Published in final edited form as:

Hum Pathol. 2010 October ; 41(10): 1405–1412. doi:10.1016/j.humpath.2010.02.010.

Merkel Cell Carcinoma: Correlation of KIT Expression with Survival and Evaluation of KIT Gene Mutational Status

Aleodor A. Andea, Raj Patel, Selvarangan Ponnazhagan, Sanjay Kumar, Patricia DeVilliers, Darshana Jhala, Isam E. Eltoum, and Gene P. Siegal
UAB Department of Pathology, Birmingham, AL

Abstract

Merkel cell carcinoma is one of the most aggressive primary cutaneous malignancies. Since some Merkel cell carcinomas express the receptor tyrosine kinase KIT, we aimed to evaluate the correlation of KIT expression with outcome and the presence of activating mutations in the KIT gene in Merkel cell carcinoma.

A total of 49 tumors from 40 patients with a diagnosis of Merkel cell carcinoma were identified of which 30 cases from 21 patients were used in the study. KIT expression was assessed by immunohistochemistry on formalin-fixed, paraffin embedded material. Cases were divided into low expressors (0-1+ staining intensity) and high expressors (2-3+ staining intensity). Direct sequencing of exons 9, 11, 13, 17, and 18 of the KIT gene spanning the extra-cellular, juxtamembrane and tyrosine kinase domains was performed from cases with high KIT expression.

Thirty tumors from 21 patients were analyzed for KIT expression. High KIT expression was seen in 67% of the patients. Five-year survival rates in tumors expressing high versus low levels of KIT were 0% versus 57.8% respectively however, this dramatic difference did not reach statistical significance ($p=0.07$). A total of 4 point mutations were identified in 18 tumors analyzed. Two of these were silent mutations involving exons 17 and 18, and 2 involved intron 16-17. Two of the identified mutations may represent novel polymorphisms.

Our work suggests a correlation between KIT expression and a worse prognosis in Merkel cell carcinoma patients, raising the possibility of an active role of this receptor in tumor progression and metastasis. We did not identify however, KIT activating mutations in any of the tumors analyzed.

INTRODUCTION

Merkel cell carcinoma (MCC), also known as primary cutaneous neuroendocrine carcinoma was originally described by Toker et al. as trabecular carcinoma because of original cases in which this pattern engendered comparison with sweat gland carcinoma [1]. Later studies demonstrated the presence of neurosecretory granules in the tumor cells, similar to those seen in non-neoplastic Merkel cells which resulted in renaming the tumor as MCC [2]. MCC is a relatively rare tumor with an incidence of 0.24-0.44 cases per 100000 person-years [3,4].

Among skin tumors, MCC is regarded as one of the most aggressive cancers with survival rates at 5 years ranging from 29% to 74% [5-11]. The most important prognostic factor is the tumor stage, particularly as determined by lymph node status or metastasis [3,5,9-12]. Recently, histologic factors such as tumor pattern, tumor depth and lymphovascular invasion have been shown to have prognostic implications independent of tumor stage [7].

The mainstay of therapy for this tumor is currently surgical excision with negative margins [5,8-11,13-16] with the possibility of radiotherapy of the tumor bed [5,8,10,14,16,17]. In addition, lymph node dissection or more recently sentinel node biopsy has been recommended with possible further radiotherapy of the draining nodal basin advised for cases with positive nodes [5,8,17]. The role of chemotherapy and radiotherapy in treating MCC remains controversial [5,8,10]. While patients with stage I (node negative and primary tumor < 2 cm) or stage II (node negative and primary tumor $\geq$ 2 cm) disease enjoy relatively long survivals with this approach (5-year survival rates for Stage I and II are 81% and 67% respectively), patients with stage III (positive nodes) and stage IV (distant metastases) do not fare as well (5-year survival rates for Stage III and IV are 52% and 11% respectively) [5]. Unfortunately after the disease spreads to distant sites there is little that can be offered to the patients in term of treatment options.

