Expression of VEGF-A/C, VEGF-R2, PDGF- α/β , c-kit, EGFR, Her-2/Neu, Mcl-1 and Bmi-1 in Merkel cell carcinoma

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Merkel cell carcinoma is a rare but very aggressive tumor of the skin. With current treatment options, Merkel cell carcinoma is associated with a high incidence of recurrence and metastasis. Targeted anticancer therapies such as receptor tyrosine kinase inhibitors and antisense oligonucleotides have been found to be a promising new type of treatment for various types of cancer. To evaluate whether the use of targeted therapies is a possible treatment option in Merkel cell carcinoma, we determined the expression of the target molecules c-kit, Mcl-1, Bmi-1, vascular endothelial growth factor (VEGF)-A, VEGF-C, VEGF-receptor 2 (VEGF-R2), platelet-derived growth factor (PDGF)- α , PDGF- β , epidermal growth factor receptor (EGFR) and Her-2/Neu in a tissue microarray of 32 samples of 29 patients with Merkel cell carcinoma. C-kit-positive samples were analyzed for mutations in exons 9 and 11. The tissue microarray was stained immunohistochemically with antibodies directed against the above-mentioned proteins, and an immunoreactivity score was calculated. DNA was extracted from c-kit-positive samples and was analyzed for exon 9 and 11 mutations using direct DNA sequencing. We found that c-kit (7%), Mcl-1 (88%), Bmi-1 (78%), VEGF-A (91%), VEGF-C (75%), VEGF-R2 (88%), PDGF- α (72%) and PDGF- β (13%) were expressed in Merkel cell carcinomas. All samples showed a lack of EGFR and Her-2/Neu expression. Analysis of c-kit revealed no mutations. As VEGF-A, VEGF-C, VEGF-R2, PDGFs and c-kit are targets of new cytostatic agents used in the treatment of other cancers, inhibition by a multitargeted chemotherapy could be a very promising treatment option. High expression of Bmi-1 and Mcl-1 warrants further studies on the use of antisense oligonucleotides in Merkel cell carcinoma.

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Most cases of Merkel cell carcinoma occur in elderly people with the average age of presentation being 69 years.¹ Increased frequency has been reported in sun-exposed parts of the body and after immunosuppression. According to the US Surveillance Epidemiology and End Results Program (SEER database), the estimated incidence per 100 000 people is 0.23 in the white population and 0.01 in the black population.^{1,2} Although many small studies have been published on the treatment of Merkel cell carcinoma, so far no gold standard has been established.³ Current treatment options are

surgery alone, radiotherapy alone or surgery with postoperative radiotherapy. Owing to the rareness of the disease, very little data are available on the efficacy of chemotherapy and the results are conflicting.^{4,5} Overall 5-year survival ranges from 31 to 74%.⁶

Targeted anticancer therapies have been found to be very promising options in the treatment of different forms of cancer such as renal cell carcinoma^{7,8} and gastrointestinal stromal tumors.^{9,10} So far no clinical trials using small molecules for Merkel cell carcinoma have been published. Moreover, data available on the expression of possible therapy targets in Merkel cell carcinoma are very limited. Only c-kit, a receptor tyrosine kinase, was found to be expressed in 15–90% of Merkel cell carcinoma samples.^{11–16}

In the present study, we have assessed the expression of the possible molecular targets vascular

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endothelial growth factor (VEGF)-A, VEGF-C, VEGF-receptor 2 (VEGF-R2), platelet-derived growth factor (PDGF)- α and PDGF- β , epidermal growth factor receptor (EGFR), Her-2/Neu (c-erbB-2), c-kit (CD117), Mcl-1 and Bmi-1 in 32 samples of Merkel cell carcinoma to determine if targeted therapy could be a possible treatment option. C-kit-positive samples were analyzed for the most common mutations in exons 9 and 11 using direct DNA sequencing.

Materials and methods

Patients

Thirty-two archival samples of Merkel cell carcinoma of 29 patients (mean age 76.5 years, 17 male, 12 female) from the Department of Otorhinolaryngology, Head and Neck Surgery and Department of Dermatology of the Vienna Medical

University were included in the study. Patients' clinical data are shown in Table 1. All samples were staged according to the most commonly used system proposed by Yiengpruksawan *et al*¹⁷ indicating (i) stage I: local disease, (ii) stage II: loco-regional disease and (iii) stage III: distant disease.

