase 3 RCT with LDE225 0.75% cream was

withdrawn before participants were enrolled. Although a follow-up phase 3 RCT comparing patidegib 2% gel with vehicle was recently completed, results are not yet available and the following open-label extension study was terminated due to low blinded event rate according to clinicaltrials.gov.

In conclusion, evidence for treatment with HPIs in patients with HF-BCC and BCNS is scarce. Continuous treatment with oral HPIs is effective, but often not suitable for long term use due to adverse events. Personalized rotational schedules for oral HPIs can be an effective and tolerable solution for a subset of patients with BCNS and HF-BCC. Topical HPIs seemed promising, as they are accompanied by fewer AEs, but efficacy and safety data to support approval are not expected to be available on short-term.

SUPPLEMENTARY INFORMATION

Available from: https://medicaljournalssweden.se/actadv/article/view/980/5544

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Chapter 4.3

Treatment of basal cell carcinomas and basaloid follicular hamartomas in children and adolescents with basal cell nevus syndrome

B.J.A. Verkouteren and K. Mosterd

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LETTER TO THE EDITOR

Patients with basal cell nevus syndrome (BCNS) can develop over hundreds of basal cell carcinomas (BCCs) in their lives, of which the first can already be seen in early childhood. In children and adolescents, typically tens of shiny, sometimes pigmented, papules can be present at once, histologically diagnosed as BCCs or basaloid follicular hamartomas (BFHs), recognised as part of the cutaneous tumour spectrum in BCNS.[1] Surgery can be painful and traumatizing, and scars can be mutilating due to the high number of lesions in BCNS patients. Left untreated, the lesions can progress and treatment can be more challenging, resulting in larger scars. Imiquimod 5% cream has proven to be a very effective non-invasive therapy for BCCs, with a cure rate of ~80%, 5 years after treatment.[2] Prior curettage can shorten imiquimod treatment duration with comparable cure rates.[3] Especially in BCNS, a large advantage is that multiple lesions can be treated at once. Here, we describe a case series of young BCNS patients with multiple small BCCs or BFH treated with curettage and imiquimod 5%.

Between January 2017 and February 2020, 100 clinical BCCs or BFHs were treated with curettage followed by imiquimod cream application in 4 BCNS patients with a confirmed germline *PTCH1* mutation. Curettage was performed under general anaesthesia (for other reasons) in a 4-year old patient and an hour after application of lidocaine/prilocaine in the older patients (14, 19 and 20 years old). Lesions were 1-5 millimetres and located on the trunk (77), neck (13), arms (6), legs (2), and face (2). In all patients, at least one lesion was histologically confirmed to be a BCC. A mean number of 14 lesions were curettaged per session (range, 4-23) and patients applied imiquimod 5% cream 5 days/week during 6 weeks, using 1 sachet (250mg) per day. Treatment results were evaluated on follow-up visits each 4-6 months based on photographs taken before treatment (Figure 1). Median follow-up time of all BCCs was 11 months (range, 5-26 months), in which 6 of 100 BCCs recurred. One patient reported mild pain during both curettage (after lidocaine/prilocaine) and imiquimod treatment, but preferred it over excision. No other side effects were mentioned and none of the patients was lost to follow-up.

Imiquimod was previously described in the treatment of BCCs in 3 BCNS-children, with partial response following application 3 days/week for a total duration of at least 8 weeks.[4] Based on the results of a recent RCT, we used the recommended schedule of 5 days per week for 6 weeks.[3] Safety data on imiquimod treatment in children are sparse, but in several phase II trials there was low systemic exposure and side effects consisted mostly of application site reactions.[5]

Based on our small case series, curettage followed by imiquimod 5% cream seems effective in the treatment of multiple small BCCs at once. The use of appropriate anaesthesia is important to prevent traumatizing procedures in young BCNS patients who will need medical care for the rest of their lives.

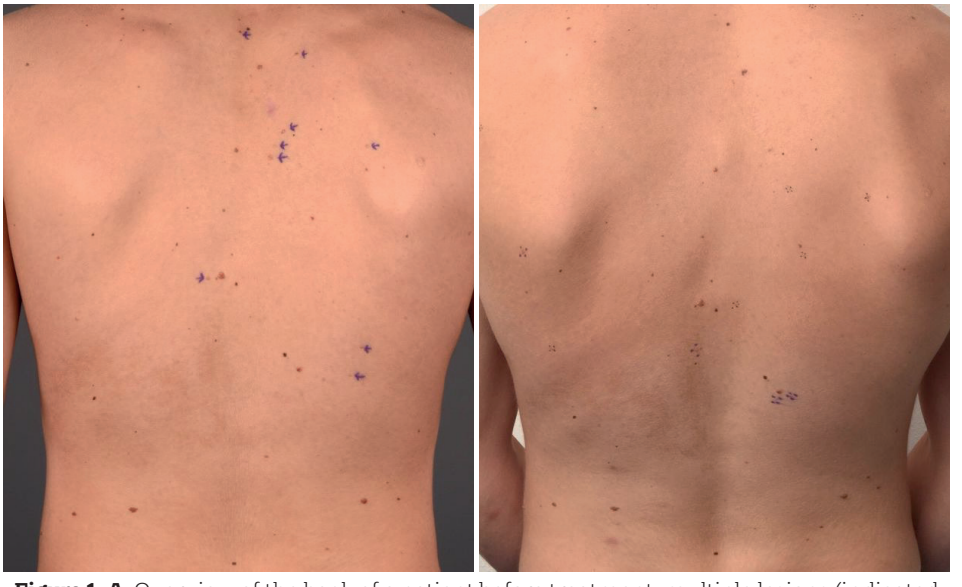

Figure 1. A. Overview of the back of a patient before treatment; multiple lesions (indicated by the arrows). **B.** Overview of the back 26 months after treatment; no recurrences but multiple new lesions (indicated by the dotted circles).

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Chapter 4.4

Prevalence of medulloblastoma in basal cell nevus syndrome patients with a *PTCH1* mutation

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LETTER TO THE EDITOR

Patients with basal cell nevus syndrome (BCNS) are at risk of developing a medulloblastoma (MB) in childhood, which usually develops before the age of 3.[1,2] The incidence of MB seems to differ between *PTCH1* and *SUFU* heterozygotes. Knowledge of the prevalence of MB is important to guide screening, which is performed by MRI. In the consensus statement of the international colloquium on BCNS published in 2011, yearly MRI screening is advised in all BCNS children.[1] However, more recently, a British cohort showed a MB prevalence of 33.3% in 9 *SUFU* heterozygotes and 2.4% in 126 *PTCH1* heterozygotes.[3] Based on this study, a recent guideline on cancer surveillance in patients with BCNS advised not to screen in *PTCH1* heterozygotes and screen *SUFU* heterozygotes more frequently (i.e. every 4 months during the first 3 years and half-yearly till the age of 5).[4] For more robust evidence on the low incidence of MB among *PTCH1* heterozygotes, we determined the prevalence of MB in a Dutch cohort.

A retrospective cohort study was conducted at the VU University Medical Centre (VUMC) and the Maastricht University Medical Centre+ (MUMC+) in the Netherlands.[5] Between April 1999 and December 2015, the laboratories of those two hospitals processed all clinical requests for *PTCH1* mutation analysis in the Netherlands and various foreign hospitals. Analysis was done by standard *PTCH1* mutation analysis (Sanger sequencing) and multiplex ligation-dependent probe amplification (MLPA). After a search for *PTCH1* analysis requests in the electronic genetic medical record system of the VUMC and MUMC+, patients with a pathogenic *PTCH1* mutation were selected. Foreign patients were excluded due to practical difficulties. Information about MB presence was retrieved from the medical records from October 2015 until December 2016.[5] The medical records of all patients aged <8 years at initial data assessment were reassessed between May 2020 and August 2020 to exclude MB development.

Clinical data were available for 83 patients (from 77 families) with a pathogenic *PTCH1* mutation. Two further cases were excluded because of intrauterine foetal death. One patient was 4 years old at time of data collection, all other patients were ≥8 years.

Of the 81 found mutations, 27 (33.3%) were nonsense, 25 (30.9%) frameshift, 11 (13.6%) splicing, 10 (12.3%) missense, 5 (6.2%) in-frame duplications and deletions, and 3 (3.7%) whole *PTCH1* gene deletions.

Only 1 of the 81 (1.2%) *PTCH1* patients was diagnosed with a MB at the age of 11 months. The patient had a germline *PTCH1* nonsense mutation in exon 12, c.1691T>G, which results in a stop at position 564 (p.Leu564*). He suffered from many other congenital birth defects, not all typical for BCNS.