Between 7-95% of MCCs express KIT as assessed by immunohistochemical methods [18-26]. KIT is a receptor protein-tyrosine kinase belonging to the PDGFR family with biological significance in the pathogenesis of various neoplasms such as gastrointestinal stromal tumors, melanomas, mast cell leukemias and seminomas [27]. In humans, it is a homologue of the oncogene *v-kit* of the Hardy-Zuckerman 4-feline sarcoma virus known as the *c-kit* protein (CD 117), which binds to cytokine stem cell factor, dimerizes, and communicates through secondary signaling pathways [28]. Mutations in KIT gene have been identified in several tumors such as gastrointestinal stromal tumors (GISTs), mast cell neoplasms or melanoma and have been characterized as gain-of function, although mutational sites vary depending on the tumor [29-33].

With the advent of small molecule tyrosine kinase inhibitors becoming available, new treatment options have opened for several tumors such as GISTs, melanoma (particular ocular depending on KIT mutation status) and dermatofibrosarcoma protuberans [34,35]. Considering the findings of initial studies which suggest altered expression of KIT protein in MCC, more comprehensive studies of this phenomenon might have therapeutic implications. To this end we aimed to investigate the correlation of KIT expression with tumor progression and to assess the presence of activating mutations in the KIT gene which could provide new insights into the role of this receptor protein-tyrosine kinase in MCC.

MATERIALS AND METHODS

Patients

The study was approved by the institutional review board (IRB). A search of the pathology electronic records from 1997 to 2008 identified 40 patients with a diagnosis of MCC. Pathology reports and all available slides and paraffin blocks were retrieved from the archives of the pathology department. For 8 patients, more than one tumor specimen was available for a total of 49 tumors. Clinical outcome data reported as no evidence of disease, alive with disease or death were collected for the entire cohort with a median follow-up interval of 20 months (range 1-108 months).

Immunohistochemical (IHC) analysis of KIT expression

IHC was performed in 30 tumors from 21 cases in which at least one block containing a representative tumor sample was available. Five μ m sections were obtained from formalin-fixed, paraffin-embedded block preparations and subjected to heat-induced epitope retrieval with 0.02M citrate buffer (pH 6.0) at 120°C for 30 minutes. The immunostaining was accomplished with a semi-automated immunostainer (Ventana Inc. Tucson, AZ) using a KIT monoclonal primary antibody (Labvision/Neomarkers). Stained slides were reviewed by two investigators (A.A. and P.D.). Each investigator reviewed the entire series. Cases with

discrepant interpretation were re-evaluated together by both pathologists and a consensus was reached. The percentage of cells staining and the intensity graded as negative (0), weak (1+) moderate (2+) and strong (3+) were recorded. Cases were divided in 2 groups based on the intensity and percent of cells expressing KIT protein: high-expressors defined as 2+ or 3+ staining intensity in at least 30% of the tumor cells and low-expressors, 0 or 1+ staining intensity as well as cases showing 2+ and 3+ staining but in less than 30% of tumor cells (Figure 1). In addition, the localization of KIT expression (cytoplasmic versus membranous) was recorded.

KIT gene mutation analysis

Cases categorized as high KIT expressors by IHC were further selected for KIT mutation analysis. For each case, multiple 10 µm thick unstained serial sections were obtained. The area on the tissue section containing tumor was marked on the unstained slides by comparison with the H&E stained slide. Depending on the surface area covered by tumor, between 3 and 10 slides were processed per case aiming for a total of 20 mg of tissue per case. Following deparaffinisation with xylene, DNA was extracted using QIAamp® DNA FFPE Tissue kit (Qiagen, Valencia, CA) following manufacturer's instructions. Reference sequences for KIT gene and mRNA were obtained from the Ensembl database (www.ensembl.org) [36]. We performed direct sequencing of exons 9, 11, 13, 17 and 18 which have been found to harbor activating mutations in GISTs and melanomas [29,30,32,33,37]. Primers used to amplify exons 9, 11, 13, 17, and 18 of the KIT gene and corresponding intron flanking regions were selected using Primer 3 software [38] (Table 1). PCR reactions were carried out using Platinum PCR supermix (Invitrogen, Carlsbad, CA) following manufacturer's recommendations. The reaction volume was 50 µl (45 µl PCR mix, 1 µl DNA, 1 µl forward primer, 1 µl reverse primer, 2 µl H₂O). The following PCR protocol was used: 98°C for 2 min, followed by 40 cycles of denaturation at 98°C for 40 sec, annealing at 58°C for 30 sec, and elongation at 72°C for 40 sec, and a final elongation at 72°C for 10 min. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). The identity of the PCR product was verified by gel electrophoresis. Direct sequencing was performed in both forward and reverse direction with the original PCR primers using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

The results of sequencing were compared to reference genomic KIT DNA using Mutation Surveyor (SoftGenetics, LLC. State College, PA) and Vector NTI (Invitrogen, Carlsbad, CA) software. A mutation was recorded only when the alteration was present in both the forward and reverse traces.

