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DIAGNOSIS IN ONCOLOGY

Response to a Novel Multitargeted Tyrosine Kinase Inhibitor Pazopanib in Metastatic Merkel Cell Carcinoma

A 69-year-old woman presented with a 2-year history of gradually increasing scalp swelling in December 2001. The patient was in good general condition, but physical examination revealed a 5-cm fleshy tumor on the right scalp, with pathology consistent with Merkel cell carcinoma on excisional biopsy. A metastatic work-up, including computed tomography (CT) of the thorax and abdomen, mammography, and gastroscopy, was negative, and the patient underwent a subsequent wide excision with superficial skin graft. No adjuvant radiotherapy was administered at this time. In August 2002, the patient had a local recurrence of a bulky 12-cm mass fixed to the skull, and biopsies of the right upper deep cervical and supraclavicular lymph nodes were inconclusive for involvement. The tumor was inoperable, and the patient underwent radical radiotherapy for a total of 70 Gy, with excellent response at all tumor sites. In March 2003, two small nodules developed in the field, which were believed to represent either soft tissue radionecrosis or possibly a second local recurrence. In October 2003, the patient underwent repeat wide excision with right level II to V neck dissection for nodal and scalp tumor recurrence. Histopathologic examination of the tissue revealed Merkel cell carcinoma with eight of 19 positive lymph nodes and negative margins. The tissue was strongly positive for neuron-specific enolase, with cytoplasmic and perinuclear dot positive for AE1/3, CAM5.2, and epithelial membrane antigen (Fig 1A). Synaptophysin was focally positive, whereas S-100 protein was weakly staining, and chromogranin was negative. CT of the thorax/abdomen revealed multiple pulmonary lesions consistent with metastatic disease. The patient was given six cycles of carboplatin with etoposide, and achieved a partial response. In July 2004, a 1.6 × 1.2 cm right neck mass developed just deep to the flap with a necrotic center at C2-C3, and the pulmonary metastases also showed progression on follow-up CT. The patient underwent repeat wide excision of the neck mass, and postoperative chemotherapy was begun with weekly paclitaxel. Stable disease was maintained until December 2004, when pulmonary metastases progressed, and a 2.3-cm subcarinal lymph node was noted on CT. The patient enrolled onto a clinical trial of tegafur, 5-chloro-2,4-dihydroxypyridine, and oxonic acid (S1) at 30 mg/m^2 twice daily. A partial response of the pulmonary metastases was achieved, and the patient continued to receive S1 for 35 cycles until relapse occurred in December 2006, with a 1.5-cm nodule above the right ear. Fine-needle aspiration of the nodule was consistent with Merkel cell carcinoma. The patient was observed closely until April 2007, when the right ear mass had grown to 3.5 cm, and pulmonary metastases had progressed on CT imaging. The patient was enrolled onto a clinical trial of a novel multitargeted tyrosine kinase inhibitor pazopanib at 800 mg daily after written informed consent was provided according to institutional guidelines. Consent to use her data for this report was obtained and

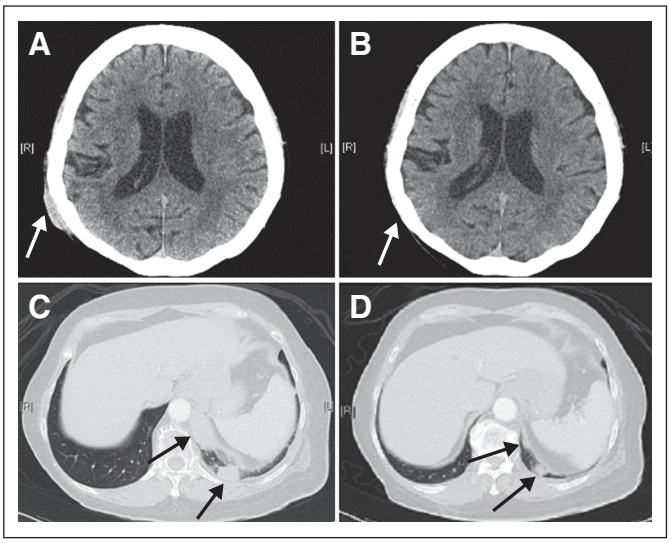

Fig 2.

