## MYELOID NEOPLASIA

# Recurrent RAS and PIK3CA mutations in Erdheim-Chester disease

Jean-François Emile,<sup>1,2</sup> Eli L. Diamond,<sup>3</sup> Zofia Hélias-Rodzewicz,<sup>1,2</sup> Fleur Cohen-Aubart,<sup>4,5</sup> Frédéric Charlotte,<sup>5,6</sup> David M. Hyman,<sup>7</sup> Eunhee Kim,<sup>8</sup> Raajit Rampal,<sup>8</sup> Minal Patel,<sup>8</sup> Chezi Ganzel,<sup>9</sup> Shlomzion Aumann,<sup>8</sup> Gladwys Faucher,<sup>1,2</sup> Catherine Le Gall,<sup>1,2</sup> Karen Leroy,<sup>10,11</sup> Magali Colombat,<sup>12</sup> Jean-Emmanuel Kahn,<sup>13</sup> Salim Trad,<sup>14</sup> Philippe Nizard,<sup>15</sup> Jean Donadieu,<sup>1,16</sup> Valérie Taly,<sup>15</sup> Zahir Amoura,<sup>4,5</sup> Omar Abdel-Wahab,<sup>8</sup> and Julien Haroche<sup>4,5</sup>

<sup>1</sup>Unité de Recherche EA 4340, Versailles University, Boulogne, France; <sup>2</sup>Pathology Department, Ambroise Paré Hospital, Assistance Publique - Hôpitaux de Paris, Boulogne, France; <sup>3</sup>Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY; <sup>4</sup>Department of Internal Medicine & French Reference Center for Rare Auto-immune & Systemic Diseases, Hôpital Pitié-Salpêtrière Hospital, Assistance Publique - Hôpitaux de Paris, Paris, France; <sup>5</sup>Pierre and Marie Curie University, Paris, France; <sup>6</sup>Department of Pathology, Hôpital Pitié-Salpêtrière, Assistance Publique - Hôpitaux de Paris, Paris, France; <sup>7</sup>Experimental Therapeutics Unit and <sup>8</sup>Human Oncology and Pathogenesis Program and Leukemia Service, Memorial Sloan-Kettering Cancer Center, New York, NY; <sup>9</sup>Department of Hematology, Shaare Zedek Medical Center, Jerusalem, Israel; <sup>10</sup>Université Paris-Est Créteil, Créteil, France; <sup>11</sup>Department of Pathology, Hospital Henri Mondor, Assistance Publique - Hôpitaux de Paris, Créteil, France; <sup>12</sup>Department of Pathology and <sup>13</sup>Department of Internal Medicine, Foch Hospital, Suresne, France; I<sup>4</sup>Department of Internal Medicine, Ambroise Paré Hospital, Assistance Publique - Hôpitaux de Paris, France; <sup>15</sup>Université Paris Sorbonne Cité, INSERM, Paris, France; and <sup>16</sup>Department of Pediatrics & French Reference Center for Langerhans Cell Histiocytosis, Trousseau Hospital, Assistance Publique - Hôpitaux de Paris, Paris, France;

### **Key Points**

- PIK3CA and NRAS mutations are recurrent in BRAFV600E wild-type ECD patients.
- 57.5% (46/80) of ECD patients have a *BRAF*V600E mutation, and an additional 10.9% and 3.7% have *PIK3CA* and *NRAS* mutations, respectively.