Statistical analysis

Analysis of disease-specific survival was performed using Kaplan-Meier curves and the log-rank statistical test. Two and 5-year survival rates were estimated using follow-up life tables. For all analyses the SPSS 9.0.0 statistical software package was used. Results with p-values < 0.05 were considered statistically significant.

RESULTS

Clinical and demographic data

Distribution of patients' demographic parameters and clinical data are presented in Table 2. The most common primary tumor site was the head and neck area (55% of cases), followed by the extremity (25%), axilla (10%), trunk (5%), and buttocks (2.5%). In one case the tumor presented as a metastasis with unknown primary. Median age at presentation was 72 years (range 44-93 years). Men were more often affected than women (87.5% versus 12.5%

respectively). Overall, there were 20 death of disease events recorded (54%) at a median follow-up of 20 months (1-108 months). Survival rates for the entire cohort at 2 and 5 years were 59.3% and 37.1%, respectively.

Expression of KIT protein by IHC

Results of KIT expression performed 30 tumors from 21 patients are presented in Table 3 along with the patients' demographic characteristics. Out of 30 MCC tumors analyzed, 25 (83.3%) demonstrated KIT protein expression and 19 (63.3%) showed high expression defined as a staining intensity of 2+ or 3+ in more than 30% of the tumor cells (Figure 1). Overall there were 18 out of 21 patients (85.7%) with primary MCC tumors demonstrating expression of KIT protein and 13 patients (62%) exhibiting high expression. There was concordance in the KIT expression profile between primary and metastatic tumors in 6 out of 8 patients in which multiple tumors were analyzed. In one patient, the intensity of KIT staining was 3+ in the primary tumor and 1+ in the subsequent cutaneous metastasis while in the other patient the primary tumor showed 1+ and the lymph node metastasis 2+ staining intensity. The staining was cytoplasmic in 18 of 19 MCC tumors (94.7%) and membranous in 1 case (5.3%). The distribution of stage I, II, III and IV in low versus high KIT expressor groups was 37.5%, 25%, 37.5% and 0% versus 50%, 16.7%, 16.7% and 16.7%, respectively, $p=0.5$.

The overall survival was compared between the groups of patients demonstrating high (13 patients) versus low (8 patients) KIT expression. There was a decreased overall survival in the high KIT expressors compared to low KIT expressors (Fig 2). The 2-year survival rates in high KIT versus low KIT expressors were 53% versus 86.7%, respectively and the 5-year survival rates in high KIT versus low KIT expressors were 0% versus 57.8%, respectively. The difference did not reach however, statistical significance ($p=0.07$).

KIT gene mutational status

A total of 17 tumors from the 13 patients with MCC demonstrating high KIT protein expression by IHC were further analyzed for KIT mutation status. In addition, a lymph node metastasis exhibiting high (2+) KIT staining intensity, from a patient in which the primary tumor showed low (1+) KIT expression, was also included for a total of 18 tumors and 14 patients. One hundred eighty electropherograms traces were generated and analyzed for genomic alterations and 4 point mutations were identified (Table 4 and Figure 3). Two of these involved exons 17 (c.2394 C>T -2 patients) and 18 (c.2586 G>C -3 patients) and were silent mutations. The other 2 mutations involved intron 16-17. One was g.55,293,886 T>A located 107 bp from the 5' end of exon 17 (5 patients) and the other one was g.55,293,916 G>A located 77 bp from the 5' end of exon 17 (7 patients). Two of the identified mutations (c.2586 G>C and g.55,293,916 G>A) correspond to previously reported polymorphisms of the KIT gene (rs3733542 and rs4864921 respectively). There were no missense mutations identified within the coding region of the KIT gene.