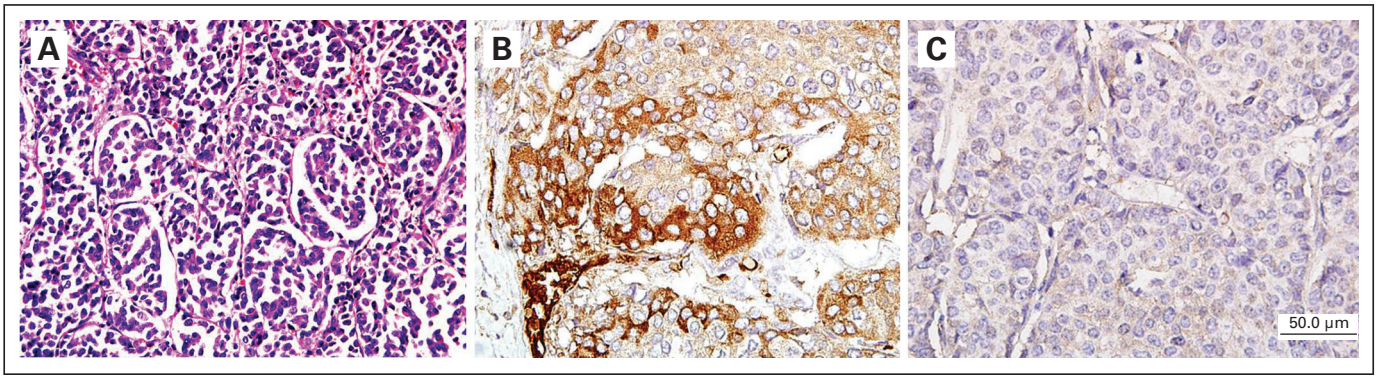

Fig 1.

studies in this report were done with the approval of the institutional review board. Two months later, repeat CT scans showed a complete response in the scalp lesion (Fig 2A, before treatment; Fig 2B, after treatment) and a partial response in the pulmonary metastases (Figs 2C, before treatment; and 2D, 2 days after treatment). The sum of the longest diameters of these target lesions in the lungs showed a reduction of 57%, according to RECIST (Response Evaluation Criteria in Solid Tumors). The patient tolerated pazopanib well, with minimal adverse effects. Because of an episode of gallstone pancreatitis 3 months into the trial, the dose of pazopanib was reduced to 400 mg daily. Despite this dose reduction, the patient maintained a partial response for a total of 6 months while receiving pazopanib, until a CT scan in October 2007 revealed progression of disease in the scalp and lungs. Pazopanib was discontinued at this time and palliative doxorubicin was administered. The patient ultimately died as a result of progressive disease of the lungs and liver in August 2008.

Merkel cell carcinoma is a rare, highly aggressive cutaneous neoplasm, typically arising as a flesh-colored, nontender nodule in the head, neck, or extremities of elderly patients. There have been no prospective studies to guide therapeutics, and most data are from retrospective analyses in single institutions. The tumor arises as a nested arrangement in the dermis and typically stains positive for cytokeratin 20 and negative for cytokeratin 7, distinguishing it from other neuroendocrine tumors, and stains negative for S-100 protein, distinguishing it from melanoma. Despite reports of 5-year disease-specific survival rates for early-stage disease of slightly more than 60%,[1] local recurrence and metastasis are common and the mortality rate is high. Wide surgical excision is the treatment of choice for nonmetastatic disease, with some institutions performing sentinel lymph node biopsy.[1,2] Although adjuvant radiotherapy to the primary site and involved lymph nodes may reduce the rate of local recurrence, the value of adjuvant chemotherapy is less clear.[3] Locally advanced

