

## Brief report

High prevalence of *BRAF V600E* mutations in Erdheim-Chester disease but not in other non-Langerhans cell histiocytoses

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**Histiocytoses are rare disorders of unknown origin with highly heterogeneous prognosis. *BRAF* mutations have been observed in Langerhans cell histiocytosis (LCH). We investigated the frequency of *BRAF* mutations in several types of histiocytoses. Histology from 127 patients with histiocytoses were reviewed. Detection of *BRAF*<sup>V600E</sup> mutations was performed by pyrosequencing of DNA extracted from**

**paraffin embedded samples. Diagnoses of Erdheim-Chester disease (ECD), LCH, Rosai-Dorfman disease, juvenile xanthogranuloma, histiocytic sarcoma, xanthoma disseminatum, interdigitating dendritic cell sarcoma, and necrobiotic xanthogranuloma were performed in 46, 39, 23, 12, 3, 2, 1, and 1 patients, respectively. *BRAF* status was obtained in 93 cases. *BRAF*<sup>V600E</sup> mutations were detected in 13 of 24 (54%) ECD, 11 of**

**29 (38%) LCH, and none of the other histiocytoses. Four patients with ECD died of disease. The high frequency of *BRAF*<sup>V600E</sup> in LCH and ECD suggests a common origin of these diseases. Treatment with vemurafenib should be investigated in patients with malignant *BRAF*<sup>V600E</sup> histiocytosis. (*Blood*. 2012;120(13):2700-2703)**

## Introduction

Histiocytoses encompass a wide range of rare and heterogeneous diseases characterized by the accumulation and/or the proliferation of histiocytes within various tissues. Since 1987, the classification for histiocytoses relies on the Langerhans and non-Langerhans cell origin.<sup>1</sup> The distinction was based on the presence of Birbeck granules and, more recently, on CD1a expression on formalin-fixed, paraffin-embedded samples.<sup>2,3</sup> The latest World Health Organization classifications has individualized Langerhans cell histiocytosis (LCH), Rosai-Dorfman disease, disseminated juvenile xanthogranuloma (JXG; synonym of Erdheim-Chester disease [ECD] and xanthoma disseminatum), interdigitating dendritic cell sarcoma, and histiocytic sarcomas.<sup>4,5</sup> Diagnosis of these conditions is mainly based on histopathology and corresponds to highly variable clinical syndromes, whose prognoses range from benign self-healing to highly malignant.

The RAS-RAF-MEK-ERK pathway is a cellular signaling pathway, which plays a major role in tumors.<sup>6</sup> *BRAF*<sup>V600E</sup> mutation,

an activating mutation of the proto-oncogene *BRAF*, is present in several human tumors.<sup>7</sup> This mutation results in an activation of RAS-ERK pathway, independently of RAS activation. Inhibition of *BRAF* activation by vemurafenib improves survival of patients with *BRAF*<sup>V600E</sup> metastatic melanomas.<sup>8</sup> *BRAF*<sup>V600E</sup> mutations have been detected in patients with LCH.<sup>9,10</sup> We thus investigated whether this mutation was present in other subsets of histiocytoses.

## Methods

## Patients and samples

Patients were retrieved from the databases of the French Registry of Histiocytoses, and of 3 teaching hospitals (Pitié-Salpêtrière, Necker-Enfants Malades, and Ambroise Paré). Thirty-nine and 12 consecutive cases of LCH and cutaneous JXG were included, respectively. For other histiocytoses, all cases available were included. Study was approved by the

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ethic committee Ile de France III (#2011-A00447-34) and conducted in accordance with the Declaration of Helsinki. Clinical follow-up of patients with LCH and ECD was prospectively recorded according to previously described methodologies.<sup>11,12</sup>

All tissue samples were reviewed by at least 4 independent pathologists, trained in the field of histiocytoses (N.B., D.C., F.C., and J.-F.E.), and classified according to the World Health Organization classification.<sup>4,5</sup> Immunohistochemistry was performed with CD1a (Beckman-Coulter), CD68 (Dako Denmark), CD163 (Thermo Scientific), CD205 (Langerin; Novocastra), S100 protein (Dako Denmark), and factor XIIIa (Novocastra) when necessary. For ECD cases and a few other difficult cases, the diagnoses were achieved taking into account the clinical and radiologic aspects of the disease.<sup>10</sup> Patients with both LCH and ECD features were excluded.