Tissue Microarray

Before analysis, H&E-stained sections from each sample were evaluated and the suitability of inclusion for the study was determined. Sections (2–3 μ m) were used for the analysis. The suitability of the tissue was evaluated using a number of inclusion criteria such as the size (1–2 mm in depth and at least 5 mm in length and width), as well as other features such as appropriate fixation, absence of significant electrosurgical device lesions, signs of acidic decalcifying agents and the presence of

Table 1 Clinical data of 29 patients with Merkel cell carcinoma

S/P	Type	Site	Stage	1st treatment	Rec. (months)	2nd treatment	3rd treatment	Status	TLF	IS
1/1	Primary	Hand	I	Surgery				DOC	7	Yes
2/2	Primary	Nose	I	RT (64)				AW	48	No
3/3	Meta	Foot	III	Surgery	Local (4)/metastasis (7)	Chemotherapy ^a	Chemotherapy ^b	DOD	24	Yes
4/4	Primary	Thigh	I	Surgery	Metastasis (4)	RT (30)		DOC	36	No
5/5	Meta	Cheek	I	Surgery	Local (6)/metastasis (11)	Surgery+RT	Endoxan	DOD	18	No
6/6	Primary	Lid	I	Surgery				AW	56	No
7/7	Meta	Cheek	II	Surgery	Metastasis (6)	RT (60)		DOD	24	Yes
8/8	Meta	Elbow	I	Surgery	Metastasis (14)	Chemotherapy ^c	RT (50)	AW	30	Yes
9/8	Meta	Chest								
10/9	Primary	Face	II	Surgery+RT (50)	Metastasis (2)		RT (30)	DOD	6	No
11/10	Primary	Cheek	I	Surgery+RT (30)				DOC	18	Yes
12/11	Primary	Lid	I	Surgery+RT (30)	Metastasis (37)	Surgery	RT (50)	AWD	53	No
13/11	Meta	Neck								
14/12	Meta	Lid	I	Surgery	Metastasis (14)	Surgery		AW	24	No
15/13	Meta	Thigh	I	Surgery	Metastasis (3)	Surgery+RT (56)	Chemotherapy ^d	DOD	50	No
16/14	Meta	Leg	II	Surgery	Metastasis (4)	RT (46)	Chemotherapy ^a	AWD	10	Yes
17/15	Meta	Leg	I	Surgery	Regional (7)	Surgery	Surgery+RT (38)	AW	54	No
18/16	Primary	Ear	I	Surgery	Local (4)	Surgery		AW	36	No
19/17	Primary	Nose	I	Surgery	Local (11)	RT (60)		DOD	49	No
					Regional (12)					
20/18	Primary	Nose	I	Surgery	Regional (4)	Surgery		AW	36	No
21/19	Meta	Leg	I	Surgery	Local (12)	Surgery	Chemotherapy ^a	DOD	44	Yes
					Regional (36)					
22/20	Meta	Arm	I	Surgery	Regional (2)	Surgery	RT (50)	DOD	24	No
					Metastasis (12)					
23/21	Primary	NA	I	Surgery				AW	25	No
24/22	Primary	Cheek	I	Surgery				AW	89	No
25/22	Meta	Chest								
26/23	Primary	Neck	I	Surgery	Local (5)	Surgery+RT (62)	Surgery+RT (25)	DOC	17	Yes
					Metastasis (15)					
27/24	Primary	Face	II	Surgery	Local (4)	Surgery		AW	82	No
28/25	Meta	Leg	III	Surgery+RT (30)				DOD	8	No
29/26	Primary	Neck	I	Surgery				DOC	17	No
30/27	Meta	Face	I	Surgery	Regional (23)	Surgery		DOD	24	No
31/28	Primary	Ear	I	Surgery	Metastasis (6)	Surgery+RT (30)		DOD	6	Yes
32/29	Meta	Neck	I	Surgery	Metastasis (5)			AW	109	No

AW, alive and well; AWD, alive with disease; DOC, died of other cause; DOD, died of disease; IS, immunosuppression; Rec., recurrence; RT (), radiotherapy (dose in Gray); S/P, sample/patient number; site, site of primary tumor; TLF, time to last follow-up or death; NA, not available.

^aCisplatin/Etoposide.

^bAdriamycin, Cyclophosphamide, Vincristine.

^cCyclophosphamide, Methotrexate, Fluorouracil.

