# Vismodegib-resistant basal cell carcinomas in basal cell nevus syndrome: Clinical approach and genetic analysis

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## INTRODUCTION

Basal cell nevus syndrome (BCNS, Gorlin syndrome) is a rare inherited disorder characterized by the development of multiple basal cell carcinomas (BCCs), odontogenic keratocysts, and palmar pits.[1]

BCC development is caused by sonic hedgehog pathway (SHH) activation caused by mutations in tumor suppressor gene patched 1 (*PTCH1*) or activating mutations in the oncogene smoothened (*SMO*).[2] Because patients with BCNS carry a germ-line mutation in *PTCH1*, one additional somatic mutation (second hit) results in BCC development at a young age.

In 2012, the US Food and Drug Administration approved vismodegib for treatment of locally advanced BCC (laBCC) or metastatic BCC (mBCC). Vismodegib prevents activation of the SHH pathway by binding and inhibiting the SMO protein.[3] Vismodegib resistance, mainly caused by *SMO* mutations, is an important problem seen in laBCC or mBCC in patients with and without BCNS.[4,5] Vismodegib resistance in smaller BCCs, which are far more frequent in BCNS patients, is only described once.[6] Here, vismodegib resistance of those smaller BCCs in a BCNS patient is genetically explained, and a clinical treatment approach is given.

*Abbreviations used:*

| | |
|---|---|
| BCC: | basal cell carcinoma |
| BCNS: | basal cell nevus syndrome |
| laBCC: | locally advanced BCC |
| mBCC: | metastatic BCC |
| SHH: | sonic hedgehog pathway |
| smMIP: | single-molecule molecular inversion probes |

## CASE REPORT

A 59-year-old man with BCNS (Gorlin syndrome) was seen at our dermatology outpatient clinic with a history of multiple BCCs, palmar pits, and hypertelorism. After former extensive mutilating surgical procedures, surgery was not feasible. Vismodegib treatment was started at 150 mg/d in a clinical trial (STEVIE; NCT01367665). There was a reduction of the amount and size of the BCCs until no BCCs were clinically detectable and also palmar pits disappeared (Fig 1, *A* and *B*). Adverse events included hair loss, muscle cramps, a total lack of taste, and weight loss of 15 kg. After 3 years of continuous vismodegib therapy, 3 lesions developed (preauricular and 2 on his back) suspect for recurrent BCC (Fig 1, *C*). After excision of the 3 lesions, 2 lesions (preauricular and on his back) were histologically confirmed to be superficial BCCs, whereas one of the lesions on his back showed no histologic signs of malignancy. Following the study protocol, treatment was discontinued (resistance). Written informed consent was obtained to perform genetic analysis on their tissue. Two months after discontinuing vismodegib treatment, multiple BCCs