DISCUSSION

The main purpose of this study was to evaluate the prognostic implication of KIT overexpression in MCC and to investigate whether activating mutations of the KIT oncogene are present in cases overexpressing the protein. According to the Ensembl database (www.ensembl.org) [36], the KIT gene, located on chromosome 4p, is composed of 21 exons spanning 87,787 base pairs that encodes for a 976 amino-acid protein containing an extracellular, transmembrane, juxtamembrane and tyrosine kinase domain. Interaction between KIT protein and its ligand SCF appears to be essential for the development of

human melanocytes, erythrocytes, germ cells, mast cells and of the interstitial cells of Cajal [29].

Previous studies have described KIT expression in 7-95% of MCC cases [18-26] (85.7% in our study). Some of the differences in reported figures are due to different cut-off points used to regard a case as positive. We have found that expression of KIT protein by immunohistochemistry correlates with a trend for worse prognosis in MCC patients. The 2-year survival rate in patients with tumors expressing high versus low levels of KIT was 53% versus 86.7%, respectively. This finding raises the possibility that KIT could play an active role in MCC progression and/or metastasis. Of note, three prior studies by Su et al., Feinmesser et al. and Llombart et al. did not find any association between the intensity of KIT expression and behavior [18,21,39]. However, Feinmesser et al observed that strong KIT expression correlates with lymphovascular invasion and a high mitotic rate, both parameters associated with a more aggressive tumor course [21]. Although our study does not reach statistical significance, the trend is intriguing and further correlative studies should be performed on larger series to validate the prognostic implications. It is important to note that, as mentioned above, there is a wide range in the reported KIT expression in MCC in the literature (7-95%). According to Yang et al., this may be due to variations in IHC techniques such as antigen retrieval [26]. These variations may be at the root of the conflicting data regarding the correlation between KIT expression and prognosis as inaccurate evaluation of protein expression will impact the strength and direction of any discovered association.

Prior studies have identified the presence of activating KIT mutations in GISTs located in exon 11 encoding the juxtamembrane domain, exons 13 and 17 encoding the tyrosine kinase domain and less commonly in exon 9 encoding the extracellular domain [29-31,40,41]. The DNA alterations include both point mutations and in-frame deletions. Two previous studies have investigated the presence of KIT mutations in MCC tumors in exons 9, 11, 13 and 17 and one in exons 9 and 11 [22-24]. The studies have failed to find any missense mutations in these exons which are frequently altered in GISTs and melanomas. A recent study has demonstrated the presence of an activating mutation involving exon 18 of the KIT gene in malignant melanoma [32]. Therefore, in additions to exons 9, 11, 13 and 17, we also sequenced exon 18 whose status was not investigated before in MCC. Similar to other studies we did not identify KIT activating mutations in the tumors analyzed. We have found 4 single nucleotide alterations. Two of these involve the coding regions of exon 17 and 18 which encode for the tyrosine kinase domain however, they do not result in an amino acid change (i.e. silent mutations). The other 2 mutations involve intron 16-17, close to the 5' end of exon 17 and do not result in mRNA or protein changes. While 2 of these mutations have been previously reported as polymorphic sites, the other 2 might represent novel single nucleotide polymorphisms of the KIT gene.

The absence of activating KIT mutations suggests that if the KIT pathway plays a role in the pathogenesis or progression of MCC, alternative mechanisms, possibly involving up- or downstream effectors of the KIT pathway could be involved. At this point, further studies are warranted to validate the prognostic implications of KIT expression and possibly, thereafter, evaluate the biologic significance of KIT protein over-expression in MCC.

REFERENCES

1. Toker C. Trabecular carcinoma of the skin. Arch Dermatol. 1972; 105:107–110. [PubMed: 5009611]
2. Tang CK, Toker C. Trabecular carcinoma of the skin: An ultrastructural study. Cancer. 1978; 42:2311–2321. [PubMed: 719609]

