

Letter to the editor

***BRAF*^{V600E} mutation in sporadic and neurofibromatosis type 1-related malignant peripheral nerve sheath tumors**

Corresponding Author: David H. Gutmann, MD, PhD, Department of Neurology, Box 8111, 660 S. Euclid Avenue, Washington University School of Medicine, St. Louis, MO, 63110 (gutmann@neuro.wustl.edu).

Keywords: microarray, MPNST, NF1, sarcoma.

Malignant peripheral nerve sheath tumors (MPNSTs) are rare, but deadly, soft tissue sarcomas that occur more commonly and at a younger age in individuals affected with the neurofibromatosis type 1 (NF1) inherited cancer predisposition syndrome. Currently, the only curative treatment for MPNSTs is surgery, and treatment of advanced disease is limited to cytotoxic chemotherapy with generally little effect on overall survival. Moreover, there is a pressing need for targeted therapeutic options for MPNSTs arising in individuals with NF1 and in the general population.

Mutations in the *NF1* gene have been reported in both NF1-related and sporadic MPNSTs,¹ such that loss of *NF1* protein (neurofibromin) function leads to increased Ras signaling.^{2–4} Given that the *BRAF* kinase molecule is also a known downstream Ras effector and that *BRAF* mutations are found in numerous nervous system cancers,^{5,6} we hypothesized that *BRAF* mutations may identify a molecularly-distinct subset of MPNST.

To explore this hypothesis, we employed immunohistochemistry using a *BRAF*^{V600E} mutation-specific antibody on formalin-fixed/paraffin-embedded samples representing 62 MPNSTs and reviewed the associated clinical data to determine NF1 status, overall survival, and time to metastasis. Representative images are shown in Fig. 1 (left panels). Confirmatory *BRAF*^{V600E} sequencing was not performed on these tumors, based on our previous experience revealing complete concordance between sequencing and immunohistochemistry results in ganglioglioma⁷ as well as in other laboratories using different tumor types.^{8,9}

First, we found that individuals with NF1 developed MPNSTs ~10–15 years earlier than their sporadic counterparts ($P < .003$; Fig. 1; right panel), which was similar to previous reports.^{10–12} Second, *BRAF*^{V600E} mutation was observed in 20% (5/25) of sporadic MPNSTs. In these *BRAF*^{V600E}-positive MPNSTs, 90%–100% of the tumor cells were *BRAF*^{V600E}-immunoreactive, suggesting that this mutation could be a primary driver of malignancy and not merely a mutation found in a subset of tumor cells. The prevalence of *BRAF*^{V600E} mutation in our series of 62 MPNSTs was slightly higher than previously reported in a smaller series of tumors (1 of 13 sporadic MPNSTs¹³) and was likewise higher than observed in

sporadic glioblastoma.¹⁴ In this regard, analysis of available The Cancer Genome Atlas (TCGA) data using the C-BIO website application (<http://cbio.mskcc.org>) revealed that *BRAF*^{V600E} mutations predominated in thyroid cancer (182/323 tumors; ~56%) and melanoma (81/228 tumors; ~36%) but were far less common in all other cancers examined (<9%). Interestingly, melanocytes and Schwann cells have neural crest origins, suggesting a susceptible cell of origin for *BRAF* mutation in the genesis of these cancers. Third, *BRAF*^{V600E} mutation was not observed in benign neurofibromas (0/11 tumors), implicating *BRAF* mutation in malignant progression rather than in neurofibroma tumorigenesis. Fourth, only one NF1-associated MPNST harbored a *BRAF*^{V600E} mutation (1/37; 2.7%). There was a statistically significant difference in the percentage of *BRAF*^{V600E} mutations in NF1-associated MPNSTs relative to their sporadic counterparts ($P = .035$). While these molecular events might be considered mutually exclusive, *BRAF* molecular alterations have also been reported in another NF1-associated nervous system tumor (pilocytic astrocytoma).¹⁵ Fifth, *BRAF*^{V600E} mutation does not appear to confer any statistically significant differences in overall patient survival or time to metastasis when patients were censored by either date of death or date of last follow-up. This may reflect the small sample size, the rarity of these tumors, and the fact that nearly one-third of the patients were lost to follow-up (and assumed to be living as they could not be found in the Social Security Death Index). While the prognostic value has yet to be established, this study identifies *BRAF* mutation as a significant molecular alteration in MPNST, potentially offering another therapeutic target for a deadly cancer with no currently efficacious treatment options.