Guidelines for patients with BCNS advise screening for MB with MRI.[1,4] However, screening with MRI often requires general anaesthesia in young children which can be stressful for parents and children. Moreover, the developmental risk of general anaesthesia at young age is still under debate and high frequency general anaesthesia should therefore be performed only with caution.[6] In this nationwide retrospective cohort study, we found a MB prevalence of 1.2% in *PTCH1* heterozygotes. Taking into account the disadvantages of MRI and the low MB prevalence in two *PTCH1* cohorts, high-frequency routine neuroimaging for MB in children with BCNS with an underlying *PTCH1* mutation is debatable. We advocate to perform MRI in *PTCH1* heterozygotes only when clinical symptoms are present. With this strategy it is essential to monitor the development and skull growth of children twice per year during the first years in life.

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Chapter 4.5

Molecular mechanism of extracutaneous tumours in patients with basal cell nevus syndrome

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SUMMARY

Basal cell nevus syndrome (BCNS) is a rare genetic disorder accompanied by a broad variety of tumours, of which basal cell carcinomas and odontogenic keratocysts are the most common. BCNS is caused by a germline or postzygotic mutation in either *PTCH1* or *SUFU*. As BCNS is a rare disease, it is difficult to establish whether less frequently occurring tumours are actually part of the syndrome. In this study the molecular mechanism behind four extracutaneous tumours in patients with BCNS was elucidated. A leiomyoma of the testis and meningioma were confirmed to be associated with BCNS in two patients by presence of a second mutation or loss of heterozygosity in *PTCH1*. In a meningioma of a patient with a mosaic postzygotic *PTCH1* mutation an association could not be conclusively confirmed. *SUFU* was probably not involved in the development of a thyroid carcinoma in a patient with a germline *SUFU* mutation. Hence, we have proven that meningioma and leiomyoma of the testis are rare extracutaneous tumours that are part of BCNS.

INTRODUCTION

Basal cell nevus syndrome (BCNS, OMIM #109400), also known as Gorlin syndrome, is a rare autosomal dominant disorder characterised by multiple basal cell carcinomas (BCCs), odontogenic keratocysts (OKCs) and calcification of the falx cerebri.[1] Besides these symptoms, multiple developmental defects and a variety of other tumours have been described.[2] BCNS is usually caused by a germline mutation in the patched-1 (*PTCH1*) gene. The *PTCH1* gene encodes for the patched-1 protein, which acts as a tumour suppressor gene (TSG) in the sonic hedgehog (SHH) signalling pathway. Patched-1 inhibits the protein smoothened (SMO). If this inhibition is released, SMO can translocate into the cell and eliminate the binding and anchoring of the glioma associated oncogene (GLI) transcription factors by the suppressor of Fused (SUFU). This, in turn, causes GLI transcription factors to become active (GLIA),[3] which leads to proliferation, suppression of apoptosis and angiogenesis. Other causative mutations for the BCNS phenotype are germline mutations in *SUFU* or postzygotic mutations in *PTCH1* or *SMO*.[2, 4, 5] According to the two-hit hypothesis[6], a mutation needs to be accompanied by a second hit in the wild-type allele of a TSG leading to its inactivation, for example, a second mutation, gene loss or a promoter hypermethylation event, in order to result in loss of functionality and subsequent induction of tumorigenesis. In several BCNS-related tumours *PTCH1* mutation with loss of heterozygosity (LOH) has been described.[7-9] Alternatively, haploinsufficiency might occur.[10] In many less frequently reported BCNS tumours, the molecular mechanism behind tumour formation has not been examined yet. In the present study, we investigated the extracutaneous tumours of four individuals with a BCNS phenotype caused by a germline/postzygotic mutation in *PTCH1* or *SUFU* using targeted next generation sequencing (NGS).

CASE 1

A male patient had numerous histopathological proven BCCs and a family history with a *PTCH1* germline mutation, c.747-2A>G, located in the splice acceptor site of intron 5 (previously published[11]). Besides BCCs, the patient also had segmentally distributed neurofibromas without other features of neurofibromatosis. DNA analysis on blood revealed no germline mutations in *NF1* or *SPRED1*. However, on DNA extraction of three independent neurofibromas, a shared *NF1* mutation was found (c.6522_6523dup p.(Thr2175Argfs*5), located in exon 43, NM_000267.3) and the patient was diagnosed with type I segmental mosaicism for neurofibromatosis.

In his late 40s, he presented with a meningothelial meningioma, which was surgically removed. SmMIP genetic analysis of the meningioma was performed to determine whether the meningioma developed as a result of a second hit in either *PTCH1*, *NF1* or occurred sporadically through mutations in for example *NF2*, frequently involved in meningioma tumour formation.[12] The variant allele frequency (VAF) of the *PTCH1* germline mutation was 91% in the sample (90% tumour cells), indicating LOH. TSO500 confirmed this finding (Table 1) and did not reveal mutations in *NF1*, *NF2,* or other high VAF of possible tumorigenesis initiating driver mutations. We, therefore, conclude LOH of *PTCH1* was the oncogenic initiating event in the meningioma.

CASE 2

A female patient presented with multiple BCCs and OKCs. Mid 40s, she presented with a mixed type meningioma, which was surgically removed. The clinical suspicion of BCNS could not be confirmed genetically, since no variant was detected after *PTCH1* and *SUFU* analysis in DNA isolated from blood. To exclude BCNS on the basis of post-zygotic mosaicism, formalin fixed, paraffin embedded (FFPE) samples from 2 BCCs and the meningioma were analysed. All samples demonstrated the same *PTCH1* mutation in exon 15 (c.2359G>T p.(Glu787*)) and the patient was diagnosed with type I segmental mosaicism for BCNS. In both BCCs either a second hit or LOH of *PTCH1* was seen (table 1). To test the hypothesis that the postzygotic mutation contributed to the development of the meningioma, we sought for other variants or possible LOH of *PTCH1* in the meningioma sample. Only the known mutation was found with the smMIP-NGS approach and TSO500 confirmed the increased presence of this *PTCH1* variant (VAF 39%, 90% tumour cells compared to an undetectable *PTCH1* mutation in blood). Furthermore, TSO500 identified a loss of function variant in *NF2*, c.301del p.(Y101Ifs*22), with a high VAF of 69%. These findings did not provide enough evidence to conclude which mutation, in *PTCH1* or *NF2*, initiated tumorigenesis of the meningioma.

CASE 3

A male patient presented with multiple BCCs and OKCs since his early teenage years. Mutation analysis of the peripheral blood detected a single heterozygous mutation in *PTCH1*, i.e., c.2308C>T p.(Arg770*), located in exon 15. In his late teenage years, he presented with a testicular leiomyoma which was surgically removed (Figure 1). To confirm the contribution of the germline *PTCH1* mutation to the development of the leiomyoma, smMIP mutation analysis of the leiomyoma (80% tumour cells) was performed and revealed the germline mutation and a second variant in *PTCH1*, ie, c.2542_2544del p.(Phe848del). These variants were also detected using the TSO500 analysis (*PTCH1*, c.2308C>T, VAF 52.4% and *PTCH1*, c.2542_2544del, VAF 36.1%) (table 1). No other explanatory driver mutations were found. These data imply that the leiomyoma was initiated due to a somatic second hit (mutation) in the *PTCH1* gene.