3. Agelli M, Clegg LX. Epidemiology of primary merkel cell carcinoma in the united states. *J Am Acad Dermatol.* 2003; 49:832–841. [PubMed: 14576661]
4. Hodgson NC. Merkel cell carcinoma: Changing incidence trends. *Journal of surgical oncology.* 2005; 89:1–4. [PubMed: 15611998]
5. Allen PJ, Bowne WB, Jaques DP, Brennan MF, Busam K, Coit DG. Merkel cell carcinoma: Prognosis and treatment of patients from a single institution. *J Clin Oncol.* 2005; 23:2300–2309. [PubMed: 15800320]
6. Allen PJ, Zhang ZF, Coit DG. Surgical management of merkel cell carcinoma. *Ann Surg.* 1999; 229:97–105. [PubMed: 9923806]
7. Andea AA, Coit DG, Amin B, Busam KJ. Merkel cell carcinoma: Histologic features and prognosis. *Cancer.* 2008; 113:2549–2558. [PubMed: 18798233]
8. Eng TY, Naguib M, Fuller CD, Jones WE 3rd, Herman TS. Treatment of recurrent merkel cell carcinoma: An analysis of 46 cases. *American journal of clinical oncology.* 2004; 27:576–583. [PubMed: 15577435]
9. Koljonen V, Bohling T, Granthorh G, Tukiainen E. Merkel cell carcinoma: A clinicopathological study of 34 patients. *Eur J Surg Oncol.* 2003; 29:607–610. [PubMed: 12943628]
10. Medina-Franco H, Urist MM, Fiveash J, Heslin MJ, Bland KI, Beenken SW. Multimodality treatment of merkel cell carcinoma: Case series and literature review of 1024 cases. *Ann Surg Oncol.* 2001; 8:204–208. [PubMed: 11314935]
11. Yiengpruksawan A, Coit DG, Thaler HT, Urmacher C, Knapper WK. Merkel cell carcinoma. Prognosis and management. *Arch Surg.* 1991; 126:1514–1519. [PubMed: 1842182]
12. Tai PT, Yu E, Tonita J, Gilchrist J. Merkel cell carcinoma of the skin. *J Cutan Med Surg.* 2000; 4:186–195. [PubMed: 11231196]
13. Ratner D, Nelson BR, Brown MD, Johnson TM. Merkel cell carcinoma. *J Am Acad Dermatol.* 1993; 29:143–156. [PubMed: 8335732]
14. Haag ML, Glass LF, Fenske NA. Merkel cell carcinoma. Diagnosis and treatment. *Dermatol Surg.* 1995; 21:669–683. [PubMed: 7633811]
15. Hitchcock CL, Bland KI, Laney RG 3rd, Franzini D, Harris B, Copeland EM 3rd. Neuroendocrine (merkel cell) carcinoma of the skin. Its natural history, diagnosis, and treatment. *Ann Surg.* 1988; 207:201–207. [PubMed: 3277546]
16. Cotlar AM, Gates JO, Gibbs FA Jr. Merkel cell carcinoma: Combined surgery and radiation therapy. *The American surgeon.* 1986; 52:159–164. [PubMed: 3954263]
17. Eich HT, Eich D, Staar S, Mauch C, Stutzer H, Groth W, Krieg T, Muller RP. Role of postoperative radiotherapy in the management of merkel cell carcinoma. *American journal of clinical oncology.* 2002; 25:50–56. [PubMed: 11823697]
18. Su LD, Fullen DR, Lowe L, Uherova P, Schnitzer B, Valdez R. Cd117 (kit receptor) expression in merkel cell carcinoma. *Am J Dermatopathol.* 2002; 24:289–293. [PubMed: 12142606]
19. Strong S, Shalders K, Carr R, Snead DR. Kit receptor (cd117) expression in merkel cell carcinoma. *Br J Dermatol.* 2004; 150:384–385. [PubMed: 14996126]
20. Yang DT, Holden JA, Florell SR. Cd117, ck20, ttf-1, and DNA topoisomerase ii-alpha antigen expression in small cell tumors. *J Cutan Pathol.* 2004; 31:254–261. [PubMed: 14984578]
21. Feinmesser M, Halpern M, Kaganovsky E, Brenner B, Fenig E, Hodak E, Sulkes J, Okon E. C-kit expression in primary and metastatic merkel cell carcinoma. *Am J Dermatopathol.* 2004; 26:458–462. [PubMed: 15618926]
22. Swick BL, Ravdel L, Fitzpatrick JE, Robinson WA. Merkel cell carcinoma: Evaluation of kit (cd117) expression and failure to demonstrate activating mutations in the c-kit proto-oncogene - implications for treatment with imatinib mesylate. *J Cutan Pathol.* 2007; 34:324–329. [PubMed: 17381803]
23. Brunner M, Thurnher D, Pammer J, Geleff S, Heiduschka G, Reinisch CM, Petzelbauer P, Erovic BM. Expression of vegf-a/c, vegf-r2, pdgf-alpha/beta, c-kit, egfr, her-2/neu, mcl-1 and bmi-1 in merkel cell carcinoma. *Mod Pathol.* 2008; 21:876–884. [PubMed: 18408656]
24. Kartha RV, Sundram UN. Silent mutations in kit and pdgfra and coexpression of receptors with scf and pdgfa in merkel cell carcinoma: Implications for tyrosine kinase-based tumorigenesis. *Mod Pathol.* 2008; 21:96–104. [PubMed: 18084259]