^dDoxorubicin, Dacarbazine, Ifosfamide.

Table 2 Antibodies and retrieval buffers for each antibody

PDGF- α	NeoMarkers	RB-9027	Rabbit	1:100	Citrate buffer
PDGF- β	NeoMarkers	RB-9032	Rabbit	1:100	Citrate buffer
VEGF-A	Pharmingen	ab3109	Mouse	1:50	Citrate buffer
VEGF-C	Zymed	34-4300	Mouse	1:100	Citrate buffer
VEGF-R2	Santa Cruz	N-931	Rabbit	1:80	EDTA buffer
Bmi-1	Upstate	05-637	Mouse	1:300	EDTA buffer
Mcl-1	NeoMarkers	MS-681	Mouse	1:200	Citrate buffer
EGFR (EGF-R Zymed)	Invitrogen	08-1205	Mouse	RTU	Citrate buffer
Her-2 (HerceptTest)	Dako	K5204	Rabbit	RTU	Citrate buffer
C-kit (c-Kit pharmDx)	Dako	K1907			

VEGF-A, vascular endothelial growth factor A; VEGF-C, vascular endothelial growth factor C; VEGF-R2, VEGF-receptor 2; PDGF- α , platelet-derived growth factor alpha; PDGF- β , platelet-derived growth factor beta; EGFR, epidermal growth factor receptor; c-erbB-2, HER-2/Neu; CD117, c-kit; Mcl-1, myeloid cell leukemia-1; Bmi-1, B-cell-specific moloney murine leukemia virus site 1; RTU, ready to use; EDTA, ethylenediaminetetraacetic acid.

usable tissue in each block. Each H&E-stained slide was revaluated and mapped to identify the specific areas for tissue acquisition to build the tissue microarrays, which were constructed using the approach described by Kononen *et al*,¹⁸ using a Beecher manual tissue arrayer (MTA-1) (Beecher Instruments Inc., WI, USA). Core diameter was 0.6 mm. Three cores were used per patient.

Immunohistochemistry

Dewaxed and rehydrated tissue microarrays were subjected to antigen retrieval in a microwave oven (600 W) employing the appropriate buffer (Table 2). Blocking of unspecific binding was achieved with 5% TBS/BSA (Sigma-Aldrich, Germany) for 1 h at room temperature. The primary antibody (Table 2) and appropriate negative and positive controls were applied at 4°C overnight. A secondary biotinylated antibody (1:200, Multilink; Dako, Denmark) in 1% TBS/BSA was included for 1 h at room temperature, followed by alkaline phosphatase-conjugated streptavidin-AP/TBS/BSA (1:250; Dako) for 1 h at room temperature. Visualization was performed by fast red (Sigma-Aldrich, St Louis, MO, USA) and counterstained by hemalaun. Samples were analyzed using an Olympus BH-2 microscope.

Specimen Classification Based on Immunohistochemical Results and Morphological Features

We used an immunoreactivity score proposed by Sinicrope *et al*¹⁹ to evaluate both the intensity of immunohistochemical staining and the proportion of the stained tumor cells. The staining intensity was subclassified as follows: 1, weak; 2, moderate; or 3, strong. The positive cells were quantified as a percentage of the total number of tumor cells and the proportions were assigned to one of five categories: 0, <5%; 1, 5–25%; 2, 26–50%; 3, 51–75%; and 4, >75%. The percentage of positivity of the tumor cells and the staining intensity were then multiplied to generate the immunoreactivity score for each of

Table 3 Primers were designed as described in Materials and methods

<i>C-kit</i> exon		
<i>Exon 9 (305 bp)</i>		
Forward	5'-ATTTATTTTCCTAGAGTAAGCCAGGG-3'	
Reverse	5'-ATCATGACTGATATGGTAGACAGAGC-3'	
<i>Exon 11 (247 bp)</i>		
Forward	5'-CCAGAGTGCTCTAATGACTG-3'	
Reverse	5'-CTGTTATGTGTACCCAAAAAGG-3'	

bp, base pairs.

the tumor specimens. Mean immunoreactivity score was calculated from the three samples per patient. The expression pattern of each marker in the complete tumor samples was determined independently by two experienced investigators (MB and BME). An attempt to avoid observer bias was made by repeating the evaluation of protein expression at two different time points and without knowledge of patients' clinical data.