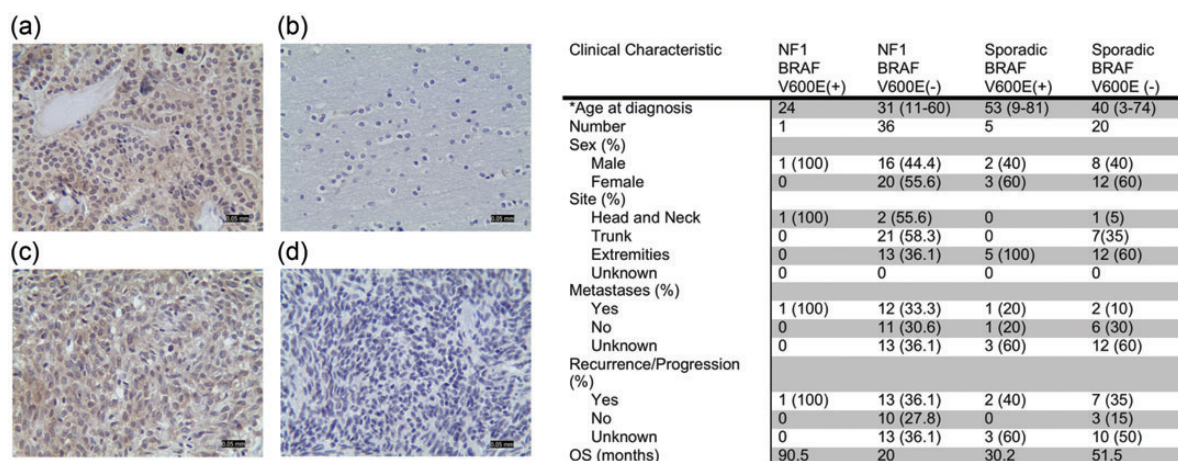
Methods

Cases with the diagnosis of MPNST were collected from UCSF (1990–2012) and Washington University (1990–2005). Clinical data were retrieved from electronic medical records, and all cases with available material were reviewed by at least one of two experienced neuropathologists for diagnostic confirmation (S.D., A.P.). Nerve sheath tumors with equivocal features of malignancy were excluded. The most representative tumor blocks were

Received 12 October 2013; accepted 2 December 2013, Advance Access publication 22 December 2013

© The Author(s) 2013. Published by Oxford University Press on behalf of the Society for Neuro-Oncology. All rights reserved.

For permissions, please e-mail: journals.permissions@oup.com.



* p=0.003 for age between all NF1 patients versus all sporadic patients

Fig. 1. Left. $BRAF^{V600E}$ immunostaining in (a) human papillary thyroid cancer, positive control; (b) normal human brain, negative control; (c) one representative $BRAF^{V600E}$ -immunopositive MPNST; and (d) one representative $BRAF^{V600E}$ -immunonegative MPNST. Magnification, 200x; Scale bar, 50 microns. Right. summary of clinical, demographic, and survival data for individuals with MPNST.

selected in each case, and microarray blocks were constructed containing two cores of each cancer tissue from the most representative area of tumors, away from necrosis.

Immunohistochemistry was performed, as previously described using a $BRAF^{V600E}$ -specific antibody (clone VE1; Spring Bioscience; 1:100 dilution).⁷ Tumors were scored as immunopositive if they displayed strong and diffuse cytoplasmic expression. Overall survival data were generated by Kaplan-Meier analysis and log-rank test using Graphpad Prism Version 5.03. A Student's *t* test was employed to determine the age differences between NF1 and sporadic MPNST patients. A Fisher exact test was employed to determine the difference in frequency of the $BRAF^{V600E}$ mutation in NF1 versus sporadic MPNST patients.

Angela C. Hirbe, Melike Pekmezci, Sonika Dahiya, Anthony J. Apicelli, Brian A. Van Tine, Arie Perry, and David H. Gutmann

Division of Medical Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri (A.C.H., B.V.T.); Department of Pathology and Immunology, UCSF School of Medicine, San Francisco, California (M.P., A.P.); Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri (S.D.); Department of Radiation Oncology, Washington University School of Medicine, St. Louis, Missouri (A.J.A.); Department of Neurology, Washington University School of Medicine, St. Louis, Missouri (D.H.G.)

Funding

None declared.

Conflict of interest statement. None declared.

References

- Gupta G, Mammis A, Maniker A. Malignant peripheral nerve sheath tumors. *Neurosurg Clin N Am.* 2008;19:533–543.
- Williams VC, Lucas J, Babcock MA, et al. Neurofibromatosis type 1 revisited. *Pediatrics.* 2009;123:124–133.
- Basu TN, Gutmann DH, Fletcher JA, et al. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. *Nature.* 1992;356:713–715.
- DeClue JE, Papageorge AG, Fletcher JA, et al. Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. *Cell.* 1992;69:265–273.
- Dienstmann R, Tabernero J. BRAF as a target for cancer therapy. *Anticancer Agents Med Chem.* 2011;11:285–295.
- Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature.* 2002;417:949–954.
- Dahiya S, Haydon DH, Alvarado D, et al. BRAF(V600E) mutation is a negative prognosticator in pediatric ganglioglioma. *Acta Neuropathol.* 2013;125:901–910.
- Colomba E, Helias-Rodzewicz Z, Von Deimling A, et al. Detection of BRAF p.V600E mutations in melanomas: comparison of four methods argues for sequential use of immunohistochemistry and pyrosequencing. *J Mol Diagn.* 2013;15:94–100.
- Capper D, Preusser M, Habel A, et al. Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathol.* 2011;122:11–19.
- Ducatman BS, Scheithauer BW, Piepgras DG, et al. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. *Cancer.* 1986;57:2006–2021.
- Ferner RE, Gutmann DH. International consensus statement on malignant peripheral nerve sheath tumors in neurofibromatosis. *Cancer Res.* 2002;62:1573–1577.
- Evans DG, Baser ME, McGaughan J, et al. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. *J Med Genet.* 2002;39:311–314.
- Serrano C, Simonetti S, Hernandez-Losa J, et al. BRAF V600E and KRAS G12S mutations in peripheral nerve sheath tumours. *Histopathology.* 2013;62:499–504.
- Dahiya S, Haydon DH, Leonard JR, et al. BRAF-V600E mutation in pediatric and adult glioblastoma. *Neuro Oncol.* 2014;16:318–319.
- Rodriguez FJ, Ligon AH, Horkayne-Szakaly I, et al. BRAF duplications and MAPK pathway activation are frequent in gliomas of the optic nerve proper. *J Neuropathol Exp Neurol.* 2012;71:789–794.