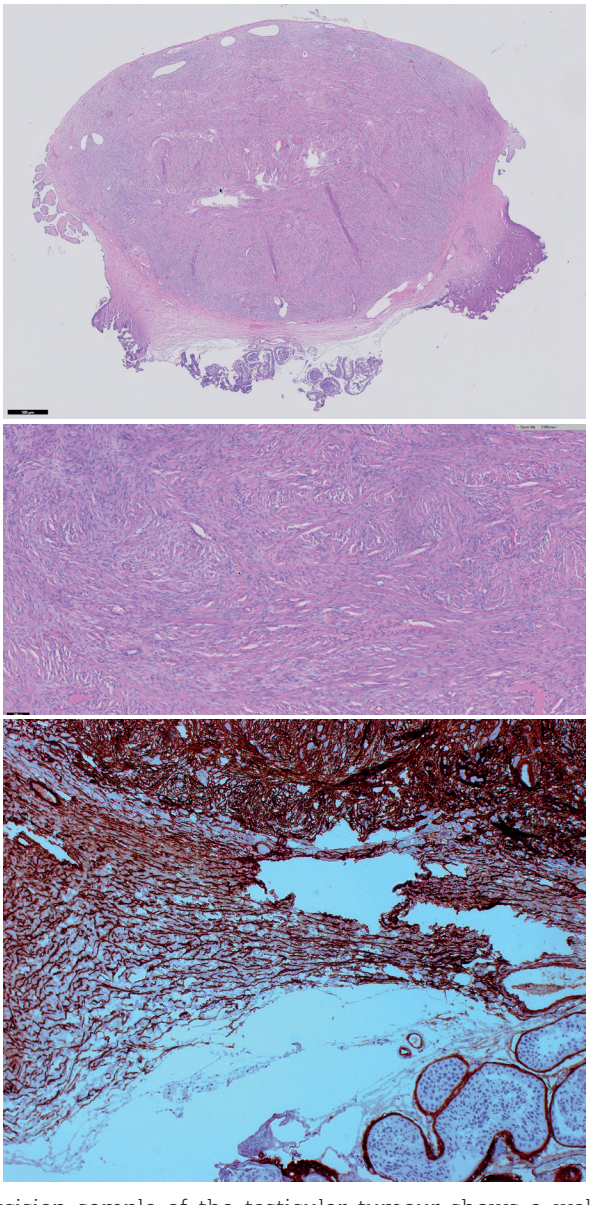

Figure 1. A. Excision sample of the testicular tumour shows a well-defined, non-encapsulated lesion originated from the tunica albuginea. Hematoxylin and eosin stain, original magnification. **B.** The lesion consists of intersecting bundles of non-atypical spindle-shaped cells in a background of collagenous fibres. There are scattered blood vessels of which the walls show continuity with the lesion. There is no mitotic activity or necrosis. Hematoxylin and eosin stain, x100. **C.** Positive alpha-smooth muscle actin staining led to the diagnosis of testicular leiomyoma.

Table 1. Results of TSO500 next-generation sequencing analysis and small molecule molecular inversion probe analysis of four extracutaneous tumours in patients with basal cell nevus syndrome.

Case	Tumour, % tumour cells	Gene	cdna	Protein
1	**Meningioma, 90% tumour cells**	PTCH1	c.747-2A>G	p.?
		MSH6	c.1052_1053 dup	p.V352Tfs*20
2	**Meningioma, 90% tumour cells**	PTCH1	c.2359G>T	p.E787*
		NF2	c.301del	p.Y101Ifs*22
	Basal cell carcinoma #1	PTCH1	c.2359G>T	p.E787*
		PTCH1	c.2524_2548delins23	p.(Lys842Aspfs*5)
		PTCH1	c.2588_2589delinsAA	p.(Trp863*)
	Basal cell carcinoma #2	PTCH1	c.2359G>T	p.E787*
3	**Leiomyoma, 80% tumour cells**	PTCH1	c.2308C>T	p.R770*
		PTCH1	c.2542_2544del	p.F848del
4	**Thyroid carcinoma, 90% tumour cells**	SUFU	c.1022+1G>A	p.?
		BRAF	c.1799T>A	p.V600E
		ARID1A	c.1558C>T	p.Q520*

LOH, loss of heterozygosity.
Only (likely) pathogenic (class 4 and 5) mutations are listed.[19]
[#]LOH confirmed with multiplex ligation-dependent probe amplification analysis.

Allele Frequency	Depth	Genomic Position (GRCh37)	Exon	Consequence
90%	82	98242872	i5	Splice Acceptor SNV
9%	148	48026172	4	Frameshift
39%	273	98229599	15	Nonsense
69%	136	30035135	3	Frameshift
38%	133	98229599	15	Nonsense
11%	114	98229434	15	Frameshift
24%	37	98224253	16	Nonsense
67%[#]	226	98229599	15	Nonsense
52%	168	98229650	15	Nonsense
36%	122	98229413	15	In Frame Deletion
44%	155	104359302	i8	Splice Donor SNV
33%	177	140453136	15	Missense
33%	300	27057850	3	Nonsense

CASE 4

A male patient presented with multiple BCCs, numerous trichoepithelioma and milia on the face, and epidermoid cysts from his early 60s. A year later, he developed a papillary thyroid carcinoma. Because of the high number of BCCs and the typical coarse facial features BCNS was suspected, but *PTCH1* analysis of the blood DNA revealed no pathogenic mutation. Additional analysis of *SUFU* detected a heterozygous germline mutation, that is, c.1022+1G>A, located in the splice donor site of intron 8, in peripheral blood. In the literature, only a few patients have been described with a *SUFU* germline mutation, including the mutation detected here[13], and in one of them a thyroid carcinoma was reported.[14] To test the hypothesis that the *SUFU* germline mutation could be underlying to thyroid carcinoma development, smMIP analysis on resection material of the thyroid carcinoma (90% tumour cells) was performed and the SUFU germline mutation was detected. No additional variants were detected in *SUFU*. TSO500 analysis performed on the thyroid carcinoma sample confirmed the germline *SUFU* mutation c.1022+1G>A with a VAF of 43.9% without an additional *SUFU* mutation. Several additional relevant mutations were found (Table 1), of which *BRAF* c.1799T>A p.(V600E) (33%) was assumed to be the most likely oncogenic driving event in the thyroid carcinoma.

DISCUSSION

In this study, the molecular mechanism underlying the development of extracutaneous tumours in four individuals with a BCNS phenotype was elucidated. Two individuals, one with a *PTCH1* germline mutation (case 1) and one with a postzygotic *PTCH1* mutation (case 2), presented with a meningioma. The meningioma that developed in the patient with a heterozygous germline *PTCH1* mutation (case 1) was initiated by LOH of *PTCH1*. This is consistent with previous findings in one patient.[15] In the other meningioma (case 2), no second hit (mutation) or LOH of *PTCH1* could be detected. Still, involvement of *PTCH1* in the tumorigenesis of this meningioma could be considered due to the fact that the postzygotic mutation is more prominently present in the meningioma. Haploinsufficiency of *PTCH1*, however, is less likely as a cause of tumorigenesis: according to the haploinsufficiency theory, mutations in TSG leading to haploinsufficiency usually occur in genes involved in DNA repair or chromosomal segregation, which is not the case for *PTCH1*.[16] TSO500 NGS analysis also identified an additional pathogenic driver variant in *NF2*, which is a commonly mutated in meningiomas.[12] Consequently, LOH of *NF2* could also have been the tumour initiating event of the meningioma.

One individual (case 3) presented with a testicular leiomyoma, a rare benign smooth muscle tumour that has not been previously reported in patients with BCNS. Leiomyomas that have been described in BCNS were located in the stomach, in the kidney and in an ovary.[17,18] Only in the ovarian leiomyoma mutation analysis was performed and it revealed a second hit in *PTCH1*, resulting in LOH.[17] We found a second variant in *PTCH1* in the testicular leiomyoma, but could not demonstrate whether both variants were located on the different alleles. The pathogenicity of this somatic second hit has not yet been proven and is based on theoretical variant classification (ACMG guidelines, variant of unknown clinical significance class 3).[19] However, no other driver gene mutations were found and it is very likely that this second variant in *PTCH1* is the second hit promoting tumorigenesis.

Thyroid carcinoma is rarely detected in patients with BCNS. After an extensive literature search we found four reports of patients with a BCNS phenotype who developed a thyroid carcinoma. One patient with a *SUFU* germline mutation received chemotherapy for a medulloblastoma and developed a papillary thyroid carcinoma, of which no further genetic analysis was performed.[14] In one patient with a medullary thyroid carcinoma at 32 years old,[20] no germline mutation in *PTCH1* was detected and *SUFU* was not analysed.[20] The patient had no OKCs

but did develop a medulloblastoma in childhood, features that are more linked to heterozygous *SUFU* patients.[21] In two other patients with BCNS features and a thyroid carcinoma, no additional information was given regarding genetic analyses.[22] In our patient, no second mutation or LOH in *SUFU* in the thyroid carcinoma was found and therefore we cannot conclude that the thyroid carcinoma is induced by *SUFU* loss of function. NGS analysis, however, did reveal an activating mutation in *BRAF*, which is a common driver of papillary thyroid carcinoma. This mutation thus is most likely the initiating oncogenic event in this case.

In conclusion, elucidating the molecular mechanisms underlying less common tumours in rare syndromes can provide evidence for associations between specific tumours and a syndrome. This is the first report proving that *PTCH1* can be responsible for the development of a leiomyoma of the testis. This information is important to completely understand the pathogenesis of BCNS and also to raise awareness for physicians treating patients with BCNS that also leiomyoma of the testis can be associated with BCNS. We confirmed previous findings that meningiomas are associated with BCNS caused by a *PTCH1* germline mutation, but did not find any evidence that thyroid carcinomas are associated with a germline *SUFU* mutation.