DNA Sequence Analysis

Isolation of DNA from paraffin-embedded tumor tissue

Genomic DNA was prepared from 4- to 8.5- μ m-thick sections of c-kit-positive paraffin-embedded tumor material using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany). Paraffin blocks were trimmed to minimize the amount of non-neoplastic tissue before sectioning. A 100 ng portion of genomic DNA was amplified in 50 μ l PCR mixtures using BD Advantage 2 (Clontech; BD Biosciences, Erembodegem, Belgium) according to the manufacturers' instructions.

Design of primers

Primers specific for c-kit exons 9 and 11 were designed with the use of Primer Designer Software (Scientific and Education Software, Durham, NC, USA). The primers used are listed in Table 3.

PCR and DNA sequence analysis

PCR was performed using the AmpliTaq Gold kit (Applied Biosystems, Darmstadt, Germany). Bidirectional sequencing of PCR products was performed using BigDye 1.1 terminator chemistry (Applied Biosystems, Foster City, CA, USA) and an ABI3130 Sequencer (Applied Biosystems). For BigDye-Terminator removal, sequenced PCR products were cleaned up before analysis using Sigma Post-Reaction Clean-Up plates (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturers' recommendations.

Statistical Analysis

To assess the correlation between target expression and tumor remission, sex and tumor stage univariate and multivariate stepwise logistic analyses were performed. The Statistical Package for the Social Sciences Software, Version 11.5 for Windows (SPSS UK Ltd, Surrey, UK), was used for analysis. A *P*-value <0.05 (two-sided) was considered as statistically significant.

Results

Clinical Data

Of the 29 patients, 17 (59%) were male and 12 (41%) were female, ranging in age from 50 to 96 years (mean 76.8 years). Nineteen tumors (66%) were located in the head and neck region and 10 (34%) on the extremities. Twenty-three patients (79%) presented with early disease (stage I), four with stage II (14%) and two with stage III (7%). Eleven (40%) are alive without disease and two (7%) are alive with disease. Eleven died of the disease (40%) and five died of other causes (13%). Table 1 shows the complete clinical data.

Microscopy

As described previously by Sur *et al*,²⁰ we observed two distinct types of Merkel cell carcinoma cells. Nine samples showed morphology similar to classical small cell carcinoma. In 18 samples, the carcinoma cells were significantly bigger (larger than 3 lymphocytes) and showed high mitotic activity and multiple nucleoli. In five samples, both morphologies were present.

Immunohistochemistry

Interestingly, with the exception of c-kit, the investigated proteins were usually uniformly expressed in all tumor cells (Figure 1).

c-kit

Two out of 32 (7%) Merkel cell carcinoma samples were positive for c-kit. Both samples belonged to the same patient and showed a membranous staining pattern in a small number of tumor cells. Immunoreactivity score was 2.7 in one and 3.7 (mean 3.2) in the other sample. Both samples belonged to the large cell carcinoma group.

Mcl-1

Out of 32 samples, 25 (88%) were positive for Mcl-1 in the cell cytoplasm. Mean immunoreactivity score of the positive samples was 6.4. There was no significant difference between small and large cells.

Bmi-1

Out of 32 samples, 24 (75%) were positive for Bmi-1 in the cell nuclei and cytoplasm. Mean immunoreactivity score of the positive samples was 8. There was no significant difference between small and large cells.

VEGF-A

Out of 32 samples, 29 (91%) were positive for VEGF-A in the cell cytoplasm. Although almost all samples expressed VEGF-A, the staining intensity was generally low with a mean immunoreactivity score of 4.7. There was no significant difference between small and large cells.

VEGF-C

Out of 32 samples, 24 (75%) were positive for VEGF-C in the cell cytoplasm. As with VEGF-A, the mean staining intensity was relatively low with an immunoreactivity score of 4.4. There was no significant difference between small and large cells.

VEGF-R2

Out of 32 samples, 28 (88%) were positive for VEGF-R2 with a membranous and cytoplasmic staining pattern. Almost all samples expressed VEGF-R2, some samples with a very high intensity. The mean immunoreactivity score of the positive samples was 8.6. VEGF-R2 expression was higher in the large cell group, although the difference failed to reach statistical significance (*P* = 0.007).

PDGF-β

Only 4 samples out of 32 (13%) expressed PDGF-β in the cell cytoplasm. All of those samples belonged to the large cell group. Two samples were with an intensity score of 4 and two with a score of 8 (mean 6).