## Introduction

Erdheim-Chester disease (ECD) is a rare histiocytic disorder that is challenging to diagnose and treat. We performed molecular analysis of *BRAF* in the largest cohort of ECD patients studied to date followed by *N/KRAS*, *PIK3CA*, and *AKT1* mutational analysis in *BRAF* wild-type patients. Forty-six of 80 (57.5%) of patients were *BRAFV*600E-mutant. *NRAS* mutations were detected in 3 of 17 ECD *BRAFV*600E wild-type patients. *PIK3CA* mutations (p.E542K, p.E545K, p.A1046T, and p.H1047R) were detected in 7 of 55 patients, 4 of whom also had *BRAF* mutations. Mutant *NRAS* was present in peripheral blood CD14<sup>+</sup> cells, but not lymphoid cells, from an *NRASQ*61R mutant patient. Our results underscore the central role of RAS-RAF-MEK-ERK activation in ECD and identify an important role of activation of RAS-PI3K-AKT signaling in ECD. These results provide a rationale for targeting mutant RAS or PI3K/AKT/mTOR signaling in the subset of ECD patients with *NRAS* or *PIK3CA* mutations. (*Blood*. 2014;124(19):3016-3019)

Erdheim-Chester disease (ECD) is a histiocyte proliferation with frequent multiorgan involvement,<sup>1</sup> and aggressive phenotypes in ECD may lead to death despite treatment.<sup>2</sup> Recently, ECD and the related disorder Langerhans cell histiocytosis (LCH) have been identified to have BRAFV600E mutations in 40% to 70% of patients.<sup>3-6</sup> The discovery of BRAFV600E mutations in ECD and LCH has provided an important therapeutic target, and treatment of BRAFV600E-mutant ECD and LCH patients with vemurafenib has demonstrated dramatic therapeutic efficacy in pilot studies.7 Therefore, accurate identification of BRAFV600E mutations in ECD and LCH is critical. However, the heterogeneous nature of ECD and LCH lesions frequently presents a challenge to the accurate identification of BRAFV600E mutations in lesional tissue.<sup>8</sup> In addition, several groups have noted that a larger proportion of LCH and ECD lesions have activation of ERK signaling than that demonstrated to have the BRAFV600E mutation.<sup>3,8,9</sup> Interestingly, Cangi et al recently identified that 18 of 18 ECD patients had a BRAFV600E mutation in DNA from whole lesional tissue if an

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J.-F.E., E.L.D., O.A.-W., and J.H. contributed equally to this study.

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ultrasensitive methodology was used, suggesting that current data underestimate the true mutational frequency of *BRAFV600E* mutations in ECD.<sup>8</sup> Concurrently, we recently identified an *NRASQ*61R mutation in an ECD patient who was definitively *BRAF*–wild-type.<sup>10</sup>

Given this, we analyzed 80 ECD patients for the *BRAF*V600E mutation followed by interrogation of 25 ECD thought to be *BRAF*-wild-type using a variety of sensitive techniques for repeat *BRAF* mutational analysis. In parallel, we analyzed these samples for *NRAS*, *KRAS*, *PIK3CA*, and *AKT1* mutations.

## Methods

## Patients

Eighty ECD patients were included in this study, approved by the Ethics Committee IIe de France III (#2011-A00447-34) and the Institutional Review

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**Figure 1.** *NRAS* and *PIK3CA* mutations in ECD histiocytes and CD14<sup>+</sup> cells from peripheral blood. (A) Detection of *NRAS* p.Q61 mutation by pyrosequencing (first 2 columns) and confirmation by Sanger sequencing (third column). The lower pyrogram corresponds to an *NRAS*—wild-type ECD case, and the 2 others correspond to patients with *NRAS* mutations. Mutants are detectable with the appearance of a new peak at the first C injection (gray arrows), and size variation of the peak at the first T injection compared with the second C injection (red arrows and dashed line). Pyrosequencing with another sequence of injection (ESGTTACTCAGTCAGCT) was used to further identify the mutations (middle column) as c.181C>A mutations. (B) Detection of *PIK3CA* E545A mutation by Sequenom and Sanger sequencing in an *NRAS/KRAS/BRAF*—wild-type ECD patient. (C) Phosphorylated MEK (pMEK) and ERK (pERK) detected by immunohistochemistry in *BRAF*V600E—wild-type, *NRAS*-mutant ECD. Histiocytes (noted by hematoxylin and eosin and CD68 stain) failed to stain for *BRAF*V600E VE1 monoclonal antibody but had high cytoplasmic expression of pMEK1/2 with 2 different antibodies as well as cytoplasmic and nuclear expression of pERK1/2 (original magnification ×600). (D) Genotyping of CD14<sup>+</sup> monocytes and CD3<sup>+</sup> T cells purified from the peripheral blood of an *NRAS*Q61R-mutant ECD patient with double-FACS sorting reveals the presence of *NRAS* mutation in CD14<sup>+</sup> cells but not in T cells.