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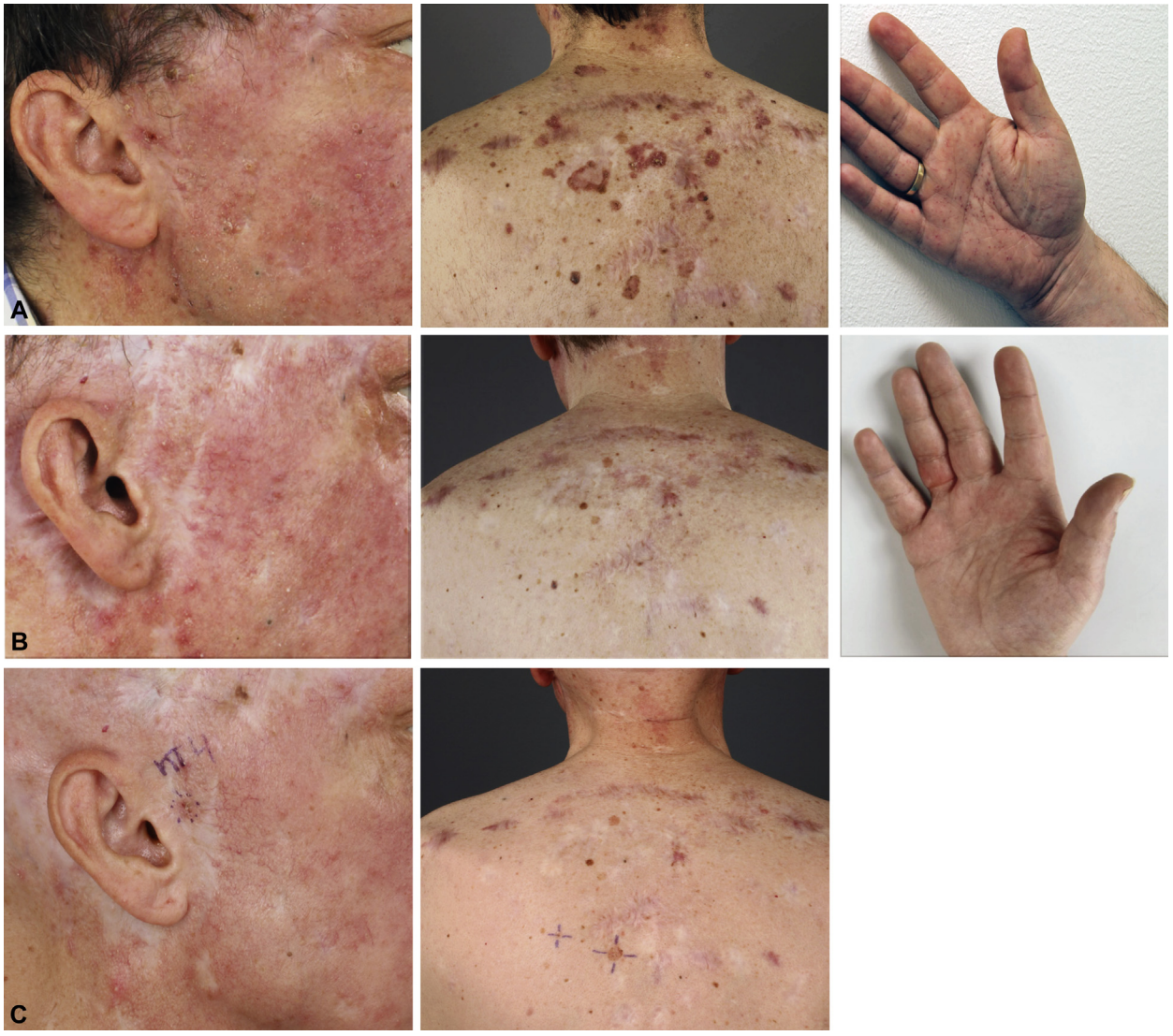


**Fig 1.** BCNS patient. **A**, Before starting vismodegib. **B**, Clearance after 3 months of vismodegib treatment. **C**, BCC redevelopment (resistance).

(re)developed on their original locations and with the exact sizes as before treatment. Currently, he is treated intermittently with vismodegib (4-5 months on and 6-7 months off), depending on response and side effects. Resistant tumors are surgically excised at the end of each treatment cycle.

## METHODS

Directly after excision, biopsy samples (3 mm) were taken from the 3 clinically suspect BCCs preauricular and on the back. The samples were freshly frozen and stored at −80°C, and subsequently DNA was extracted (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) and analyzed using single-molecule molecular inversion probes (smMIPs).[7] An smMIP-based library preparation was used to target coding sequences of genes involved in the SHH pathway: *PTCH1*, *PTCH2*, *SMO*, *SUFU*, *GLI2*, and *TP53* (resp. NCBI RefSeq: NM_000264.3, NM_003738.4, NM_005631.4, NM_016169.3, NM_005270.4, NM_000546.5/NM_001126113.2/NM_001126114.2). Subsequently, the samples were sequenced on a MiSeq system (Illumina, San Diego, CA) next-generation sequencer.