25. Krasagakis K, Kruger-Krasagakis S, Eberle J, Tsatsakis A, Tosca AD, Stathopoulos EN. Co-expression of kit receptor and its ligand stem cell factor in merkel cell carcinoma. *Dermatology* (Basel, Switzerland). 2009; 218:37–43.
26. Yang Q, Hornick JL, Granter SR, Wang LC. Merkel cell carcinoma: Lack of kit positivity and implications for the use of imatinib mesylate. *Appl Immunohistochem Mol Morphol*. 2009; 17:276–281. [PubMed: 19276970]
27. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature*. 2001; 411:355–365. [PubMed: 11357143]
28. Taniguchi M, Nishida T, Hirota S, Isozaki K, Ito T, Nomura T, Matsuda H, Kitamura Y. Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. *Cancer Res*. 1999; 59:4297–4300. [PubMed: 10485475]
29. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Tunio G Muhammad, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998; 279:577–580. [PubMed: 9438854]
30. Antonescu CR, Besmer P, Guo T, Arkun K, Hom G, Koryotowski B, Leversha MA, Jeffrey PD, Desantis D, Singer S, Brennan MF, Maki RG, DeMatteo RP. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin Cancer Res*. 2005; 11:4182–4190. [PubMed: 15930355]
31. Antonescu CR, Sommer G, Sarra L, Tschernyavsky SJ, Riedel E, Woodruff JM, Robson M, Maki R, Brennan MF, Ladanyi M, DeMatteo RP, Besmer P. Association of kit exon 9 mutations with nongastric primary site and aggressive behavior: Kit mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. *Clin Cancer Res*. 2003; 9:3329–3337. [PubMed: 12960119]
32. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of kit in distinct subtypes of melanoma. *J Clin Oncol*. 2006; 24:4340–4346. [PubMed: 16908931]
33. Hodi FS, Friedlander P, Corless CL, Heinrich MC, Mac Rae S, Kruse A, Jagannathan J, Van den Abbeele AD, Velazquez EF, Demetri GD, Fisher DE. Major response to imatinib mesylate in kit-mutated melanoma. *J Clin Oncol*. 2008; 26:2046–2051. [PubMed: 18421059]
34. Fiorentini G, Rossi S, Lanzaova G, Biancalani M, Palomba A, Bernardeschi P, Dentico P, De Giorgi U. Tyrosine kinase inhibitor imatinib mesylate as anticancer agent for advanced ocular melanoma expressing immunoistochemical c-kit (cd 117): Preliminary results of a compassionate use clinical trial. *J Exp Clin Cancer Res*. 2003; 22:17–20. [PubMed: 16767900]
35. Llombart B, Sanmartin O, Lopez-Guerrero JA, Monteagudo C, Serra C, Requena C, Poveda A, Vistos JL, Almenar S, Llombart-Bosch A, Guillen C. Dermatofibrosarcoma protuberans: Clinical, pathological, and genetic (coll1a1-pdgfb) study with therapeutic implications. *Histopathology*. 2009; 54:860–872. [PubMed: 19635106]
36. Flicek P, Aken BL, Beal K, Ballester B, Caccamo M, Chen Y, Clarke L, Coates G, Cunningham F, Cutts T, Down T, Dyer SC, Eyre T, Fitzgerald S, Fernandez-Banet J, Graf S, Haider S, Hammond M, Holland R, Howe KL, Howe K, Johnson N, Jenkinson A, Kahari A, Keefe D, Kokocinski F, Kulesha E, Lawson D, Longden I, Megy K, Meidl P, Overduin B, Parker A, Pritchard B, Prlic A, Rice S, Rios D, Schuster M, Sealy I, Slater G, Smedley D, Spudich G, Trevanion S, Vilella AJ, Vogel J, White S, Wood M, Birney E, Cox T, Curwen V, Durbin R, Fernandez-Suarez XM, Herrero J, Hubbard TJ, Kasprzyk A, Proctor G, Smith J, Ureta-Vidal A, Searle S. Ensembl 2008. *Nucleic acids research*. 2008; 36:D707–714. [PubMed: 18000006]
37. Agaram NP, Besmer P, Wong GC, Guo T, Socci ND, Maki RG, DeSantis D, Brennan MF, Singer S, DeMatteo RP, Antonescu CR. Pathologic and molecular heterogeneity in imatinib-stable or imatinib-responsive gastrointestinal stromal tumors. *Clin Cancer Res*. 2007; 13:170–181. [PubMed: 17200352]
38. Rozen S, Skaletsky H. Primer3 on the www for general users and for biologist programmers. *Methods in molecular biology* (Clifton, NJ. 2000; 132:365–386.
39. Llombart B, Monteagudo C, Lopez-Guerrero JA, Carda C, Jorda E, Sanmartin O, Almenar S, Molina I, Martin JM, Llombart-Bosch A. Clinicopathological and immunohistochemical analysis of 20 cases of merkel cell carcinoma in search of prognostic markers. *Histopathology*. 2005; 46:622–634. [PubMed: 15910593]