Board at Memorial Sloan-Kettering Cancer Center. Informed consent was provided according to the Declaration of Helsinki.

#### **Genetic analyses**

Genomic DNA was extracted from formalin-fixed, paraffin-embedded samples after histologic review and enrichment by macrodissection to  $\geq$ 10% histiocytes. All samples derived from patients before any therapy. Workflow of genetic analysis is depicted in supplemental Figure 1 (available on the *Blood* Web site). Detection of *BRAF*V600 and *NRAS*Q61 mutations was performed by pyrosequencing.<sup>11</sup> *BRAF* status of 41 of the 80 patients was already reported (*BRAF*V600E mutational frequency was 51% in this initial series).<sup>5,6,12</sup> Fifty-eight cases, in which 25 initially did not have BRAF p.V600 mutation detected, were further analyzed for *BRAF* mutations with other methods including multiplex picodroplet digital polymerase chain reaction (PCR)<sup>13</sup> (Raindance Technologies; details in the supplemental Methods). Screening for mutations in other genes was performed with Sequenom mass spectrometric–based genotyping assays as previously described<sup>14</sup> (*NRAS, KRAS, PIK3CA*, and *AKT1* hotspot mutations), and next-generation targeted sequencing analysis for regions of mutations in *BRAF, N/KRAS*, and *PIK3CA* by Illumina MiSeq as described in the supplemental Methods.

Age† (y/Gender)	Principal organs involved by histiocytosis	Tissue biopsy site	% Histiocytes in biopsy	BRAF p.V600	Other genes	Follow up (mo, status at last follow-up)
67/M	Heart, aorta, RPF, pleura	Peritoneum	70	Wild-type	NRAS p. G12D	43, DoD
56/M	Aorta, RPF, pleura, bone	Pleura	70	Wild-type	<i>NRAS</i> p. Q61K	108, AwD
65/M	Aorta, pleura	Pleura	40	Wild-type	NRAS p.Q61R	26, AwD
32/M	Aorta, paraparesis	Paravertebral	10	Wild-type	<i>PIK3CA</i> p.E542K	100, AwD
32/M	CNS, visual loss, bone	Bone	10	Wild-type	<i>PIK3CA</i> p.E545K	Not available
29/M	RPF, bone	Bone	20	Wild-type	<i>РІКЗСА</i> р.Н1047R	50, AwD
62/M	CNS, aorta, bone, sinus	Perirenal	30	p.V600E	<i>PIK3CA</i> p.A1046T	60, AwD
52/M	DI, bone, RPF	Skin	60	p.V600E	<i>PIK3CA</i> p.H1047L	24, AwD
58/M	Lung, CNS, X, aorta	Skin	50	p.V600E	<i>PIK3CA</i> p.H1047L	15, AwD
42/M	E, bone, aorta, RPF	Orbital	60	p.V600E	<i>РІКЗСА</i> р.Н1047L	30, AwD
53/M	CNS, bone	Bone	10	Wild-type	None detected	17, AwD
49/M	RPF, DI, sclerosing cholangitis, sinus	Perirenal	20	Wild-type	None detected	48, AwD
56/M	Lung, bone	Bone	40	Wild-type	None detected	44, AwD
81/F	Aorta, X, skin	Skin	50	Wild-type	None detected	52, AwD
70/M	Aorta, RPF, pleura, skin, arthritis	Skin	30	Wild-type	None detected	18, AwD
65/M	Lung, bone	Bone	40	Wild-type	None detected	72, AwD
55/M	Heart, RPF, lung, bone, X	Skin	70	Wild-type	None detected	33, AwD
54/M	Mesenteric	Mesentery	40	Wild-type	None detected	27, AwD
39/M	CNS, E, skin	Skin	90	Wild-type	None detected	134, AwD
42/F	Not available	Bone	40	Wild-type	None detected	Not available
30/M	Heart, aorta, paraparesis, liver	Paravertebral	30	Wild-type	None detected	20, AwD

