Molecular testing in metastatic basal cell carcinoma

Verkouteren, Babette J A; Wakkee, Marlies; van Geel, Michel; van Doorn, Remco; Winnepenninckx, Véronique J; Korpershoek, Esther; Mooyaart, Antien L; Reyners, An K L; Terra, Jorrit B; Aarts, Maureen J B

Published in:
Journal of the American Academy of Dermatology

DOI:
10.1016/j.jaad.2019.12.026

Molecular testing in metastatic basal cell carcinoma

Babette J. A. Verkouteren, MD,[a,b] Marlies Wakkee, MD, PhD,[c] Michel van Geel, PhD,[a,b,d] Remco van Doorn, MD, PhD,[c] Véronique J. Winnepenninckx, MD, PhD,[f] Esther Korpershoek, PhD,[g] Antien L. Mooyaart, MD, PhD,[g] An K. L. Reyners, MD, PhD,[h] Jorrit B. Terra, MD, PhD,[i] Maureen J. B. Aarts, MD, PhD,[b,j] Marie G. H. C. Reinders, MD, PhD,[a,b] and Klara Mosterd, MD, PhD[a,b]
Maastricht, Rotterdam, Leiden, Groningen, and Zwolle, the Netherlands

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 to explore which hedgehog pathway-related mutations are involved in mBCC.

Methods: Genetic analysis was conducted in 10 primary BCCs and their metastases. Genes relevant for BCC development were analyzed in tumor 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 8 of 10 patients, identical gene mutations could be demonstrated in the primary tumors and their metastases. A broad spectrum of mutations was found. Four patients had *SMO* mutations in their tumor or metastasis, or both. All *SMO* mutations found were known to cause resistance to targeted therapy with vismodegib.

Limitations: In 2 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. (J Am Acad Dermatol 2021;85:1135-42.)

Key words: basal cell carcinoma; hedgehog pathway; metastatic; molecular genetics; targeted therapy; vismodegib.

Basal cell carcinoma (BCC) is the most common skin cancer among white individuals, 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,

From the Department of Dermatology,[a] and GROW School for Oncology and Developmental Biology,[b] Maastricht University Medical Center; the Department of Dermatology, Erasmus University Medical Center Cancer Institute, Rotterdam[c]; the Department of Clinical Genetics, Maastricht University Medical Center[d]; the Department of Dermatology, Leiden University Medical Center[e]; the Department of Pathology, Maastricht University Medical Center[f]; the Department of Pathology, Erasmus University Medical Center Cancer Institute[g]; the Department of Medical Oncology, University Medical Center Groningen[h]; the Department of Dermatology, Isala Dermatologic Center, Zwolle[i]; and the Department of Medical Oncology, Maastricht University Medical Center.[j]

Funding sources: None.

Conflicts of interest: Dr Aarts has advisory relationships with BMS. Drs Verkouteren, Wakkee, van Geel, van Doorn, Winnepenninckx, Korpershoek, Mooyaart, Reyners, Terra, Reinders, and Mosterd have no conflict of interest to declare.

IRB approval status: Reviewed and approved by the Maastricht University Medical Center+ Ethics Committee (#15-4-231).

Accepted for publication December 12, 2019.

Correspondence and reprint requests to: Babette J. A. Verkouteren, MD, P. Debyelaan 25, 6229 HX Maastricht, the Netherlands. E-mail: babette.verkouteren@mumc.nl.

Published online December 21, 2019.

0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2019.12.026

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-signaling pathway.[6] Approximately 85% of sporadic BCCs harbor mutations in 1 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 harbor vismodegib-resistant mutations. Mutations in *SMO*, either primarily present in the tumor 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.

CAPSULE SUMMARY

- Metastatic basal cell carcinoma can be difficult to diagnose. Molecular testing can help to diagnose metastatic basal cell carcinoma and may be useful in tailoring the treatment.
- We advise to obtain fresh tumor biopsy samples for molecular testing if metastatic basal cell carcinoma is suspected.

METHODS

Between April 2016 and May 2019, genetic analysis was performed for 8 patients with mBCC in the Maastricht University Medical Center+ (Maastricht UMC+) and the Erasmus University Medical Center Cancer Institute (Erasmus MC). The Maastricht UMC+ also received requests for genetic analysis of 3 patients with mBCC from 2 other centers. In the Maastricht UMC+, DNA was extracted and analyzed 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* (National Center for Biotechnology Information [NCBI] RefSeq: NM_000546.5/NM_001126132.2/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 NGS 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*, and *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, and 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 tumors and metastases was 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 tumor.[17] All patients included gave written informed consent for genetic analysis except one. Only histologic analysis was performed on this patient.