### Detection of BRAF<sup>V600</sup> mutations

Tumor DNA was extracted from formalin-fixed, paraffin-embedded tissues as described.<sup>13</sup> Four serial sections were performed for each sample. The first section was used for histology and selection of the areas of highest histiocyte density and the 3 others for dissection at  $\times 10$  magnification. When histiocyte infiltration was lower than 20%, sensitivity was considered as insufficient for *BRAF* heterozygous mutation detection. Detection of *BRAF* V600 mutations was performed by pyrosequencing with PyroMark Q24 (QIAGEN).

### Immunohistochemistry with BRAF<sup>V600E</sup>

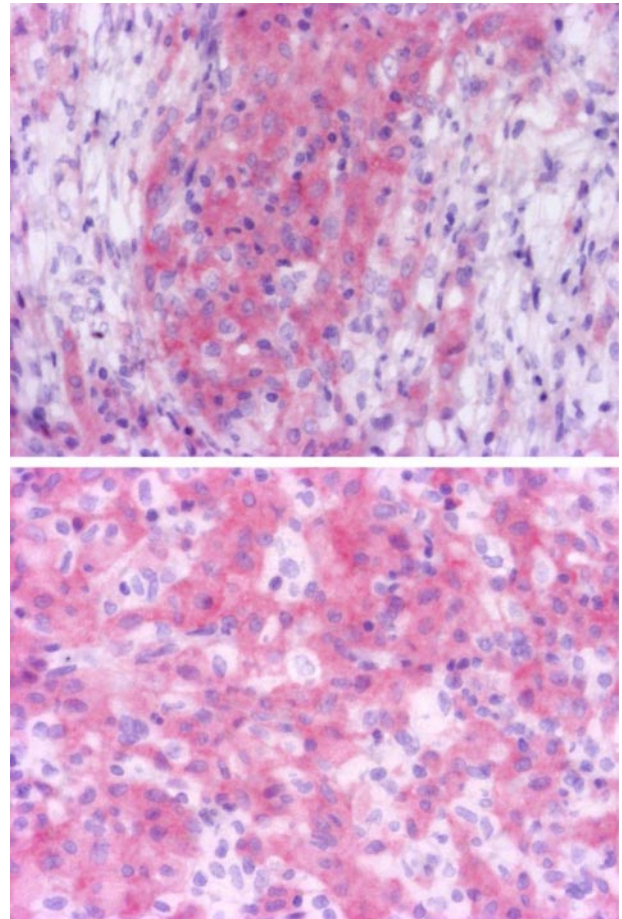
Mouse monoclonal antibody VE1 was shown to be specific of *BRAF*<sup>V600E</sup> mutation.<sup>14</sup> Stainings were performed with Bond-Max (Leica Biosystems). Antigen retrieval was performed during 60 mn at 96°C in pH9 buffer Bond Epitope Retrieval Solution 2 (Leica Biosystems). VE1 hybridoma supernatant was diluted one-third and incubated at 37°C for 32 mn. Staining was revealed with Bond polymer refine red detection kit (Leica Biosystems). Staining was scored according to previously published criteria<sup>14</sup> by a pathologist who was not aware of genetic results.

### Statistical analysis

Differences between groups of patients were tested using Mann-Whitney or Kruskal-Wallis tests for continuous data, and Fisher exact or  $\chi^2$  tests for categorical data. These analyses were followed by Bonferroni correction for multiple testing, when needed. All *P* values were 2-tailed, and statistical significance was defined as *P* < .05. Statistical analyses were performed using JMP8 (SAS Institute).

## Results and discussion

Samples of the 127 patients mainly originated from bone (*n* = 29), skin (*n* = 27), lymph node (*n* = 18), perirenal infiltration (*n* = 12), and lung (*n* = 9; supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Diagnosis of ECD (*n* = 46), LCH (*n* = 39), Rosai-Dorfman disease (*n* = 23), JXG (*n* = 12), histiocytic sarcomas (*n* = 3), xanthoma disseminatum (*n* = 2), interdigitating dendritic cell sarcoma (*n* = 1), and NXG (*n* = 1) were established. In all ECD cases, histiocytes were CD68<sup>+</sup>, CD1a<sup>-</sup>, S100<sup>-</sup>. Histiocytes were positive for factor XIII in 5 of 9 of ECD cases. All LCH cases contained CD1a<sup>+</sup>, S100<sup>+</sup> histiocytes. *BRAF* mutational status was obtained by pyrosequencing in 93 (73%) of available cases. Failure to determine *BRAF* status was more frequent in bone and perirenal fat than in other sites of biopsies (49% vs 17%, *P* = .0005; supplemental Tables 1-3).