## Table 1. Characteristics of NRAS- and PIK3CA-mutant ECD patients and those not identified as having BRAF, NRAS, or PIK3CA mutations\*

AwD, alive with disease; CNS, central nervous system; DI, diabetes insipidus; DoD, dead of disease; E, exophthalmos; RPF, retroperitoneal fibrosis; X, xanthelasma. \*The 42 ECD patients with *BRAF* p.V600E mutations, but without *PIK3CA* mutation, are not included in this table. For 17 other *BRAF*V600E/*NRAS*-wild-type ECD patients, *PIK3CA*, *KRAS*, and *AKT1* hotspot mutations were not investigated, because biopsies and tumor DNA samples were exhausted.

†Age at time of diagnosis.

#### Immunohistochemistry

Details of immunohistochemical methods and analyses are provided in the supplemental Methods.

## **Results and discussion**

A *BRAF* c.1799T>A, p.V600E mutation was initially detected in 38 of 80 (47.5%) ECD patients using direct pyrosequencing of DNA obtained from lesional tissue enriched for histiocytes. Of note, there was no correlation between *BRAF*V600E allele burden from pyrosequencing and percent of histiocytes in these samples. As was mentioned earlier, recent work from our group identified a single ECD patient as having an *NRASQ*61R mutation without any evidence of *BRAF*V600E mutation based on pyrosequencing, Sanger sequencing, or locked nucleic acid PCR followed by pyrosequencing.<sup>10</sup> Given this result, we screened with pyrosequencing all patients for mutations in *NRASQ*61 and detected 2 of 80 *NRASQ*61-mutated cases (Figure 1 and Table 1).

Although a *BRAF*V600E mutational frequency of 47.5% is roughly consistent with the frequency of *BRAF*V600E mutation demonstrated in ECD previously,<sup>5,6</sup> given the results of Cangi et al,<sup>8</sup> we performed further detailed analyses for *BRAF*V600E mutations in the subset of ECD patients thought to be *BRAF*–wildtype based on direct pyrosequencing analysis. Using both allelespecific real-time PCR, next-generation sequencing of lesional DNA (mean depth of  $66\times$ ), and multiplex picodroplet digital PCR in a subset of 25 samples without initial *BRAF*V600E mutation detection, we were able to identify an additional 8 patients as being *BRAF*V600E-mutant, increasing the frequency of *BRAF*V600E mutations in ECD to 57.5% (46/80) (supplemental Figure 1). We cannot exclude the possibility that the frequency of *BRAF*V600E mutations found here (46/80) differs from that of Cangi et al  $(18/18)^8$  because of technical differences in modalities used for *BRAF*V600E mutation detection between the 2 studies.

Because both the PI3K/AKT/mTOR and RAF/MEK/ERK pathways are downstream effectors of RAS signaling, we also screened for mutations in commonly mutated genes in the PI3K/AKT pathway using a combination of mass spectrometric–based genotyping as well as next-generation sequencing analysis of frequently mutated regions of *NRAS*, *KRAS*, *PIK3CA*, and *AKT1*. We identified a third patient as having an *NRAS* mutation (Table 1). Immunohistochemical analysis for phosphorylated MEK1/2 (pMEK1/2) and ERK1/2 (pERK1/2) in *NRAS*-mutant ECD revealed that histiocytes were clearly positive for pMEK1/2 Ser217 and Ser217/221 as well as pERK1/2 Thr202/Tyr204 (Figure 1C). Positive staining with pERK1/2 was detected in 11 cases without *BRAF*, *NRAS*, or *PIK3CA* mutations as well as 3 *BRAF*V600E and 3 *NRAS* mutant samples (supplemental Figure 2).