METHODS

DNA extraction

DNA was extracted from peripheral blood and FFPE tumour samples using the DNeasy Blood&Tissue Kit (Qiagen) and The Maxwell® RSC-DNA-FFPE-Kit (Promega), respectively. DNA from peripheral blood was analyzed with the BigDye v1.1 sequencing kit, ABI3730 DNA analyser (Applied Biosystems, primer sequences on request) and multiplex ligation-dependent probe amplification analysis for the *PTCH1* gene (kit-P067-B3, MRC Holland). DNA from tumour samples was analysed using single molecule molecular inversion probes (smMIPs) limited to genes of the SHH signaling pathway followed by NGS and NGS with TruSight™ Oncology500 panel (TSO500, Illumina).

SmMIPs (826 probes, available on request) were limited to *PTCH1, PTCH2, SMO and SUFU* (resp. NCBI RefSeq: NM_000264.3,NM_003738.4,NM_005631.4 and NM_01619.3). Also *NF1* and *SPRED1* were analysed using smMIPs (resp. NCBI RefSeq: NM_000267.3 and NM_152594.3). Mutation detection was performed using the NextSeq-500 (Illumina) with manufacturer's materials and protocols. Library preparations (ThermoFisherScientific) and sequencing was performed as described earlier.[23] Variant filtering and interpretation was achieved with Alamut v2.11 (Interactive Biosoftware) and included public databases like the Genome Aggregation Database (gnomAD) and the Catalogue Of Somatic Mutations in Cancer (COSMIC). Variant classification was performed according to the ACMG guidelines.[22] SmMIPs were used to identify germline/postzygotic mutations and identify a shared mutation in the different tumour samples from an individual patient.

TSO500

The TSO500 panel (20028216;Illumina) was used to confirm mutations found and identify other relative pan-cancer genes in the tumour samples. The TSO500 panel included full coding of 523 pan-cancer genes and detected single nucleotide variants, indels, copy number variations, fusions and immune-oncology biomarkers as well as tumour mutational burden and microsatellite instability. Library preparations were performed using genomic DNA according to the manufacturers' instructions. Data analysis was performed using the TSO500 local app, and variants were classified subsequently using the inline Varsome application (https://varsome.com).

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ETHICS APPROVAL

After approval by the medical ethics committee of the Maastricht University Medical Centre+, the Netherlands(15-4-231), all patients provided written informed consent for genetic analysis. Clinical information was retrieved from the electronic patient files.

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Chapter 5

General discussion and summary

GENERAL DISCUSSION and SUMMARY

The incidence of BCC is very high and still increasing.[1,2] One in five to six Dutch people will develop a BCC.[2] This high incidence leads to a high burden on dermatological practice as surgical excision is the main treatment for BCC.[3,4] Evaluation and optimisation of non-invasive and preferably self-administered treatment modalities for BCC contribute to decrease the burden on dermatological practice by reducing attendant workload and possibly the health care costs. Furthermore, it might increase patient satisfaction. The research described in this thesis evaluates treatment and care for patients with basal cell carcinoma (BCC), advanced BCC (aBCC), basal cell nevus syndrome (BCNS) and high-frequency BCC (HF-BCC).

Treatment of sporadic basal cell carcinoma

The gold standard treatment for BCC is surgical excision.[3,4] However, non-invasive treatment modalities, such as imiquimod cream, 5-fluourouracil cream, and photodynamic therapy, are also available, of which imiquimod cream has been proven to be the most effective in superficial BCC.[5] For superficial BCC, treatment with imiquimod is recommended in the European and American guidelines.[3,4] We aimed to investigate whether imiquimod is also an effective and safe treatment modality for small, nodular BCCs.

We conducted a non-inferiority randomized controlled trial (**Chapter 2.1**) comparing the efficacy of imiquimod with prior curettage with that of surgical excision in patients with nodular BCC, between 4-20 mm, not located in the H-zone of the face. Five years after treatment, the probability of tumour-free survival was 77.8% (95% confidence interval (CI): 65.7-86.0) for curettage and imiquimod cream and 98.2% (95% CI: 88.0-99.8) for surgical excision.

Although the efficacy of imiquimod does not equal that of surgical excision, the lack of efficacy may be outweighed by other advantages such as better cosmetic outcome. We therefore investigated the cosmetic outcome five years after treatment, scored by both physicians and patients. Five years after treatment, the cosmetic appearance of the treated area, as scored by physicians was significantly better after imiquimod treatment than after surgery. However, patients themselves regarded the cosmetic outcome of both procedures as good/excellent and no significant difference could be detected. However, it is known that patients are often satisfied with the result of the randomized treatment. Furthermore, patients cannot compare their own scar with that

of patients that received the other treatment, whereas physicians can. This supports the assumption that physicians are better in discriminating between more and less favourable cosmetic outcomes.

In our study, the probability of tumour-free survival after curettage and imiquimod at 5-year follow-up was 77.8%. Similar response rates were found in one other randomized controlled trial on the effectiveness of imiquimod for nodular BCC and in a large randomized controlled trial in superficial BCC.[5,6] Why this treatment does not work approximately 20% of BCCs is still not clarified. Identification of the cause of treatment failure could help in finding a target for improving the treatment or adjuvant treatment. Furthermore, imiquimod could be specifically prescribed for patients who are expected to respond to this treatment if it could be predicted in what patients the treatment will fail.

We retrospectively analysed data from two randomized controlled trials (**Chapter 2.2**) to find prognostic factors associated with treatment failure in imiquimod cream. The risk of treatment failure was significantly higher in males compared to females and for tumours located on the lower extremities compared to the head- and neck area. No histologic factors, including tumour thickness and invasion depth, were associated with a higher risk of treatment failure. We also found that an absent skin reaction in response to treatment led to a significantly higher risk of treatment failure compared to the occurrence of a severe skin reaction. However, as this latter determinant cannot be identified before the start of treatment, it cannot be used to select the appropriate treatment.

In international guidelines, non-invasive treatment modalities are already accepted as standard care for superficial BCC.[3,4] Based on our research, there is no reason to follow another approach for nodular BCCs <20mm not located in the H-zone of the face. Despite a lower efficacy compared to surgical excision, curettage and imiquimod can be a valuable treatment alternative as the high incidence of BCC puts a burden on the workload of dermatologists. Especially in patients with multiple lesions, this treatment increases capacity and might be more cost-effective. Furthermore, imiquimod treatment has a better cosmetic outcome and can be performed at home, which is an advantage for patients who are unable or unwilling to visit the hospital.

Treatment of advanced basal cell carcinoma

Until 2012, treatment options for advanced BCC predominantly consisted of surgery, radiotherapy or a combination of the two.[3,4] However, in some cases those treatment modalities are not feasible because they are associated with extensive

morbidity. In those cases, chemotherapy was sometimes prescribed, although it had disappointing outcomes.[7] In 2012, the first hedgehog pathway inhibitor (HPI) vismodegib was approved for the treatment of aBCC, which comprises both locally advanced BCC (laBCC) and metastatic BCC (mBCC).[8] In the Dutch guidelines, the indication for vismodegib treatment is "reserved only for patients with an aBCC in whom surgery and radiotherapy are ineffective or encounter major objections".[9] Similar statements are also incorporated in the European and American BCC guidelines.[3, 4] Data on effectiveness in real-life clinical practice is lacking and therapeutic options after treatment failure with a HPI are sparse.

We performed a national, retrospective cohort study (**Chapter 3.1**) which included all patients with BCC that received treatment with vismodegib between July 2011 and September 2019 in the Netherlands. Three different indications were defined: laBCC (n=48), mBCC (n=11) and BCNS (n=19). Median progression-free survivals (PFS) of 10.3 months (95% CI: 7.5-22.6) for laBCC and 11.7 months (95% CI: 5.2-17.5) for mBCC were found. Our cohort study was the first, non-industry driven, real world data study on the efficacy of vismodegib for aBCC. The results of our study were in line with two industry-driven trials investigating the efficacy and safety to vismodegib for aBCC.[10, 11] The only exception is the world-wide STEVIE-trial that reported a much longer PFS of 23.2 months in the laBCC group.[10] We hypothesize that the difference in outcome between our cohort and the STEVIE-trial is caused by defining a tumour "irresectable and not suitable for radiotherapy" at a more advanced stage in the Netherlands compared to other countries. Unfortunately, data on tumour size and time of presence of the BCC from the STEVIE trial that could support this hypothesis are not available. In the Netherlands, all patients are discussed in a multidisciplinary tumour board including a head- and neck surgeon, a radiotherapist, an oncologist and a dermatologist.[9] Head- and neck surgeons are able to perform surgery on very extensive tumours which leads to higher advanced cases in our cohort study. More advanced cases may have a shorter progression-free survival, but other data to further support this are lacking. Today, it is advised by the European BCC guidelines to discuss all patients with laBCC in a multidisciplinary tumour before the start of treatment.[3] Another reason for the difference in outcome between our cohort and the STEVIE-trial in laBCC patients is the retrospective nature of our study. This may have led to less meticulous measurements and a less delineated definition of tumour progression.