**Figure 1. Identification of cells with BRAF mutation.** Immunohistochemistry with *BRAF*<sup>V600E</sup> specific antibody VE1 disclosed cytoplasmic staining of histiocytes, whereas lymphocytes and fibroblasts were negative (top, original magnification  $\times 100$ ). Some histiocytes were not stained and correspond to reactive macrophages (bottom, original magnification  $\times 200$ ). Microphotographs were performed with a microscope BX41, eyepiece (WH 10 $\times$ /22), objectives Olympus UPlanFI 10 $\times$  and Olympus UPlanFI 20 $\times$  (Olympus), Camera Axopcam ICc1, and AxioVision Rel Version 4.8 software (Carl Zeiss).

A *BRAF*<sup>V600E</sup> mutation was detected by pyrosequencing in 13 of 24 (54%) patients with ECD. For 2 patients, 2 different samples were available and both harbored the *BRAF*<sup>V600E</sup> mutation. The exact frequency of *BRAF* mutations in ECD patients remains to be confirmed in other series, as the present series may be biased by the fact that only 52% could be evaluated for *BRAF* status. The pathophysiology of histiocytoses remains to be determined. LCH was shown to be a clonal proliferation by HUMARA<sup>15</sup>; this result was confirmed with other methods. By contrast, there is an ongoing debate as to whether ECD should be considered as a tumor or an abnormal immune response. Indeed, the complex network of cytokines and chemokines associated with ECD underlines an intense systemic Th-1-oriented immune activation.<sup>16</sup> However, ECD was shown to be clonal in 5 patients, with either HUMARA<sup>17</sup> or cytogenetic.<sup>18</sup> The detection in the present study of *BRAF* mutations in 13 other ECD cases confirms that ECD is a clonal proliferation.

To determine which cells were mutated in ECD and further confirm the presence of the *BRAF*<sup>V600E</sup> mutation, we performed confirmatory immunohistochemistry analysis with *BRAF*<sup>V600E</sup> specific antibody on wild-type and mutated ECD samples. We recently confirmed in a series of melanomas that VE1 antibody was highly specific of *BRAF*<sup>V600E</sup> mutation, and shown it was more sensitive

**Table 1. Main clinical characteristics of the 46 patients with ECD according to *BRAF*<sup>V600E</sup> status**

	WT (n = 11)	BRAF V600E (n = 13)	NA (n = 22)	P	
				BRAF V600E versus WT*	Across all 3 categories†
Median age at diagnosis, y (range)	55 (39-81)	55 (37-72)	57 (16-73)	.62	.83
Sex, male/female	9/2	8/5	16/6	.28	.54
<b>Involvement</b>					
CNS, n (%)	2 (18)	6 (46)	11 (50)	.15	.20
Heart, n (%)	4 (36)	7 (54)	10 (45)	.39	.73
Large vessels, n (%)	5 (45)	11 (85)	14 (64)	.04	.13
Exophthalmos, n (%)	3 (27)	7 (54)	7 (32)	.19	.32
Diabetes insipidus, n (%)	2 (18)	3 (23)	7 (32)	.77	.67
Lung, n (%)	4 (36)	4 (31)	10 (45)	.77	.67
Perirenal infiltration, n (%)	3 (27)	7 (54)	13 (59)	.19	.21
Xanthelasma, n (%)	4 (36)	4 (31)	6 (27)	.77	.87
Bone pain, n (%)	5 (45)	7 (54)	11 (50)	.68	.92
Death of disease progression, n (%)	2 (18)	2 (15)	5 (23)	.85	.86

\*P values computed using Mann-Whitney test,  $\chi^2$  test, or Fisher test, as appropriate.