Abbreviations used:

BCC:	basal cell carcinoma
Erasmus MC:	Erasmus University Medical Center Cancer Institute
FFPE:	formalin-fixed, paraffin-embedded
Maastricht UMC+:	Maastricht University Medical Center+
mBCC:	metastatic basal cell carcinoma
NCBI:	National Center for Biotechnology Information
NGS:	next-generation sequencing
smMIP:	small molecule molecular inversion probe

RESULTS

The clinical characteristics of the 11 included patients are summarized in Table I. The median age at diagnosis of the primary BCC was 63 years (range, 42-80 years), and 7 patients (64%) were women. The primary BCC was located on the trunk in 5 patients, in the head and neck region in 4, and on the lower extremity in 2. The primary BCC of 6 patients was initially treated with surgery. The excision in 2 of those patients did not lead to tumor-free margins. One of these patients was treated with radiation therapy afterward, and the other patient did not receive adjuvant treatment. The primary locally advanced BCC of 1 patient was treated with vismodegib.

The metastases in 4 patients were already present at the time of the primary BCC diagnosis, and 3 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, eighth edition). Four patients only had regional lymph node metastases. Distant metastases were present in 7 patients: 5 in the lungs, 1 in the pleural cavity, and 1 in the bones. Three patients with distant metastases also had proven regional lymph node metastases. Apart from 1 patient with basal cell nevus syndrome and 1 patient with HIV, there were no other patients with a genetic syndrome or immunosuppression.

The results of histologic characteristics can be found in Table II. Of the 11 patients, 7 had an infiltrative subtype of their primary BCC, 3 had a mixed nodular and infiltrative subtype, and 1 patient had a primary nodular BCC (Table II). Squamous differentiation was observed in 4 metastases and 3 primary tumors. 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 tumors and metastases. Histologic samples were available for both the primary tumor and metastasis in 9 patients. Cell type and differentiation differed between the primary tumor and metastasis in 7 of these 9 patients. For example, the primary tumor in patient 1 showed a typical BCC histology, but the lung metastasis showed more squamous differentiation (Fig 1). Owing to differences in histopathology, additional immunohistochemical staining was performed in the metastasis of all these patients (Table II).

Genetic analysis of the primary tumors and metastases was performed in 10 of the 11 patients, because 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 3 patients (Table III). In 1 of those 3 patients, genetic analysis of the FFPE material of the primary tumor and metastasis failed with targeted NGS. In a different patient, genetic analysis of FFPE material with smMIP was successful in the primary tumor biopsy but failed in the cytologic puncture of the metastasis.

In all 8 patients in whom genetic analysis was successful for both samples, the mutations found in the metastases were identical to those found in the primary tumors. Four of those patients had distant metastases, 3 patients only had regional lymph node metastases, and 1 had a parotid gland metastasis. All 4 patients with distant metastases had a known vismodegib-resistant *SMO* mutation, 2 of them received vismodegib therapy before material for genetic analysis was obtained. Specifications of the tumor mutation profiles and corresponding clinical courses are provided in Table III.

Nine patients were treated with vismodegib for their mBCC, of which 2 attained complete response. Progressive disease developed in the remaining 7 within 1 year under this therapy, and vismodegib treatment was discontinued. Of those, 3 died, 2 are currently in between treatments, and 2 are being treated with a checkpoint inhibitor in a clinical trial setting.[19]

DISCUSSION

In this case series we demonstrated the presence of identical gene mutations in 8 primary BCCs and

Table I. Clinical characteristics

Patient	Age (y)/ sex	Primary site	TNM/ stage*	Size of primary (cm)	Deep invasion†	Treatment primary tumor	Recurrence	Interval to metastasis (y)	Site of metastasis
1	68/F	Scapula	T3N0M1/IVB	15	Yes	Vismodegib, excision	Yes	4.6	Lung
2‡	54/F	Head	T4N3bM0/IVA	20	Yes	Excision	NA	0	Cervical LN
3	63/F	Abdomen	T3N3M0/IVA	>10	Yes	Vismodegib	NA	0	Axillary & inguinal LN
4	49/F	Head	T3N1M1/IVB	10	Yes	Excision,§ RT	Yes	11	Preauricular LN & lung
5	52/F	Scapula	T3N2M1/IVB	5	No	Excision	Yes	10	Axillary LN & lung
6	57/M	Back	T2N3M0/IVA	3	No	Excision	Yes	7	Axillary LN
7	70/F	Sternum	T4N0M1/IVB	7	Yes	Vismodegib	NA	0	Lung
8	80/M	Head	T3N2AM0/IVA	4	Yes	Excision	Yes	3.4	Parotid gland
9‖	72/M	Leg	T4N1M1/IVB	20	Yes	Excision§	No	1	Inguinal LN & lung
10	42/M	Head	T3NxM1/IVB	>5	Yes	Excision	Yes	10	Pleural cavity
11	76/F	Leg	T3NxM1/IVB	15	Yes	Vismodegib	NA	0	Bones