40. Lasota J, Corless CL, Heinrich MC, Debiec-Rychter M, Sciort R, Wardelmann E, Merkelbach-Bruse S, Schildhaus HU, Steigen SE, Stachura J, Wozniak A, Antonescu C, Daum O, Martin J, Del Muro JG, Miettinen M. Clinicopathologic profile of gastrointestinal stromal tumors (gists) with primary kit exon 13 or exon 17 mutations: A multicenter study on 54 cases. *Mod Pathol.* 2008; 21:476–484. [PubMed: 18246046]
41. Prakash S, Sarraf L, Socci N, DeMatteo RP, Eisenstat J, Greco AM, Maki RG, Wexler LH, LaQuaglia MP, Besmer P, Antonescu CR. Gastrointestinal stromal tumors in children and young adults: A clinicopathologic, molecular, and genomic study of 15 cases and review of the literature. *J Pediatr Hematol Oncol.* 2005; 27:179–187. [PubMed: 15838387]

Figure 1. KIT immunohistochemical staining in MCC (Hematoxylin and eosin, X400). A. Negative staining (0). B. 1+ staining intensity, cytoplasmic pattern. C. 2+ staining intensity, cytoplasmic pattern. D. 3+ staining intensity, cytoplasmic pattern. E. 3+ staining intensity, membranous pattern.

Figure 2.

The impact of KIT protein expression on survival in MCC. Interrupted line indicates low KIT expression (0 and 1+ intensity), solid indicates high KIT expression (2+ and 3+ intensity). P-value was calculated using the log-rank test.

Figure 3. Mutational analysis of KIT gene in MCC. A. Representative pictures of agarose gel electrophoresis with the PCR products for exons 9, 11, 13, 17 and 18 of the KIT gene. B, C, D, E. Electropherograms of the 4 mutations identified. Top electropherogram corresponds to the forward trace, bottom to the reverse trace. Arrows point to the mutated nucleotide.

Table 1

Primers used for KIT gene PCR amplification and sequencing

Exon	Forward primer (5'-3')	Tm (°C)	Reverse primer (5'-3')	Tm (°C)	Product size (bp)
9	AGAGTAAGCCAGGGCTTTTG	58.6	GGTAGACAGAGCCCTAAACATCC	57	279
11	CCAGAGTGTCTCTAATGACTG	53.4	ACCCAAAAAGGTGACATGGA	60.2	236
13	CATCAGTTTGCCAGTTGTGC	60.3	GCAAGAGAGAAACAACAGTCTGG	59.1	294
17	TGTGAACATCATTTCAAGGCGGTAC	62.1	CAGGACTGTCAAGCAGAGAAATGG	63.4	331
18	CCACATTTCAGCAACAGCAG	60.4	CAAGGAAGCAGGACACCAAT	60.1	290

Table 2

Demographic parameters and clinical data.