PDGF-α

Out of 32 samples, 23 (72%) were positive for PDGF-α with a partially cytoplasmic and partially membranous staining pattern. The mean immunoreactivity score of the positive samples was 4.7. There was no significant difference between small and large cells.

Immunohistochemistry showed no positive staining for EGFR and Her-2/Neu. Staining pattern of all antibodies is shown in Figure 2.

DNA Sequence Analysis

Paraffin-embedded samples of the two c-kit-positive tumors were screened for mutations in hot-spot

Figure 1 Immunostaining of target proteins in a tissue microarray of Merkel cell carcinomas. C-kit IHC showed the typical membranous staining pattern and was slightly positive in only two samples. PDGF- α , PDGF- β , VEGF-C, VEGF-A, VEGF-R2 and Mcl-1 were mainly expressed in the cell cytoplasm, whereas Bmi-1 showed a predominantly nuclear staining pattern. The PDGF- β stain represents one of the few samples where the protein was expressed only in parts of the tumor—in the majority of samples, we found a very uniform expression throughout the cores. IHC, immunohistochemistry.

regions of c-kit (exons 9 and 11). However, no mutations leading to amino-acid changes in the mature protein could be detected.

Correlation of Target Expression and Clinical Outcome

Statistical analysis revealed no correlation between target expression and tumor remission, gender and tumor stage.

Discussion

Merkel cell carcinoma, a neuroendocrine neoplasm, is a highly proliferative epidermal tumor with a high rate of recurrence and metastasis and poor survival. Current treatment options did not significantly improve patients overall survival. Thus, advances in therapy will depend on novel therapeutic approaches. During the last decade,

Figure 2 A schematic representation of the expression pattern of the tested antigens in Merkel cell carcinoma: (a) Bmi-1, (b) Mcl-1, (c) VEGF-A, (d) VEGF-C, (e) VEGF-R2, (f) PDGF- α , (g) PDGF- β , (h) c-kit, (i) EGFR and (j) Her-2/Neu. The expression level of individual cases represented by immunoreactivity score (IS) is shown in colors.

several potential marker proteins as potential anticancer targets have been discovered.

A frequently utilized group of targets of new signal-transduction agents is the protein kinase signalling network. One mode of attenuation is to block the ligand binding domain. This domain is the target of two antibodies in clinical use, Trastuzumab and Cetuximab. Trastuzumab (Herceptin[®]) is directed against the erbB-2 receptor, which is also known as Her-2/Neu oncogene. Cetuximab (Erbix[®]) is active against the EGFR. In our samples, Merkel cell carcinoma cells did neither express EGFR nor Her-2/Neu. Thereby, we could confirm the findings of Maubec *et al*,²¹ who also could not detect any expression of these two receptors in Merkel cell carcinoma by immunohistochemistry.

The next group of targets in the protein kinase signalling network is receptor tyrosine kinases. Receptor tyrosine kinases are single-pass membrane proteins with an extracellular ligand binding and an intracellular kinase domain. Receptor tyrosine kinases function in cell signalling and transmit signals regulating growth, differentiation, adhesion, migration and apoptosis.^{22–26} The receptor tyrosine kinase family consists of several subfamilies, including the epidermal growth factor receptors (EGFRs or ErbBs), the platelet-derived growth factor receptors (PDGFRs) and the VEGF receptors.²³ All of the

above-mentioned subfamilies are known to be expressed in many cancers and play an important role in angiogenesis and tumor progression.^{27,28} In this study, we observed that VEGF-A, VEGF-C, VEGF-R2, PDGF- α and PDGF- β are expressed in Merkel cell carcinomas. Interestingly, all of the examined proteins were uniformly expressed throughout the tumors and their expression levels were rather low. There was a tendency toward higher expression in large cell tumors, although the difference did not reach statistical significance. This discrepancy was most pronounced in VEGF-R2. Furthermore, in most of the investigated samples VEGF-R2 was detectable in the cell cytoplasm and its expression level was significantly higher than that of the above-mentioned proteins. The reason for the differential expression of receptor tyrosine kinases in Merkel cell carcinoma is still unknown.

C-kit, a proto-oncogene involved in the activation of cell proliferation, is expressed not only in normal cells²⁹ but also in tumors of the solid aerodigestive tract.^{30–32} The expression of c-kit (CD117) in Merkel cell carcinoma has been examined previously in five studies with a range of 15–95%.^{11–16} In our study, 7% of Merkel cell carcinoma samples expressed c-kit. We hypothesize that the discrepancy between the five studies can be explained by the small sample numbers and by different staining and evaluation methods utilized.