Analysis of regions of recurrent mutations in *PIK3CA* in a subset of 30 *BRAF*V600E-mutant ECD revealed 7 of 58 ECD patients overall with *PIK3CA* mutations, of whom 4 were *BRAF*-mutated (Figure 1 and Table 1). This finding is consistent with data demonstrating a frequent overlap of *PIK3CA* mutations with mutations in the mitogen-activated protein kinase pathway.<sup>14</sup>

The cell-of-origin for histiocytic disorders including LCH and ECD has been long debated, and recent work from both Berres et al<sup>4</sup> and Cangi et al<sup>8</sup> demonstrated the presence of *BRAF*V600E in peripheral blood CD14<sup>+</sup> cells in a proportion of *BRAF*V600E-mutant LCH and ECD patients, respectively. Genetic analysis of CD14<sup>+</sup> cells purified by double fluorescence-activated cell sorting (FACS) of peripheral blood mononuclear cells from an *NRAS*Q61R-mutant ECD patient clearly revealed the presence of the *NRAS* mutation in CD14<sup>+</sup> cells but not in CD3<sup>+</sup> cells (Figure 1D). These

data further suggest that the histiocytic proliferations in ECD are derived from genetically aberrant circulating myeloid hematopoietic cells.

The discovery of recurrent BRAFV600E mutations in ECD as well as the consistent activation of ERK regardless of BRAF mutational status confirms the central role of RAF/MEK/ERK activation in ECD. The identification of recurrent NRAS and PIK3CA mutations in ECD further affirms activation of both the PI3K-AKT and RAF/MEK/ERK pathways in a proportion of ECD patients. Although larger series of patients are necessary to determine whether the clinical characteristics of NRAS- or PIK3CA-mutated ECD patients differ from BRAF-mutated or BRAF/NRAS/PIK3CA-wildtype ECD patients, several of the NRAS- and PIK3CA-mutant patients here had multiorgan disease requiring treatment (Table 1). We suspect that ECD patients with RAS mutations may benefit from targeted anti-MEK therapy, as have benefitted *RAS* mutant metastatic melanoma patients.<sup>15-17</sup> Likewise, therapeutic targeting of a PI3K/AKT/mTOR pathway may be important for BRAFwild-type/PIK3CA-mutant ECD patients. Although pharmacologic targeting of this pathway has been pursued for advanced cancer patients<sup>14,18,19</sup> and LCH,<sup>20</sup> this treatment approach has only recently been initiated in ECD with a study of sirolimus for ECD patients (ACTRN12613001321730).<sup>21</sup> Correlation of clinical response to PI3KCA mutational status may be critical to interpreting results of this ongoing study and in future studies targeting this pathway in ECD.

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### Authorship

Contribution: J.-F.E., E.L.D., Z.H.-R., F.C.-A., O.A.-W., and J.H. designed the study, collected the data, contributed to data interpretation, wrote the manuscript, and approved the manuscript; and F.C., D.M.H., E.K., R.R., M.P., S.A., C.G., Z.A., G.F., C.L.G., K.L., M.C., J.-E.K., S.T., P.N., J.D., and V.T. collected the data, contributed to data interpretation, and approved the manuscript.

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Correspondence: Jean-François Emile, EA4340 & Pathology Department, Ambroise Paré Hospital, 9 Av Ch de Gaulle, F-92104 Boulogne, France; e-mail: jean-francois.emile@uvsq.fr; and Omar Abdel-Wahab, Human Oncology and Pathogenesis Program and Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065; e-mail: abdelwao@mskcc.org.