In our retrospective cohort study, the probability of achieving partial response after three months of treatment was 94.6% (95% CI: 84.4-99.0) for laBCC and 52.0% (95% CI: 25.5-83.9) for mBCC. The lower response rate to HPIs in mBCC is also seen in

other studies.[10, 12, 13] There are some possible explanations for a low response rate to HPIs in mBCC. First, more HPI-resistant mutations in smoothened (*SMO*) may be present in the metastases.[14] These mutations can be present already or develop during treatment and have been proven to cause resistance to vismodegib in aBCC.[15-17] Secondly, misdiagnosis could be an explanation for the low response rate in mBCC. Identifying the primary tumour of a metastasis is often difficult. In the presence of squamous or poor differentiation, confirmation of the origin of the metastasis can be difficult.[18, 19] In some cases a different squamous tumour, for example a primary lung carcinoma, can be mistaken for a BCC metastasis. Molecular confirmation of the origin of the metastasis is therefore a valuable addition to the diagnostic strategy.

We performed genetic analysis of ten BCCs and their putative metastases to identify mutual gene mutations and demonstrate a clonal relationship between the primary BCCs and the distant metastasized tumours (**Chapter 3.2**). A clonal relationship was confirmed in eight out of ten mBCCs. In one mBCC, only cytological material was available and genetic analysis performed on cytologic material failed. The other mBCC without a confirmed clonal relationship developed in a patient with BCNS. Only the germline patched-1 (*PTCH1*) mutation was found with loss of heterozygosity (LOH) and no additional mutations in both the primary and metastatic BCC were present. LOH is a frequently occurring event in sporadic tumour formation and therefore common LOH in both the primary and metastatic BCC may be a coincidental event. Distinction between clonality and occurrence of independent LOH could not be made. In four cases, a *SMO* mutation known to cause resistance to vismodegib was found. Despite the presence of vismodegib-resistant *SMO* mutations, two of these patients were treated with vismodegib. One might presume that vismodegib would be unsuccessful because of the vismodegib-resistant mutation. However, a biopsy sample represents only a small part of the tumour and the found vismodegib-resistant *SMO* mutations are not necessarily representative for the complete tumour mass. Partial response is still possible to achieve and can lead to a clinically significant reduction of symptoms. Genetic profiling of the metastases before treatment may be helpful in providing information on expected treatment response. Furthermore, genetic profiling in the context of diagnosis and staging can confirm the diagnosis and differentiate BCC metastases from other metastases or even primary tumours.

In three patients with aBCC who developed progressive disease during/after vismodegib treatment, genetic analysis was also performed for purposes of diagnosis and in order to find possible treatment targets (**Chapter 3.3**). Genetic analysis was performed on material from the metastasis or laBCC in the centre for personalized cancer treatment 02 (CPCT-02) and drug rediscovery protocol (DRUP) trials.[20, 21]

Two patients had disease progression and one experienced too many side effects to continue treatment with vismodegib. All BCCs had a very high tumour mutational burden (TMB), which is consistent with findings from literature.[22] Tumours with a high TMB are known to respond very well to programmed cell death-1 (PD-1) inhibitors.[23] All three aBCCs therefore received treatment with nivolumab or pembrolizumab (both PD-1 inhibitors). All patients showed partial response or stable disease following treatment with second line PD-1 inhibitors. One patient discontinued because of progressive disease during treatment and another patient discontinued treatment because of an immune-related adverse event. In our experience, PD-1 inhibitors can be used for treatment of aBCC after failure with vismodegib, but large studies to its effectiveness as second-line treatment are missing.

Not long after treatment of the three aBCCs with PD-1 inhibitors, a world-wide open-label trial that studied the efficacy of the PD-1 inhibitor cemiplimab in aBCC was executed.[24] To date, only results for laBCC (n=84) are available as data on mBCC are not mature yet. Overall response rate (ORR) was 31% (26/84) and grade 3-4 treatment emergent adverse events occurred in 40% of the patients.[24] After this study, cemiplimab was approved by the European Medicine Agency (EMA) for the treatment of patients with aBCC that progressed or are intolerant to treatment with HPIs, but it has not been approved in the Netherlands yet. Combination treatment with a PD-1 and HPI might lead to longer progression-free survival in aBCC. A proof-of-concept study investigating combination therapy was executed in 16 patients with aBCC.[25] Nine patients received pembrolizumab monotherapy and seven received vismodegib and pembrolizumab. Groups were not directly compared but ORR after 18 weeks was 44% (4/9, 95% CI: 14-79) in the pembrolizumab monotherapy and 29% (2/7, 95% CI: 4-71) in the pembrolizumab and vismodegib group.[25] Different studies on combination therapy in patients with aBCC are currently executed and results will have to be awaited (NCT04679480, NCT03521830, NCT02834013). PD-1 inhibitors are a valuable addition to the treatment of aBCCs in patients that are unresponsive or intolerant to treatment with oral HPIs and can be potentially combined with a HPI by parallel or alternating cycles to lengthen the treatment response.

Guideline for the clinical management of patients with BCNS

Patients with BCNS present with a broad variety of dermatological and non-dermatological symptoms and different types of tumours. The last guideline on BCNS was published in 2011 and did not take genetic analysis into account.[26] There was a need for an up-to-date, multidisciplinary, practical guideline for the clinical management of patients with (suspicion of) BCNS.

We developed a guideline using the Appraisal of Guidelines Research and Evaluation II (AGREE II) and Grading of Recommendations Assessment, Development and Evaluation (GRADE) instruments (**Chapter 4.1**). In this guideline we presented recommendations on diagnostic strategies for genetic analysis in patients that are suspected to have BCNS. The diagnostic criteria for BCNS did not change compared to the previous guideline by Bree et al. in 2011.[26] Our guideline does emphasize that physicians should be aware of the existence of postzygotic mutations as a cause of BCNS and know how postzygotic mosaicism can be diagnosed.[27-29] Furthermore, one of the main issues that is addressed in the guideline is the difference in screening for different symptoms, such as odontogenic keratocysts of the jaw and medulloblastoma, in patients with a *PTCH1* mutation compared to patients with a *SUFU* mutation. This will be discussed in more detail later on. The increase in genetic confirmation of the diagnosis BCNS has led to the ability to differentiate between *PTCH1* and *SUFU* patients in screening and follow-up schedules. We therefore advocate to confirm the diagnosis of BCNS with genetic testing. Furthermore, genetic confirmation is helpful in providing (pre)symptomatic testing for family members. The guideline also describes treatment modalities for the high number of BCCs that can develop in this population. Specifically oral and topical hedgehog pathway inhibitors are included in the guideline, which are more elaborately discussed below. Lastly, the guideline emphasizes the need for psychological support for patients and patient carers, who were also part of the guideline development group. Although the phenotype of BCNS varies to a great extent, many patients will require multiple hospital visits and (mutilating) surgical procedures. As research about the impact of BCNS on the quality of life is scarce[30, 31], we initiated a nationwide cohort study to determine this impact in relation to different symptoms. Gaining more insight in the quality of life in this group of patients will lead to improved care and more attention for the psychological well-being of patients with BCNS.

Treatment of BCCs in patients with basal cell nevus syndrome and high-frequency basal cell carcinoma

Development of numerous BCCs can be caused by a genetic syndrome, for example basal cell nevus syndrome (BCNS), or can occur as a solitary symptom, so called high-frequency BCC (HF-BCC) patients. Conventional treatment options for BCC might not always be feasible in both groups due to the high number of BCCs and HPIs could be of added value in decreasing the treatment burden.