Parameter	No of patients (%)
Sex (N=40)	
Male	35 (87.5%)
Female	5 (12.5%)
Age (years) (N=40)	
Median	71.6
Range	43.7-92.7
Location of primary tumor (N=40)	
Head and neck	22 (55%)
Extremity	10 (25%)
Axilla	4 (10%)
Trunk	2 (5%)
Buttocks	1 (2.5%)
Unknown	1 (2.5%)
Stage at presentation (N=27)*	
I	11 (40.7%)
II	8 (14.8%)
III	9 (33.3%)
IV	3 (11.1%)
Status at last follow-up (N=37)	
DOD	20 (54.1%)
NED	10 (27%)
AWD	7 (18.9%)
Follow-up (months)	
Median	20
Range	1-108

Abbreviations: DOD –died of disease, NED –no evidence of disease, AWD –alive with disease, N –number of patients in which the parameter was available.

* The four tiered staging system was used defined as: stage I - node negative disease and tumor < 2 cm, stage II - node negative disease and tumor ≥ 2 cm, stage III - node positive, stage IV - presence of distant metastatic disease. [6]

Table 3
KIT protein expression by immunohistochemistry in 30 MCC tumors (21 patients)

Pt #	Age (years)	Sex	Stage*	F/U (months)	Site	KIT protein expression by IHC**		
						%	int	High/Low expressor
1	61	M	II	AWD, 73.5	right knee	60	1	Low
2	56	M	I	NA	left thigh	100	2	High
3	65	M	IV	DOD, 4.5	left ear	30	2	High
4	72	M	I	NED, 38.2	right temple	50	3	High
5	79	M	I	NED, 9.5	left jaw	0	0	Low
6	67	M	I	NED, 24.9	right thigh	90	1	Low
7	53	F	III	DOD, 8.5	left lower leg	60	1	Low
					left lower leg	0	0	Low
8	63	M	I	AWD, 21.4	right cheek	60	3	High
					left face met	50	1	Low
9	73	F	IV	DOD, 0.6	right axillary LN	80	3	High
10	51	F	I	NED, 27.8	left zygoma	80	1	Low
11	93	M	II	NED, 20.3	right malar eminence	90	3	High
12	73	M	I	DOD, 27.4	Right temple	90	2	High
					forehead	100	2	High
13	77	M	I	NED, 39	left temporo-parietal scalp	100	3	High
					parotid	60	3	High
					left neck LN	80	3	High
14	81	M	II	DOD, 62	right forehead	0	0	Low
15	81	F	III	DOD, 58	left forearm	0	0	Low
					left axilla LN	0	0	Low
16	78	M	III	DOD, 6.8	left knee	80	3	High
					left groin LN	80	2	High

Pt #	Age (years)	Sex	Stage*	F/U (months)	Site	KIT protein expression by IHC**		
						%	int	High/Low expressor
17	78	M	I	AWD, 8.2	right forehead	100	3	High
18	74	M	III	NED, 20.2	right infraorbital area	50	1	Low
					right face LN	70	2	High
19	77	M	II	DOD, 59	left upper lip	50	2	High
20	72	M	III	DOD, 19.4	left postauricular	100	3	High
					parotid	40	3	High
21	73	M	NA	DOD, 0.9	right cheek	100	3	High

Abbreviations: DOD –died of disease, NED –no evidence of disease, AWD –alive with disease, NA –data not available.

*The four tiered staging system was used defined as: stage I - node negative disease and tumor < 2 cm, stage II - node negative disease and tumor ≥ 2 cm, stage III - node positive, stage IV - presence of distant metastatic disease. [6]

** KIT expression evaluated as percentage of cells staining and intensity of staining (0, 1+, 2+, 3+). High-expressors - 2+ or 3+ staining intensity in at least 30% of the tumor cells; Low-expressors - negative, 1+ staining intensity in any percentage, 2+ and 3+ intensity in less than 30% of tumor cells.

Table 4

KIT gene mutations identified in 18 MCC tumors (14 patients).

Exon/ intron	Position on chr 4	Position on coding mRNA	Base change	Type	Reported polymorphism	No patients	Frequency N=14
Exon 17	55,294,025	2,394	C>T	Silent	No	2	14.3%
Intron 16-17	55,293,886	-	T>A	Silent	No	5	35.7%
Intron 16-17	55,293,916	-	G>A	Silent	rs4864921	7	50%
Exon 18	55,297,522	2,586	G>C	Silent	rs3733542	3	21.4%