F, Female; *LN*, lymph node, *M*, male; *NA*, not applicable; *RT*, radiotherapy.
*Union for International Cancer Control TNM classification, eighth edition.
†Defined as invasion in structures beyond subcutaneous tissue.
‡Patient with basal cell nevus syndrome.
§No clear margins.
‖Patient with HIV.

Table II. Histologic characteristics

Patient	Cell type/differentiation of primary tumor	Growth pattern of primary tumor	Metastasis material	Cell type/differentiation of metastasis	Stains in metastasis (+/−)
1	Basaloid	Infiltrative	Histology	Lung: basaloid/squamous	+ p40, BerEP4 −TTF1, CK7
2*	Basaloid	Nodular & infiltrative	Histology Histology	LN1: tumor cells LN2: tumor cells	Not performed Not performed
3	Basaloid/undifferentiated	Infiltrative	Histology	Axillary LN: basaloid	+ BerEP4 −EMA
4	Basaloid	Nodular & infiltrative	Cytology Histology	LN: tumor cells Lung: basaloid	Not performed + p40, GATA-3 −CEA, TTF1
5	Basaloid/squamous	Infiltrative	Histology Histology	LN: basaloid Lung: basaloid	Not performed + p40, p63, GATA-3 −EMA, CD10, ER, PR
6	Basaloid/squamous	Infiltrative	Histology	LN: basaloid	+ BerEP4
7†	Atypical epithelioid	Nodular	Histology	Lung: non-small cell carcinoma/squamous	+ BerEP4, p40, CD10 −TTF1, napsin A
8	Basaloid	Nodular & infiltrative	Histology	Parotid gland: basaloid	+ BerEP4
9	Basaloid	Infiltrative	Cytology	LN: tumor cells	Not performed
10	Basaloid	Infiltrative	Histology	Pleura: large cell carcinoma/squamous and adenoid	+ BerEP4, p40, p63 −TTF1, CD68, PD-L1, vimentin
11	Basaloid/squamous	Infiltrative	Histology	Bone marrow: basaloid/squamous	+ BerEP4, p63, CK7 −TTF1, CK20, ER, PR, PAX8, OCT3/4

ER, Estrogen receptor; *LN*, lymph node; *PR*, progesterone receptor; +, positive stain; −, negative stain.
*Patient with basal cell nevus syndrome.
†Material obtained during treatment with sonidegib.

their metastases, providing strong evidence for a clonal relationship. In most patients, there was a discrepancy in histologic features of the primary tumors and the metastases, resulting in some uncertainty about the origin of the metastases. The ability to confirm a clonal relationship with genetic analysis can aid tumor staging. Knowledge of the mutation may be helpful in the decision to prescribe