In **chapter 3.1**, we describe 24 patients who received vismodegib for multiple BCCs in the Netherlands. Nineteen of these patients had BCNS, three had xeroderma pigmentosum and two were patients with HF-BCC. Median PFS was 19.1 months

(95% CI: 7.4-20.2) in the BCNS group and probability of partial response after 3 months of treatment was 93.3% (95% CI: 74.0-99.6). Numbers were too small to perform analyses in the non-BCNS group. The main reason for treatment discontinuation was toxicity. Thirteen patients received two or more treatment sequences (with a maximum of four) and all achieved at least partial response in all following sequences.

The incentive to prescribe HPIs in these patient groups differs from patients with aBCC, for which HPIs are the 'last' treatment option. In patients with BCNS and HF-BCC, the development of new BCCs will continue throughout life and life-long treatment with HPIs would be preferred. The goal of HPI treatment in these patients is to improve quality of life by reducing the need for conventional treatments. Side effects, experienced by all patients in our cohort, unfortunately make life-long treatment with vismodegib impossible in patients with BCNS. Over the last years, several oral and topical HPIs became available for the treatment of BCC.

Vismodegib as well as sonidegib have been proven to be effective in BCC treatment. Sonidegib is available for treatment of BCC since 2021 in the Netherlands. We systematically reviewed all data on HPIs for patients with BCNS and HF-BCC (**Chapter 4.2**) and focussed on efficacy, side effects, quality of life and tumour reoccurrence. There is limited evidence for oral and topical HPI treatment in these populations. Oral HPIs are very effective but side effects such as alopecia, muscle spasms, dysgeusia and weight loss are very common. Data on quality of life is sparse, but two randomized controlled trials reported improvement in quality of life during and shortly after treatment with oral HPIs.[32,33] There are no studies that compare vismodegib to sonidegib, but due to differences in the molecule (sonidegib is more lipophilic and has a higher volume distribution) it is hypothesized that sonidegib leads to less adverse events.[33] In order to sustain treatment with an oral HPI, different dosing schedules are applied. Several case reports and series on dosing schedules have been published, providing more evidence that treatment with adjusted dosing schedules indeed is a sustainable solution for a subset of patients. However, the published studies are mostly of retrospective nature with very small sample sizes and per study usually only the experiences of one centre are included. It is important to collect uniform data, preferably in a prospective setting, in larger cohorts to provide more insight on effectiveness, side effects, different dosing schedules and quality of life in patients treated with vismodegib or sonidegib. We therefore initiated a nationwide, prospective, cohort study that includes all patients with vismodegib or sonidegib for aBCC, BCNS or HF-BCC

in which uniform, predefined outcome measurements are used. Eventually, this cohort study will provide more data on dosing schedules and differences in daily practice efficacy and side effects between sonidegib and vismodegib.

In order to bypass side effects and still achieving high efficacy in patients with BCNS and HF-BCC,3 topical HPIs have been developed. Of those, sonidegib 0.75% cream and patidegib 2% gel, showed promising results in phase-2 trials.[34-36] One follow-up RCT of patidegib 2% gel has been completed but results have not been published yet (NCT03703310). A second follow-up trial of patidegib 2% gel and a first follow-up trial of LDE225 0.75% cream were both withdrawn before the trials were completed (NCT04308395 and NCT03070691 respectively). In the first trial (NCT03703310) the primary endpoint in difference between the placebo group and patidegib 2% group was not met. Currently, no new trials of topical HPIs are registered at clinicaltrials.gov. Efficacy and safety data to support approval of topical HPIs are not expected to be available in the short term.

Unfortunately, a subset of patients with BCNS will develop numerous BCCs and basaloid follicular hamartomas (BFHs) already from an early age. Clinically, differentiation between BCCs and BFHs can be difficult. To biopsy all lesions is not a patient-friendly option. We investigated treatment with curettage followed by imiquimod cream of 100 BCCs/BFHs in four children and adults with BCNS (**Chapter 4.3**) and only 6 of 100 lesions recurred after a median follow-up time of 11 months (range, 5-26 months). Curettage and imiquimod was performed under local anaesthesia (lidocaine/procaine cream) and only one patient reported mild pain during treatment but still preferred curettage and imiquimod over local excision. This case series demonstrates that curettage and imiquimod is a valuable treatment option for multiple lesions in young patients with BCNS. Advantages of this treatment strategy are the possibility to treat multiple lesions at once under local, topical anaesthesia and that it has a minimal amount of scarring. Preventing traumatizing and disfiguring procedures is very important in these young patients who will need medical care for the rest of their lives.

Non-dermatological tumours in patients with basal cell nevus syndrome

Different screening schedules in patients with a *PTCH1* and *SUFU* mutation were proposed. Over the past few years, two cohorts from England and the Netherlands provided evidence for differences in prevalence of symptoms in patients with a *PTCH1* and *SUFU* mutation.[37, 38] One cohort reported a lower prevalence of medulloblastoma in patients with a *PTCH1* mutation (2.4%, 3/126 patients) compared to patients with a *SUFU* mutation (33.3%, 3/9 patients). The 2011

guidelines recommended a yearly screening with MRI in children with BCNS up until the age of six years. Unfortunately, general anaesthesia is often necessary at this young age to obtain a good quality MRI scan. Validation of prevalence numbers is necessary to make adjustments to screening protocols for medulloblastoma in patients with BCNS. We performed a retrospective cohort study in a Dutch *PTCH1* database (**Chapter 4.4**) and found a prevalence of medulloblastoma of 1.2% (1/81 patients). Taking into account the disadvantages of MRI and the low MB prevalence in two *PTCH1* cohorts, high-frequency routine imaging for MB in children with BCNS with an underlying *PTCH1* mutation is debatable. We therefore advocate to perform MRI in *PTCH1* heterozygotes only when clinical symptoms, such as morning headache/nausea/vomiting and motor skill problems, are present. With this strategy, it is essential to monitor this development during the first years of life.

Besides the English cohort that included nine patients (from three families) with a *SUFU* mutation, only some case reports on patients with a *SUFU* mutation have been published.[38-43] None of the patients from these reports has developed any odontogenic keratocysts (OKCs) of the jaw, whereas the incidence of OKCs in *PTCH1* heterozygotes is 62.7%. Previously, screening for OKCs of the jaw by an oro-maxillofacial surgeon was advised in all patients with BCNS. Because of the absence of OKCs in *SUFU* heterozygotes, we abandoned this recommendation in the new guideline.

Many other tumours have been reported in patients with BCNS besides medulloblastoma and OKCs. For several tumours the molecular relationship between tumour development and the germline or postzygotic *PTCH1* or *SUFU* mutation has not been elucidated yet. We performed molecular analysis on several different extracutaneous tumours (**Chapter 4.5**) and proved the molecular relationship between meningiomas and a testicular leiomyoma and BCNS in three patients with a germline or postzygotic *PTCH1* mutation. Elucidating the molecular mechanisms of the development of less common tumours in rare syndromes can provide evidence for associations between specific tumours and a syndrome. This information is important to raise awareness for physicians treating patients with BCNS that also leiomyoma of the testis and meningioma can be associated with BCNS. It may furthermore be helpful in completely understanding the pathogenesis of BCNS. Moreover, this information regarding specific tumours may increase the knowledge on those tumours and possibly lead to the recognition of potential targets for treatment of these tumours.

In conclusion, the aim of this thesis was to optimize treatment and care for patients with (a)BCC, BCNS and HF-BCC. In this thesis we showed that imiquimod 5% cream is an effective treatment option for children and adolescents with BCNS who have multiple BCCs and that it is a good alternative to surgery in nodular BCC. Furthermore, we demonstrated that vismodegib is a suitable treatment option for patients with aBCC, BCNS and HF-BCC, but over 50% of patients with aBCC will develop disease progression within one year of treatment with vismodegib. After vismodegib failure, PD-1 inhibitors appeared to be a valuable treatment option in some patients. In patients with BCNS and HF-BCC, side effects often led to discontinuation of daily vismodegib administration. For some patients, alternating dosing schedules were a successful strategy to continue vismodegib treatment. Regarding patients with BCNS, we found that the prevalence of some (non-)dermatological symptoms depended on the causative germline mutation. We therefore advocate to confirm the diagnosis of BCNS with genetic testing.

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Chapter 6

Dutch summary

SAMENVATTING

De etiologie, pathogenese, diagnostiek en behandeling van basaalcelcarcinoom worden uitgebreid toegelicht in de algemene introductie (**hoofdstuk 1**) van dit proefschrift. Daarnaast wordt er uitleg gegeven over lokaal uitgebreid basaalcelcarcinoom, gemetastaseerd basaalcelcarcinoom, basaalcelnaevus syndroom en hoogfrequente basaalcelcarcinomen. Aan het einde van het hoofdstuk worden de doelstellingen van het proefschrift genoemd. Het proefschrift is opgedeeld in drie delen: 1. het sporadisch basaalcelcarcinoom, 2. het lokaal uitgebreid en gemetastaseerd basaalcelcarcinoom, en 3. basaalcelnaevus syndroom en hoogfrequente basaalcelcarcinomen.