C-kit and VEGF-R2, together with VEGF-R1, VEGF-R3 and PDGFRs, are targets of two multi-targeted inhibitors Sorafenib (Nexavar[®]) and Sunitinib (Sutent[®]). Sunitinib has been successfully tested in the treatment of renal cell carcinoma^{7,8} and gastrointestinal stromal tumors.^{9,10} Sorafenib is an effective anticancer agent directed against renal cell carcinoma^{33,34} and has been evaluated in phase I trials in solid tumors, melanoma and non-small-cell lung cancer.^{35–37} In gastrointestinal stromal tumors, different c-kit mutations are known to predict survival and even more importantly response to treatment with tyrosine kinase inhibitors.^{38,39}

The exons of c-kit that are most commonly affected by mutation are exons 9 and 11. Gastrointestinal stromal tumors with exon 11 mutations respond more frequently to targeted treatment than exon 9 mutations, while wild-type c-kit has the worst response rate.^{38,39} In two c-kit-positive samples of our study, no mutations leading to amino-acid changes in the mature protein could be detected. Based on our analysis of c-kit expression and mutation, c-kit does not seem to be a very promising candidate for targeted anticancer therapy in Merkel cell carcinoma.

Another way to affect tumor growth is to inhibit the expression of genes involved in cell proliferation and cell death by introduction of microRNAs or antisense oligonucleotides.⁴⁰ Recently, several antisense oligonucleotides targeting genes involved in neoplastic progression have been evaluated as potential therapeutic agents, one of them being

Mcl-1.^{41–43} Mcl-1, a member of the Bcl-2 protein family, is an antiapoptotic protein^{44–46} and functions by avoiding cell damage-induced mitochondrial cytochrome *c* release.^{47,48} Although the definite mechanisms of how Mcl-1 promotes cell survival is not yet fully elucidated, the biological significance of Mcl-1 protein expression in supporting cell survival has been well documented in a number of solid and non-solid tumors.^{41,49–55} Therefore, the next logical step was specific inhibition of Mcl-1 expression by antisense oligonucleotides or micro-RNAs.⁵⁵ So far no study has investigated the expression of Mcl-1 in Merkel cell carcinoma. In our cohort, the majority of tumor samples showed a very high expression of Mcl-1. That makes Mcl-1 a very auspicious candidate for targeted therapy and validates further *in vivo* studies on the effect of Mcl-1 antisense oligonucleotides in Merkel cell carcinoma.

Bmi-1 is a transcriptional repressor that belongs to the polycomb-group family of proteins involved in hematopoiesis, regulation of proliferation and axial patterning.^{56,57} It has been found to be an important factor of self-renewal and senescence of various stem cells,^{58–60} and its overexpression was shown to immortalize human mammary epithelial cells.⁶¹ Bmi-1 is highly expressed in various human malignant tumors.^{62–69} The recently developed antisense Bmi-1 expression plasmid was able to inhibit proliferation of human leukemia cells *in vitro*.⁷⁰ Interestingly, we were able to demonstrate intense Bmi-1 expression in almost all Merkel cell carcinoma specimens of our cohort. That makes Bmi-1 another promising candidate for future antisense oligonucleotide therapies in patients with Merkel cell carcinoma.

Owing to the small number of tumor specimens, we could not detect any statistically relevant correlations between patients' clinical data and target expression. Also, we could not demonstrate an impact of the morphology on the course and aggressiveness of the disease. However, further studies will be needed to fully determine the impact of targeted therapy in Merkel cell carcinoma *in vitro* and *in vivo*, as the presence of a target does not always predict response to a targeted agent.⁷¹

In summary, in this study we show for the first time that the therapy targets c-kit, Bmi-1, Mcl-1, VEGF-A and VEGF-C, VEGF-R2, PDGF- α and PDGF- β are expressed in Merkel cell carcinoma. Considering the good results of targeted anticancer and antiangiogenic therapies in recent clinical trials, these results are very promising and validate further clinical studies on the use of multitargeted tyrosine kinase inhibitors and antisense oligonucleotides in Merkel cell carcinoma.

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Disclosure/conflict of interest

The authors have no conflict of interest to declare.

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