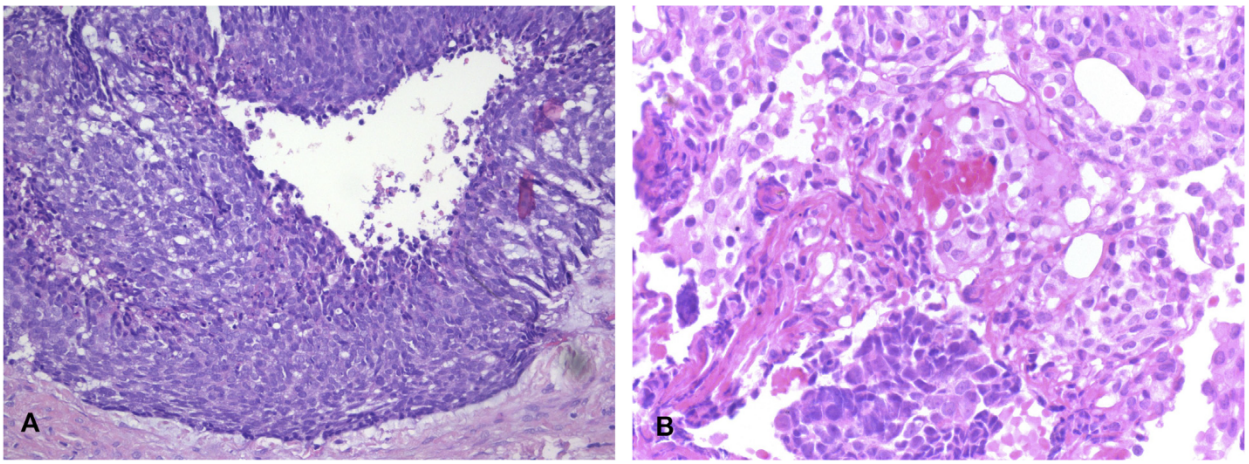

Fig 1. **A**, Biopsy sample of primary skin tumor shows deep dermal nests of basaloid epithelial cells with cystic degeneration, mucin deposits, and central apoptosis. **B**, Biopsy sample of lung metastasis shows the tumor contains few basaloid cells but is particularly composed of nests of squamous cells with abundant cytoplasm and enlarged nuclei with prominent nucleoli. (**A** and **B**: hematoxylin and eosin stain; original magnification: ×200).

targeted therapies with hedgehog inhibitors or checkpoint inhibitors in case of mBCC.[20]

Notably, all patients with *SMO* mutations had distant metastases. This could indicate that *SMO* mutations are responsible for more aggressive behavior 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.[21] 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 2 other patients should be interpreted with care, because material for molecular testing was obtained after previous treatment with vismodegib, which might have caused selection of a subpopulation in the tumor.

Among the 9 patients in our cohort who were treated with vismodegib, progressive disease eventually developed under this treatment in 7 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 1 year during treatment in only 21%.[11] This may be explained by the fact that this cohort only included patients with mBCC, who consequently have tumors with a more aggressive behavior.

As we see in our case series, primary tumors and metastases sometimes differ histologically. Also, when a metastasis is diagnosed in a clinical setting, the primary tumor 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, adverse effects can significantly affect the quality of life.[22] Also, the costs for 3.7 months of treatment may be a 100-fold higher than the costs for genetic analysis.[14,18] We do have to keep in mind that a biopsy sample represents only a small part of the tumor and, consequently, found vismodegib-resistant *SMO* mutations may not be representative of the entire tumor. A temporary tumor load reduction, improving a patient's quality of life, cannot be excluded. A different aspect of consideration is 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.[19]

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 centers was mostly FFPE, which probably caused the failure of analysis in 2 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 1 biopsy

Table III. Genetic characteristics

Patient	Origin of sample	Gene	Mutation	Frequency (%)	Protein change	Treatment of metastasis	Outcome	Currently under treatment
1[†]	BCC	*PTCH1*	c1728_1728+1delinsAA	64	r.spl?	Vismodegib, checkpoint inhibitor	SD	Yes
	Lung	*PTCH1*	c.1728_1728+1delinsAA	16	r.spl?			
		SMO	c.722C>T	11	p.(Thr241Met)[‡]			
2[§]	BCC *FFPE*	*PTCH1*	c.533A>C	56	p.(His178Pro)	CLND, vismodegib	CR	No
	Cervical LN1 *FFPE*	*PTCH1*	c.533A>C	91	p.(His178Pro)			
	Cervical LN2 *FFPE*	*PTCH1*	c.533A>C	82	p.(His178Pro)			
3	BCC	*PTCH1*	c.3053G>A	83	p.(Trp1018*)	Vismodegib, checkpoint inhibitor	SD	Yes
		TP53	c.722C>T	44	p.(Ser241Phe)			
	Axillary LN	*PTCH1*	c.3053G>A	42	p.(Trp1018*)			
		TP53	c.722C>T	13	p.(Ser241Phe)			
4	BCC	*SMO*	c.1234C>T	43	p.(Leu412Phe)[‡]	CLND, local surgery	SD	No
		TP53	c.637C>T	74	p.(Arg213*)			
		PTCH1	c.2048C >T	46	p.(Ser638Phe)			
		TERT prom	C250T	38				
	Pre-auricular LN	*SMO*	c.1234C>T	45	p.(Leu412Phe)[‡]			
		TP53	c.637C>T	94	p.(Arg213*)			
		PTCH1	c.2048C >T	45	p.(Ser638Phe)			
		TERT prom	C250T	60				
	Lung	*SMO*	c.1234C>T	39	p.(Leu412Phe)[‡]			
		TP53	c.637C>T	58	p.(Arg213*)			
		PTCH1	c.2048C >T	42	p.(Ser638Phe)			
		TERT prom	C250T	43				
5	BCC	*SMO*	*c.1234C>T*	20[‖]	p.(Leu412Phe)[‡]	ALND, RT axilla	SD	No
		TERT prom	C228T	63				
	Axillary LN	*SMO*	c.1234C>T	44	p.(Leu412Phe)[‡]			
		TERT prom	C228T	49				
	Lung	*SMO*	c.1234C>T	28	p.(Leu412Phe)[‡]			
		TERT prom	C228T	41				
6	BCC	*PTCH1*	c.466C>T	61	p.(Gln156*)	Vismodegib, ALND, RT axilla	SD	No[¶#]
		PTCH1	c.3261_3262insTGACC	27	p.(Ala1099*)			
	Axillary LN	*PTCH1*	c.466C>T	43	p.(Gln156*)			
		PTCH1	c.3261_3262insTGACC	31	p.(Ala1099*)			
7[†]	BCC	*PTCH1*	c.2839G>T	90	p.(Glu947*)	Vismodegib, surgery, RT, nivolumab, sonidegib	PD	Yes
		TERT prom	C250T	59				
		SETD2	c.2002C>A	53	p.(Pro668Thr)			
	Lung	*SMO*	*c.722C>T*	54	p.(Thr241Met)[‡]			
		PTCH1	c.2839G>T	72	p.(Glu947*)			
		TERT prom	C250T	46				
		SETD2	c.2002C>A	53	p.(Pro668Thr)			