†P values computed using Kruskal-Wallis test or  $\chi^2$  test, as appropriate. None of these P values remains significant after Bonferroni correction for multiple testing.

than Sanger sequencing (E. Colomba, Z.H.-R., A.v.D., C. Marin, N. Terrones, D. Pechaud, S. Surel, J.-F. Côté, F. Peschaud, D. Capper, H. Blons, U. Zimmermann, T. Clerici, P. Saiag, J.-F.E., Detection of *BRAF* p.V600E mutations in melanomas: comparison of four methods argues for sequential use of immunohistochemistry and pyrosequencing, manuscript submitted, August 10, 2012). Seven positive and 6 negative ECD cases identified with pyrosequencing were tested, and immunohistochemistry with VE1 confirmed the *BRAF* status in all cases. Only histiocytes were stained, whereas lymphocytes, fibroblasts, and endothelial cells were negative (Figure 1). Both mononucleated histiocytes and Touton cells were positive, confirming that both mononucleated and multinucleated histiocytes derive from the same tumor progenitor. In some areas, *BRAF*-negative histiocytes were admixed with positive cells (Figure 1), probably corresponding to reactive inflammatory cells. Validation of the detection of *BRAF* mutations with immunohistochemistry for diagnostic use would be helpful for cases with low histiocyte infiltration.

A *BRAF*<sup>V600E</sup> mutation was detected in 11 of 29 (38%) patients with LCH. This frequency was not statistically different from 13 of 24 (54%) that we observed in patients with ECD nor from 35 of 61 (57%) in the Badalian-Very series.<sup>9</sup> No mutations were detected in patients with Rosai-Dorfman disease (n = 23), cutaneous JXG (n = 12), histiocytic sarcomas (n = 3), xanthoma disseminatum (n = 2), interdigitating dendritic cell sarcoma (n = 1), or NXG (n = 1). Thus, ECD and LCH share similar oncogenic pathways, which are distinct from other histiocytoses. Interestingly, associations of ECD and LCH have been reported,<sup>19</sup> suggesting that both proliferations could derive from a common progenitor.

The treatment of ECD and LCH remains a challenge. Although some forms of LCH are benign and self-healing, some patients with ECD and LCH are resistant to several lines of chemotherapies.<sup>12,20</sup> Disease-related death occurred in 6 of 46 ECD and 3 of 39 LCH cases. Within this small series, the clinical characteristics of ECD patients did not appear to depend on *BRAF* status (Table 1); however, this outcome should be checked in a larger series.

Targeted therapies have recently been tested in both conditions<sup>21,22</sup>; however, it has limited efficacy. Prognosis of ECD has been substantially improved by IFN- $\alpha$  therapy, but many refractory forms subsist, especially those with CNS and cardiovascular involvements. Verumafenib, an inhibitor of *BRAF*, was recently approved for treating patients with metastatic melanoma and

*BRAF*<sup>V600</sup> mutations.<sup>8</sup> The poor prognosis of a substantial number of patients with multisystemic ECD and LCH warrants new therapeutic approaches that could involve *BRAF* inhibitors.

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## Authorship

Contribution: J.H., F.C., J.D., Z.A., and J.-F.E. designed research; J.H., F.C., L.A., Z.H.-R., B.H., F.C.-A., D.L., A.L., K.M., D.C., L.G., C.R., M.S., S.C., M.K., M.-C.C., S.F., N.B., Z.A., J.D., and J.-F.E. collected data; L.A. performed statistical analysis; J.H., F.C., Z.A., and J.-F.E. analyzed and interpreted data; A.v.D., F.S., and J.-F.E. performed anti-VE1 immunohistochemical analysis; J.H., F.C., Z.H.-R., and J.-F.E. analyzed data; J.H., F.C., L.A., Z.A., and J.-F.E. wrote the manuscript; and all authors approved the final manuscript.

Conflict-of-interest disclosure: J.-F.E. received honoraria for counseling on diagnosis and/or treatment with *BRAF* inhibitors of patients with melanomas from Roche and Glaxo Smith Kline. The laboratory of J.-F.E. received grants from Roche for organization and external quality control assessment of *BRAF* mutation detection in France. D.C. and A.v.D. applied for a patent on the diagnostic use of *BRAF* V600E mutant-specific antibody VE1. All terms are being managed by the German Cancer Research Center in accordance with its conflict of interest policies. The remaining authors declare no competing financial interests.

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