Deel 1 – sporadisch basaalcelcarcinoom

In **hoofdstuk 2.1** beschrijven we de bevindingen van een gerandomiseerd onderzoek naar de behandeling van nodulair basaalcelcarcinoom met curettage en imiquimod 5% crème versus chirurgische excisie in 145 patiënten. De kans op recidief-vrije overleving 5 jaar na behandeling was 77.8% in de curettage met imiquimod groep en 98.2% in de chirurgische excisie groep. Chirurgische excisie, de gouden standaard voor behandeling van nodulaire basaalcelcarcinomen, is hiermee effectiever dan imiquimod. Desondanks is het percentage succesvol behandelde patiënten met curettage en imiquimod na 5 jaar aanzienlijk en is er zeker een plaats voor deze behandeling in een geselecteerde patiëntengroep. Imiquimod 5% crème geeft een beter cosmetisch resultaat, patiënten hoeven voor deze behandeling niet extra naar het ziekenhuis te komen en er kunnen meerdere tumoren tegelijkertijd worden behandeld. In **hoofdstuk 2.2** gaan we verder in op mogelijke prognostische factoren die van invloed zijn op het behandelsucces van imiquimod in basaalcelcarcinomen. Mannen en basaalcelcarcinomen op de benen hebben een hogere kans op therapie falen ten opzichte van vrouwen en basaalcelcarcinomen elders op het lichaam. Daarnaast was een heftigere huidreactie op behandeling met imiquimod gecorreleerd met een hogere kans op behandelsucces.

Deel 2 – lokaal uitgebreid en gemetastaseerd basaalcelcarcinoom

Hoofdstuk 3.1 bespreekt de resultaten van een retrospectieve, nationale cohortstudie naar de toepassing van vismodegib voor de behandeling van lokaal uitgebreid of gemetastaseerd basaalcelcarcinoom en multipele basaalcelcarcinomen in Nederland. Van alle lokaal uitgebreide basaalcelcarcinomen had 95% binnen 3 maanden respons op de behandeling, terwijl dit voor de gemetastaseerde basaalcelcarcinomen voor 52% gold. De mediane ziektevrije overleving voor

patiënten met een lokaal uitgebreid basaalcelcarcinoom was 10.3 maanden en voor patiënten met gemetastaseerd basaalcelcarcinoom was het 11.7 maanden. De respons van lokaal uitgebreid baaalcelcarcinoom was hoger in onze cohortstudie ten opzichte van de eerdere gerandomiseerde studies, maar de duur van de respons was korter. We denken dat dit wordt veroorzaakt doordat in Nederland een tumor pas in een later stadium als 'lokaal uitgebreid' wordt gedefinieerd vergeleken met andere landen in de gerandomiseerde studies. In Nederland worden namelijk alle patiënten vooraf aan de behandeling besproken in een multidisciplinair overleg waarbij ook een radiotherapeut, oncoloog en oncologisch chirurg aanwezig zijn. De uitkomsten voor gemetastaseerd basaalcelcarcinoom in onze cohortstudie kwamen overeen met eerder gemelde resultaten in de literatuur. Twaalf van de negentien patiënten met basaalcelnaevus syndroom die waren behandeld met vismodegib hebben 2 of meer behandel cycli gehad. De meeste patiënten met basaalcelnaevus syndroom stopten de behandeling in verband met bijwerkingen. In alle volgende behandelcycli werd wederom in alle patiënten respons op de behandeling gezien.

In **hoofdstuk 3.2 en 3.3** gaan we vervolgens in op moleculair onderzoek van gemetastaseerd basaalcelcarcinoom en de mogelijke rol van moleculaire analyse van de tumor in relatie tot de behandelkeuze. **Hoofdstuk 3.2** bevestigt de klonale relatie tussen metastasen en het primaire basaalcelcarcinoom in acht patiënten. Vervolgens bespreken we in **hoofdstuk 3.3** één patiënt met lokaal uitgebreid en twee patiënten met gemetastaseerd basaalcelcarcinoom, welke na falen van behandeling met vismodegib, behandeld zijn met immunotherapie. Moleculair onderzoek werd verricht en therapeutische consequenties van de bevindingen werden besproken. Evenals de indicatie, bijwerkingen en effectiviteit.

Deel 3 – basaalcelnaevus syndroom en hoog-frequente basaalcelcarcinomen

De zorg voor patiënten met basaalcelnaevus syndroom is complex door de zeldzaamheid van de aandoening en de verscheidenheid van de symptomen. Naast de vele basaalcelcarcinomen gaat basaalcelnaevus syndroom gepaard met meerdere, niet-dermatologische symptomen. In **hoofdstuk 4.1** lichten we de richtlijn toe die we hebben ontwikkeld voor patiënten met basaalcelnaevus syndroom en hun zorgverleners met input van meerdere specialismen en meerdere (ouders/verzorgers van) patiënten. Er wordt uitgebreid ingegaan op diagnostische mogelijkheden om het syndroom te bevestigen en symptomen uit te sluiten. Wij ontwikkelden een checklist welke aangeeft welke controles plaats moeten vinden en wanneer dit dient te gebeuren. Het onderscheid tussen patiënten met een *patched-1* (*PTCH1*) en *suppressor of fused homolog* (*SUFU*) mutatie is hierbij essentieel.

Hoofdstuk 4.2 weidt verder uit over de behandeling met orale en topische hedgehog signaalroute remmers voor patiënten met basaalcelnaevus syndroom en hoogfrequente basaalcelcarcinomen aan de hand van een uitgebreid, systematisch overzicht van de literatuur. Op basis van de tot nu toe beschreven literatuur kan men concluderen dat orale hedgehog signaalroutes remmers erg effectief zijn, maar bijwerkingen voorkomen dat levenslange behandeling een optie is. In de praktijk worden vaak verschillende behandelschema's toegepast om bijwerkingen dragelijk te maken, maar onderzoeken met een hoge bewijslast die behandelschema's in kaart hebben gebracht zijn zeer beperkt. De resultaten van twee fase 2 onderzoeken naar verschillende topische hedgehog signaalroute remmers lieten zien dat ook via deze toedieningsroute er een antitumor reactie was, met daarbij als voordeel dat er geen systemische bijwerkingen optraden. Op dit moment lopen er geen fase 3 studies naar topische hedgehog signaalroute remmers en voorlopig laat deze therapie dus nog op zich wachten. **Hoofdstuk 4.3** omvat een retrospectief onderzoek naar de behandeling met curettage gevolgd door imiquimod 5% crème voor basaalcelcarcinomen in kinderen en adolescenten met basaalcelnaevus syndroom. Deze minimaal invasieve behandeling leidde tot een relatief hoge effectiviteit. Van de honderd tumoren die werden behandeld kwamen er zes terug na een follow-up duur van gemiddeld 11 maanden. Hiermee toonden wij aan dat deze minimaal invasieve behandeling een goed alternatief kan zijn in deze patiëntenpopulatie.

Eén van de major criteria voor de diagnose BCNS is, naast basaalcelcarcinomen, de aanwezigheid van een medulloblastoom. Voor de screening van medulloblastoom, dat in patiënten met BCNS altijd voorkomt op de jonge kinderleeftijd, is een MRI nodig. Er zijn aanwijzingen in de literatuur dat de prevalentie van medulloblastoom in patiënten met een *PTCH1* mutatie een stuk lager is dan bij patiënten met een *SUFU* mutatie. In **hoofdstuk 4.4** hebben we daarom de prevalentie van medulloblastoom in een Nederlands cohort van *PTCH1* patiënten bepaald. De prevalentie was 1.2% (1/81 patiënten) en dit kwam overeen met het enige andere cohort in de literatuur (2.4%). De prevalentie van medulloblastoom in patiënten met een *SUFU* mutatie wordt tot 20x hoger geschat, waardoor alleen in deze groep screening met MRI wordt aanbevolen.

Bij BCNS worden behoudens basaalcelcarcinomen ook tumoren in andere organen gezien. Een deel van deze tumoren wordt veroorzaakt door een mutatie in het *PTCH1* gen van het niet aangedane allel, maar voor een aantal tumoren bestond hiervoor nog geen bewijs. In **hoofdstuk 4.5** tonen we dit ook aan bij een meningeoom en testiculair leiomyoom in patiënten met een kiembaanmutatie

in het *PTCH1* gen. Bij een patiënt met een schildkliercarcinoom en een kiembaanmutatie in het *SUFU* gen werd in het schildkliercarcinoom geen extra mutatie in het *SUFU* gen gevonden en deze tumor is dus niet geassocieerd met het syndroom.