8	BCC	*PTCH1*	c.767G>A	52	p.(Trp256*)	Vismodegib	CR	No
	Parotid gland	*PTCH1*	c.767G>A	40	p.(Trp256*)			
9	BCC FFPE	*TP53*	c.742C>T	24	p.(Arg248Trp)	Vismodegib cyclic	PD	No[#]
		SUFU	c.187G>A	46	p.(Gly63Ser)			
		SUFU	c.1165C>T	40	p.(Leu389Phe)			
	Inguinal LN FFPE		Failed					
10	BCC FFPE		Failed			Vismodegib	PD	Yes
	Pleura FFPE		Failed					

ALND, Axillary lymph node dissection; *BCC*, basal cell carcinoma; *CLND*, cervical lymph node dissection; *CR*, complete response; *FFPE*, formalin-fixed, paraffin-embedded material; *LN*, lymph node; *PD*, progressive disease; *RT*, radiotherapy; *SD*, stable disease; *LN*, lymph node.
Patients 1, 2, 3 8, and 9 were analyzed with small molecule molecular inversion probes, and patients 4, 5, 6, 7, and 10 were analyzed with targeted next-generation sequencing.
[†]Material obtained after treatment with vismodegib.
[‡][§]*SMO* mutations p.(Leu412Phe) and p.(Thr241Met) are known to cause vismodegib resistance.
[§]Patient with basal cell nevus syndrome and a germline mutation in *PTCH1* c.533A>C.
[‖]Confirmed with Sanger sequencing analysis.
[¶]Unrelated to disease.
[#]Deceased.

sample, but the quality of the FFPE material from a cytologic puncture was too low to perform a 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 tumor and metastasis.

One of the included patients had basal cell nevus syndrome caused by a germline mutation in *PTCH1*. In both the primary tumor (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 tumor 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.