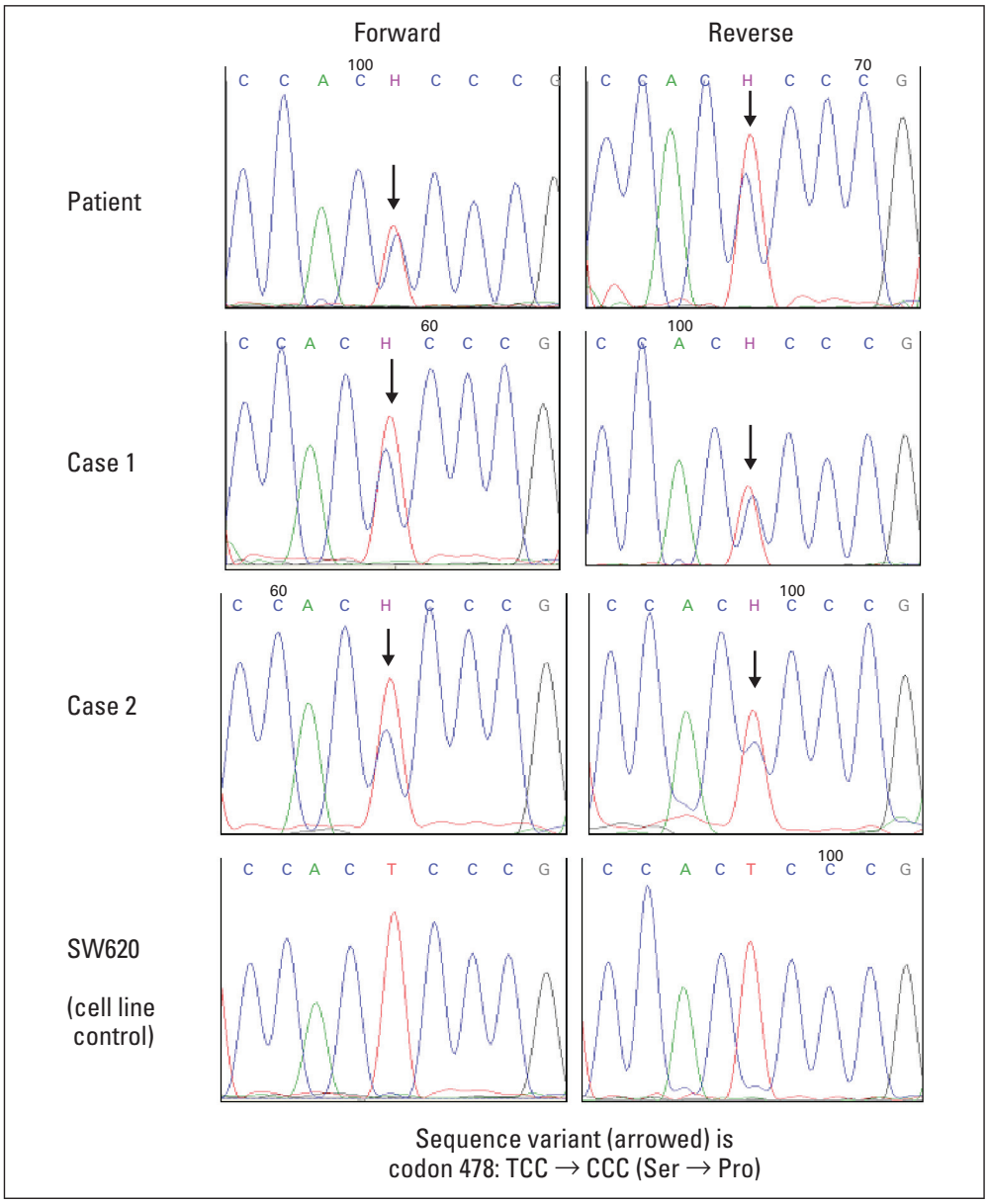

Fig 3.