In **hoofdstuk 5** bespreken we de belangrijkste conclusies van het onderzoek en wordt de relevantie voor artsen en patiënten toegelicht. Daarnaast beschrijven we mogelijkheden voor toekomstig onderzoek.

Chapter 7

Impact paragraph

Impact paragraph

The incidence of basal cell carcinoma (BCC) is high and expected to grow explosively in the next ten years. This may cause a major health issue in the near future, as a lot of surgical excisions will have to be performed to treat all the BCCs. Over the past decades, non-invasive treatments have been approved as therapy for BCC. The most effective, non-invasive, self-administered treatment for superficial BCC (sBCC) is imiquimod 5% cream, which is broadly accepted as standard treatment for sBCC. Imiquimod is an immunomodulator that binds to toll-like receptors 7 and 8.

We investigated the long-term effectiveness of imiquimod 5% cream in nodular BCC. Five years after treatment, the probability of remaining tumour-free in nodular BCC was 77.8% after treatment with curettage followed by imiquimod 5% cream and 98.2% after treatment with surgical excision. Although surgical excision is superior in efficacy, non-invasive therapy has several advantages, such as a better cosmetic outcome and the ability to treat multiple tumours at once in an at home setting. In nBCC patients who highly value cosmetic outcome, burden from surgery or have a preference for a treatment at home, imiquimod 5% cream is a good treatment option.

For a better understanding of the cause of treatment failure of imiquimod in approximately 20% of the patients, we analysed patient, tumour and treatment characteristics. Several predictors of treatment failure were found. Knowledge on predictors of treatment failure eventually leads to the possibility of determining which BCCs cannot be treated with imiquimod 5% cream before therapy is initiated. This knowledge may also be helpful in future studies aiming at improving treatment efficacy.

The high incidence of BCC will also lead to a rising incidence of patients with advanced BCC, which comprises both locally advanced and metastatic BCC. In part two of this thesis I describe a study on non-invasive treatment options in patients with advanced BCC. In this retrospective cohort study we investigated different aspects of treatment with the hedgehog pathway inhibitor vismodegib in all BCCs that received this treatment in the Netherlands. With this study, the real world use of this therapy was demonstrated, leading to a more strict protocol for treatment with hedgehog pathway inhibitors. This eventually resulted in a national prospective registry study in patients treated with hedgehog pathway inhibitors. Communication between different prescribing physicians and consequently more uniformity in prescription enables optimal treatment regimens for all patients.

In part three of this thesis I discuss basal cell nevus syndrome (BCNS), a rare genetic disorder based on either a *PTCH1* or a *SUFU* mutation and characterized by multiple BCCs and a broad variety of other symptoms. We developed an up-to-date guideline with emphasis on the value of genetic testing and differences between patients with a *PTCH1* and *SUFU* mutation. We furthermore proposed a diagnostic plan to detect all patients with a germline or postzygotic mutation. The multidisciplinary guideline offers a practical guide for physicians when screening for all possible BCNS symptoms and treatment of BCCs. Treatment with hedgehog pathway inhibitors is shortly addressed in the guideline and more elaborately discussed in an extensive review which was also included in part three. The review provides a clear overview of all available data on treatment with different hedgehog pathway inhibitors in relation to their effectiveness, side effects, improvement of quality of life and tumour recurrence after treatment discontinuation. The guideline and review can be used in daily practice for all patients with BCNS and by physicians that encounter a patient with BCNS.

Addendum

CURRICULUM VITAE

LIST OF PUBLICATIONS AND PRESENTATIONS

ACKNOWLEDGEMENTS / DANKWOORD

Addendum

CURRICULUM VITAE

CURRICULUM VITAE

Babette Verkouteren werd geboren op 10 mei 1993 te Tholen in Zeeland. In 2011 behaalde zij haar gymnasiumdiploma aan de Regionale Scholengemeenschap 't Rijks in Bergen op Zoom. In hetzelfde jaar begon zij aan de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Vanaf 2012 tot 2018 was Babette werkzaam als medisch student en teamleider (2015-2017) van het medisch studententeam op de verpleegafdeling Urologie en Gynaecologie in het Erasmus Medisch Centrum te Rotterdam. Op 12 maart 2018 rondde zij cum laude haar studie geneeskunde af. Nadat zij een klein jaar als arts niet in opleiding tot specialist had gewerkt op de afdeling dermatologie in het Amphia Ziekenhuis te Breda, startte ze in april 2019 met haar promotieonderzoek op de afdeling dermatologie van het Maastricht Universitair Medisch Centrum+ (Maastricht UMC+). Met haar onderzoek won ze in 2023 de prijs voor beste poster presentatie op het dermato-oncologiecongres in Rome, Italië. Sinds april 2021 combineert Babette haar werkzaamheden met veel enthousiasme met de opleiding tot dermatoloog. Zij vervult daarnaast sinds april 2022 de functie van voorzitter van de arts-assistenten vereniging van het Maastricht UMC+. Naast haar werkzaamheden in het ziekenhuis is ze ook medeoprichter van de dermatologische podcast: 'Onder de Loep'.

Addendum

LIST OF PUBLICATIONS AND PRESENTATIONS

LIST OF PUBLICATIONS AND PRESENTATIONS

Publications related to this thesis

Cosgun B, **Verkouteren BJA**, Kessler PAWH, Mosterd K. Het basaalcelnaevussyndroom: raakvlak tussen tandheelkunde en dermatologie.
Nederlands Tijdschrift voor Tandheelkunde, 2023, volume 130, issue number 5

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List of oral and poster presentations

Surgery versus imiquimod 5% cream for nodular basal cell carcinoma: a randomized, controlled trial with 5 years follow-up. Poster presentation at the 18th European Association of Dermato Oncology (EADO) congress, Rome, Italy, April 2023

Eight years of experience with vismodegib for advanced and multiple basal cell carcinoma patients in the Netherlands. Oral presentation at the 21st Annual Meeting of the Dutch Society for Experimental Dermatology (NVED), Lunteren, the Netherlands, June 2022

A guideline for the clinical management of basal cell nevus syndrome (Gorlin-Goltz syndrome). Poster presentation at the 17th European Association of Dermato Oncology (EADO) congress, Sevilla, Spain, April 2022

A guideline for the clinical management of basal cell nevus syndrome (Gorlin-Goltz syndrome). Hybrid oral presentation at the 356th Scientific Meeting of Dutch Society for Dermatology and Venereology (NVDV), Maastricht, the Netherlands, November 2021

Vismodegib for metastasized basal cell carcinoma and basal cell nevus syndrome. Oral presentation at the Dermatology @C congress, Egmond aan Zee, the Netherlands, November 2021

Prognostic factors for treatment failure after imiquimod treatment in basal cell carcinoma. Oral video pitch presentation at the Pélerìn symposium, Maastricht, the Netherlands, October 2021

A guideline for the clinical management of basal cell nevus syndrome (Gorlin-Goltz syndrome). Virtual oral presentation at the National Basal Cell Nevus Syndrome Patient Conference Day, Maastricht, the Netherlands, May 2021

A guideline for the clinical management of basal cell nevus syndrome (Gorlin-Goltz syndrome). Virtual oral presentation at the National Workgroup Genodermatoses, Maastricht, the Netherlands, April 2021

Eight years of experience with vismodegib for advanced and multiple basal cell carcinoma patients in the Netherlands. Virtual poster presentation at the Grow Science Day, Maastricht, The Netherlands, November 2020

Eight years of experience with vismodegib for advanced and multiple basal cell carcinoma patients in the Netherlands. Virtual oral presentation at the 16th European Association of Dermato Oncology (EADO) congress, Vilnius, Lithuania, October 2020

Molecular testing in metastatic basal cell carcinoma. Virtual poster presentation at the Dutch Dermatology Days, the Hague, the Netherlands, September 2020

Molecular testing in metastatic basal cell carcinoma. Oral presentation at the 20th Annual Meeting of the Dutch Society for Experimental Dermatology (NVED), Lunteren, the Netherlands, January 2020

Molecular testing in metastatic basal cell carcinoma. Poster presentation at the Grow Science Day, Maastricht, The Netherlands, November 2019

Addendum

DANKWOORD

DANKWOORD

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