REFERENCES

1. Flohil SC, Seubring I, van Rossum MM, Coebergh JW, de Vries E, Nijsten T. Trends in basal cell carcinoma incidence rates: a 37-year Dutch observational study. *J Invest Dermatol*. 2013;133(4):913-918.
2. Paver K, Poyzer K, Burry N, Deakin M. Letter: the incidence of basal cell carcinoma and their metastases in Australia and New Zealand. *Australas J Dermatol*. 1973;14(1):53.
3. von Domarus H, Stevens PJ. Metastatic basal cell carcinoma. Report of five cases and review of 170 cases in the literature. *J Am Acad Dermatol*. 1984;10(6):1043-1060.
4. Wysong A, Aasi SZ, Tang JY. Update on metastatic basal cell carcinoma: a summary of published cases from 1981 through 2011. *JAMA Dermatol*. 2013;149(5):615-616.
5. McCusker M, Basset-Seguin N, Dummer R, et al. Metastatic basal cell carcinoma: prognosis dependent on anatomic site and spread of disease. *Eur J Cancer*. 2014;50(4):774-783.
6. Axelson M, Liu K, Jiang X, et al. U.S. Food and Drug Administration approval: vismodegib for recurrent, locally advanced, or metastatic basal cell carcinoma. *Clin Cancer Res*. 2013;19(9):2289-2293.
7. Bonilla X, Parmentier L, King B, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nat Genet*. 2016;48(4):398-406.
8. Basset-Seguin N, Hauschild A, Kunstfeld R, et al. Vismodegib in patients with advanced basal cell carcinoma: primary analysis of STEVIE, an international, open-label trial. *Eur J Cancer*. 2017;86:334-348.
9. Atwood SX, Sarin KY, Whitson RJ, et al. Smoothened variants explain the majority of drug resistance in basal cell carcinoma. *Cancer Cell*. 2015;27(3):342-353.

10. Sharpe HJ, Pau G, Dijkgraaf GJ, et al. Genomic analysis of smoothened inhibitor resistance in basal cell carcinoma. *Cancer Cell*. 2015;27(3):327-341.
11. Chang AL, Oro AE. Initial assessment of tumor regrowth after vismodegib in advanced basal cell carcinoma. *Arch Dermatol*. 2012;148(11):1324-1325.
12. Laga AC, Schaefer IM, Sholl LM, French CA, Hanna J. Metastatic basal cell carcinoma. *Am J Clin Pathol*. 2019;152(6):706-717.
13. Weissferdt A, Kalhor N, Moran CA. Cutaneous basal cell carcinoma with distant metastasis to thorax and bone: a clinicopathological and immunohistochemical study of 15 cases. *Virchows Arch*. 2017;470(6):687-694.
14. Eijkelenboom A, Kamping EJ, Kastner-van Raaij AW, et al. Reliable next-generation sequencing of formalin-fixed, paraffin-embedded tissue using single molecule tags. *J Mol Diagn*. 2016;18(6):851-863.
15. Geurts-Giele WR, Rosenberg EH, Rens AV, Leerdam MEV, Dinjens WN, Bleeker FE. Somatic mosaicism by a de novo *MLH1* mutation as a cause of Lynch syndrome. *Mol Genet Genomic Med*. 2019;7(7):e00699.
16. van Riet J, Krol NMG, Atmodimedjo PN, et al. SNPitty: an intuitive web application for interactive B-allele frequency and copy number visualization of next-generation sequencing data. *J Mol Diagn*. 2018;20(2):166-176.
17. National Institute for Health and Care Excellence. Metastatic malignant disease of unknown primary origin in adults: diagnosis and management. 2010. Available at: https://www.nice.org.uk/guidance/cg104/resources/metastatic-malignant-disease-of-unknown-primary-origin-in-adults-diagnosis-and-management-pdf-35109328970437; 2010. Accessed September 25, 2019.
18. The Drug Rediscovery Protocol (DRUP Trial). Available at: https://ClinicalTrials.gov/show/NCT02925234. Accessed September 24, 2019.
19. Nikanjam M, Cohen PR, Kato S, Sicklick JK, Kurzrock R. Advanced basal cell cancer: concise review of molecular characteristics and novel targeted and immune therapeutics [published correction appears in *Ann Oncol*. 2019;30(10):1675]. *Ann Oncol*. 2018;29(11):2192-2199.
20. Brinkhuizen T, van Geel M, Denil SL, et al. Locally advanced basal cell carcinoma has a distinct methylation and transcriptomic profile. *Exp Dermatol*. 2016;25(4):316-318.
21. Xie P, Lefrancois P. Efficacy, safety, and comparison of sonic hedgehog inhibitors in basal cell carcinomas: a systematic review and meta-analysis. *J Am Acad Dermatol*. 2018;79(6):1089-1100.e17.
22. Fellner C. Vismodegib (Erivedge) for advanced basal cell carcinoma. *P T*. 2012;37(12):670-682.
23. Hwang DH, Garcia EP, Ducar MD, Cibas ES, Sholl LM. Next-generation sequencing of cytologic preparations: an analysis of quality metrics. *Cancer Cytopathol*. 2017;125(10):786-794.
24. Jayaraman SS, Rayhan DJ, Hazany S, Kolodney MS. Mutational landscape of basal cell carcinomas by whole-exome sequencing. *J Invest Dermatol*. 2014;134(1):213-220.