and metastatic disease is often responsive to first-line chemotherapy; cyclophosphamide in combination with platinum or anthracycline-based regimens are used most commonly. In a retrospective study of 107 patients, there was overall objective response rate of 61% to first-line chemotherapy. However, chemotherapy-related toxicity in this elderly population was high, and the median overall survival for patients with metastatic disease was only 9 months from the initiation of chemotherapy, with a 3-year overall survival of 17%.[4] Therefore, alternative treatment approaches are needed. We report impressive tumor regression in a patient with metastatic Merkel cell carcinoma during treatment with the novel small-molecule multitargeted tyrosine kinase inhibitor pazopanib, and explore possible mechanisms of response to this agent through immunohistochemical analyses of targets relevant to receptor tyrosine kinase inhibition. Pazopanib is a second-generation multitargeted tyrosine kinase inhibitor against vascular endothelial growth factor receptor (VEGFR) -1, -2, and -3; platelet-derived growth factor receptor (PDGFR) -α and -β; and c-kit. Preclinical studies demonstrated potent antitumor and antiangiogenic activity, with good oral bioavailability. Phase I clinical trials revealed favorable pharmacokinetics and a benign adverse effect profile, with activity in renal cancer and other tumors.[5] To correlate the favorable clinical response in the context of tyrosine kinase targets of pazopanib, we studied the expression of c-kit, VEGF, flt-1/kdr, flt 4, PDGFR-α, and PDGFR-β in this patient and two other patients with Merkel cell carcinoma. We also sequenced known mutations in exon 9 and 11 of c-kit, and a recently described 1432T>C mutation in PDGFR-α.[6] Immunohistochemistry showed similar characteristics for all three patient samples: c-kit negative (DAKO Polyclonal, Carpinteria, CA); VEGF (Clone C-1, sc-7269; Santa Cruz Biotechnology, Santa Cruz, CA) positive (Fig 1B); VEGFR-1 (sc-74007; Santa Cruz Biotechnology), VEGFR-2 (A-3; Santa Cruz Biotechnology), and VEGFR-3 (Clone C1.1, DM3512P; Acris Antibodies, Herford, Germany) all negative; PDGF-A negative (sc-9974; Santa Cruz Biotechnology); and PDGFR-β (sc-339; Santa Cruz Biotechnology) weakly positive (Fig 1C). No activating mutations were found in exon 9 and 11 for c-kit. However, a 1432T>C mutation in *PDGFR-α* (ser478pro) gene was found in all three patient tumor samples, as well as in their germline DNA (Fig 3, tumor mutation analysis of patient and two other historical control patients with Merkel cell carcinoma). We found the allelic frequencies of this mutation to be 13.4% in Chinese (n = 75), 17.9% in Malays (n = 14), and 25% in Indian (n = 8) healthy individuals. Previous expression studies have revealed frequent expression of VEGF-A, VEGF-C, VEGFR-2, and PDGF-A in Merkel cell carcinoma, and suggested a possible role of multitargeted receptor tyrosine kinase inhibitors in the management of this cancer. Initial investigations of the classic mitogen-activated protein kinase pathway in Merkel cell carcinoma failed to detect activating mutations in the *BRAF* gene, suggesting that drugs in the sorafanib family might be less effective.[7] In vitro studies have shown a decrease in proliferation of Merkel cell carcinoma with the tyrosine kinase inhibitor imatinib.[8] In addition, Merkel cell carcinoma has been shown to overexpress several of the targets of receptor tyrosine kinase inhibitors. Tissue microarray data have suggested that VEGF is upregulated, particularly in aggressive tumors.[9] VEGFR-2 is also overexpressed in Merkel cell carcinoma by immunohistochemistry.[10] The data for the involvement of c-kit in Merkel cell carcinoma is less clear, with some studies showing positive c-kit expression, and others, as in our patients, showing a lack of c-kit staining or activating mutations.[11] PDGFR-α and PDGF-A have been shown to be present in Merkel cell carcinoma, with one study finding coexpression in 25 (81%) of 31 samples.[6] The investigators also found the mutation in codon 478 of the *PDGFR-α* gene of one patient.[6] Intriguingly, another study showed identical mutation in three additional patients with Merkel cell carcinoma.[12] Our study demonstrates that this mutation appears to be frequent in Merkel cell carcinoma in the germline DNA, suggesting a possible causative association with Merkel cell carcinoma. Functional studies are needed to determine the impact of this mutation on the function of PDGFR-α. It is also plausible that this mutation may lead to ligand-independent PDGFR-α activation, and responsive to pazopanib. We conclude that inhibition of the VEGF and possibly mediation of angiogenesis in PDGF signaling pathways with drugs that inhibit multiple receptor tyrosine kinases could be important new treatment options in metastatic Merkel cell carcinoma. The *PDGFR-α* mutation in codon 478 should be explored further as a genetic predisposing factor to development of Merkel cell carcinoma, and also as a response marker to receptor tyrosine kinase inhibitors.

Matthew Davids
Department of Haematology/Oncology, National University Health System, Singapore, Singapore

Amanda Charlton
Department of Pathology, National University Health System, Singapore, Singapore

Swee-Siang Ng, Mei-Ling Chong
Oncology Research Institute, National University Health System, Singapore, Singapore

Kevin Laubscher, Mohammed Dar, and Jeffrey Hodge
GlaxoSmithKline, Research Triangle Park, NC

Richie Soong
Oncology Research Institute, National University Health System, Singapore, Singapore

Boon Cher Goh
Department of Haematology/Oncology and Oncology Research Institute, National University Health System, Singapore, Singapore

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

1. Allen PJ, Brown WB, Jaques DP, et al: Merkel cell carcinoma: Prognosis and treatment of patients from a single institution. J Clin Oncol 23:2300-2309, 2005

2. Eng TY, Boersma MG, Fuller CD, et al: A comprehensive review of the treatment of Merkel cell carcinoma. Am J Clin Oncol 30:624-636, 2007