## RESULTS

All 3 samples showed a germ-line mutation in *PTCH1*, located in the splice acceptor site of intron 5, c.747-2A>G and predicted to affect the conical splice site, which putatively results in aberrant splicing of the *PTCH1* transcript and is presumably causal to BCNS. The represented percentage of this *PTCH1* mutation was 47.0% for histologically normal skin, and no additional mutations were found

**Table I.** Mutational analysis: Resistant basal cell carcinomas analyzed using molecular inversion probes

| Sample | Mutation | Gene | Protein change | Type |
|---|---|---|---|---|
| Sample 1 (BCC) | c.747-2A>G 59.8% | *PTCH1* | p.?† | Germ-line splice site mutation with loss of heterozygosity of other allele (skewed %) |
| Sample 1 (BCC) | c.1417G>A 14.1% | *SMO* | p.Asp473Asn | Somatic missense mutation (responsible for vismodegib resistance) |
| Sample 2 (BCC) | c.747-2A>G 47.4% | *PTCH1* | p.?† | Germ-line splice site mutation |
| Sample 2 (BCC) | c.1804C>T 26.8% | *PTCH1* | p.Arg602* | Somatic nonsense mutation Second hit |
| Sample 2 (BCC) | c.1406G>A 23.5% | *SMO* | p.Cys469Tyr | Somatic missense mutation (responsible for vismodegib resistance) |
| Sample 2 (BCC) | c.722C>T 26.4% | *TP53* | p.Ser241Phe | Somatic missense mutation |
| Sample 3 (histologically normal skin) | c.747-2A>G 47% | *PTCH1* | p.?† | Germ-line splice site mutation |

†The germ-line mutation is located at the splice acceptor site of intron 5 (at the exon 6 border). Splice site software tools (integrated in the Alamut V2.10 software) predict the acceptor splice site to be lost by the mutation. Because the mRNA cryptic splicing needs to be experimentally verified, the resulting putative protein is unknown (p.?).

(sample 3). In sample 1 (BCC), the germ-line *PTCH1* mutation skewed to 59.8%. Furthermore, a *SMO* mutation c.1417G>A (p.(Asp473Asn)) was detected, representing 14.1% in the sample and a known mutation causing vismodegib resistance.[4] In sample 2 (BCC) the *PTCH1* germ-line mutation percentage was 47.4%, similar to that of normal skin. Additionally, a second nonsense mutation in *PTCH1* c.1804C>T (p.(Arg602*)) was detected as second hit (26.8%). In this sample, a different causal SMO mutation c.1406G>A (p.(Cys469Tyr)) was found (23.5%).[5] Furthermore, a *TP53* mutation c.722C>T(p.(Ser241Phe)), previously described as germ-line mutation in sarcoma, was found (Table I).[8]

## DISCUSSION

Here, a BCNS patient with vismodegib resistance in small, BCNS-related BCCs and the clinical course are described. To our knowledge, resistance of non-laBCCs was only documented in 1 of the 41 included BCNS patients in a phase 2 trial by Tang et al[6] This resistance is probably much less frequent than vismodegib resistance in laBCC, which occurs in approximately 20% of patients.[9]

Both detected *SMO* mutations in the resistant BCCs were found before.[4,5] The few different *SMO* mutations reported to date suggest the presence of hotspot regions in *SMO*, responsible for resistance.[4,5] We used smMIP-based analysis, because it is relatively easy in determining SMO-associated tumor resistance with low costs.[7] In a clinical setting, this may be valuable and even cost effective if the decision to continue therapy depends on one or a few lesions. Vismodegib resistance in BCNS does not have the same clinical implications as in laBCC/mBCC, because the few resistant BCCs can easily be treated otherwise.

Vismodegib discontinuation led to reoccurrence of BCCs at their original locations, suggesting that tumors are not completely eliminated. Indolent cancer stem cells, capable of redeveloping cancer cells when treatment is discontinued are hypothetically debit.[4] The fact that treatment with vismodegib is only of suppressive nature is important to discuss with the patient.

In BCNS patients with non-laBCCs only, 17% tolerate continuous vismodegib treatment during 3 years, and side effects are the major reason for discontinuation of treatment.[6] In these patients with long-term treatment need, rotational schedules have been applied (12 weeks of 150 mg/d vismodegib rotated with 8 weeks of placebo, or starting with 24 weeks vismodegib followed by 8 weeks of placebo rotated with 8 weeks vismodegib), but even then, adverse events cause high treatment discontinuation (23%).[10] Probably, these schedules are still too stringent. Because BCNS patients need lifelong treatment, intermittent vismodegib therapy seems preferable and can be combined with excision or topical treatment of the few resistant tumors. In our experience, the development of response and severity of side effects varies, so discontinuation and restart of the therapy should be guided by the burden of the patient.

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