3. Garneski KM, Nghiem P: Merkel cell carcinoma adjuvant therapy: Current data support radiation but not chemotherapy. J Am Acad Dermatol 57:166-169, 2007

4. Voog E, Biron P, Martin JP, et al: Chemotherapy for patients with locally advanced or metastatic Merkel cell carcinoma. Cancer 85:2589-2595, 1999

5. Sonpavde G, Hutson TE: Pazopanib: A novel multitargeted tyrosine kinase inhibitor. Curr Oncol Rep 9:115-119, 2007

6. 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 21:96-104, 2008

7. Houben R, Michel B, Vetter-Kauczok CS, et al: Absence of classical MAP kinase pathway signaling in Merkel cell carcinoma. J Invest Dermatol 126:1135-1142, 2006

8. Kondapalli L, Soltani K, Lacouture ME: The promise of molecular target therapies: Protein kinase inhibitors in the treatment of cutaneous malignancies. J Am Acad Dermatol 53:291-302, 2005

9. Fernandez-Figueras MT, Puig L, Musulen E, et al: Expression profiles associated with aggressive behavior in Merkel cell carcinoma. Mod Pathol 20:90-101, 2007

10. Kukko H, Koljonen V, Lassus P, et al: Expression of vascular endothelial growth factor receptor-2 in Merkel cell carcinoma. Anticancer Res 27:2587-2589, 2007

11. Feinmesser M, Halpern M, Kaganovsky E, et al: C-kit expression in primary and metastatic Merkel cell carcinoma. Am J Dermatopathol 26:458-462, 2004

12. Swick BL, Ravdel L, Fitzpatrick JE, et al: Platelet-derived growth factor receptor alpha mutational status and immunohistochemical expression in Merkel cell carcinoma: Implications for treatment with imatinib mesylate. J Cutan Pathol 35:197-202, 2008

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CORRECTIONS

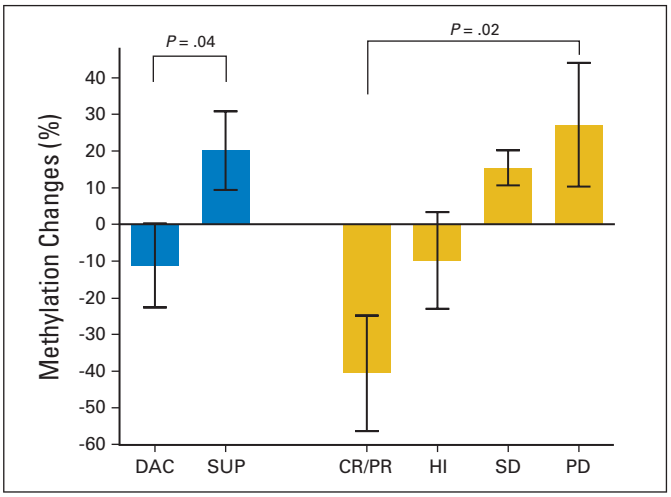
Author Corrections

The September 10, 2009, Diagnosis in Oncology article by Davids et al, entitled "Response to a Novel Multitargeted Tyrosine Kinase Inhibitor Pazopanib in Metastatic Merkel Cell Carcinoma" (J Clin Oncol 27:e97-e100, 2009), contained an error in the spelling of the first author's name. It was originally given as Matthew Davids and should have been Matthew S. Davids. The authors apologize to the readers for the mistake.

DOI: 10.1200/JCO.2010.30.5680

The February 1, 2010, article by Shen et al, entitled "DNA Methylation Predicts Survival and Response to Therapy in Patients With Myelodysplastic Syndromes" (J Clin Oncol 28:605-613, 2010), contained an error.

In Figure 3, some of the data depicted in the bar graph were incorrect for decitabine (DAC), complete remission and partial remission (CR/PR), hematologic improvement (HI), stable disease (SD), and progressive disease (PD). The corrected figure is reprinted below in its entirety.



The online version has been corrected in departure from the print. The authors apologize to the readers for the mistake.

DOI: 10.1200/JCO.2010.30.5698

Journal Correction

The May 1, 2010, article by Sekeres et al, entitled "Phase I Combination Trial of Lenalidomide and Azacitidine in Patients With Higher-Risk Myelodysplastic Syndromes" (J Clin Oncol 28:2253-2258, 2010), contained an error in the spelling of the fifth author's name. It was originally given as Rebecca Ganetsky and should have been Rebecca Ganetzky. *Journal of Clinical Oncology* apologizes to the authors and readers for the mistake.

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