BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy

Sébastien Héritier, Jean-François Emile, Mohamed-Aziz Barkaoui, Caroline Thomas, Sylvie Fraitag, Sabah Boujemaa, Florence Renaud, Anne Moreau, Michel Peuchmaur, Catherine Chassagne-Clémente, Frédérique Djouad, Valérie Rigaou, Despina Moschou, Anne Lambilliotte, François Mazinge, Kamila Kebaili, Jean Miron, Eric Jezierski, Geneviève Plat, Nathalie Aladjidi, Alina Ferster, Hélène Raguennet, Claire Galambrun, Laurence Brugières, Guy Leverger, Ludovic Mamdy, Catherine Paillard, Anne Deville, Corinne Armiari-Alla, Anne Lutun, Marion Gillibert-Yvert, Jean-Louis Stephan, Fleur Cohen-Aubart, Julien Haroche, Isabelle Pellier, Frédéric Millot, Brigitte Lescoeur, Virginie Gandemer, Christine Bodemer, Roger Lacave, Zofia Hélias-Rodzewicz, Valérie Taly, Frédéric Geissmann, and Jean Donadieu

ABSTRACT

Purpose
Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasia with a broad spectrum of clinical manifestations and outcomes in children. The somatic BRAFV600E mutation occurs frequently, but clinical significance remains to be determined.

Patients and Methods
BRAFV600E mutation was investigated in a French LCH cohort. We analyzed associations between mutation status and clinical presentation, extent of disease, reactivation rate, response to therapy, and long-term permanent sequelae.

Results
Among 315 patients with successfully determined BRAF status, 173 (54.6%) carried a BRAFV600E mutation. Patients with BRAFV600E manifested more severe disease than did those with wild-type BRAF. Patients with BRAFV600E comprised 87.8% of patients (43 of 49) with multisystem LCH with risk organ involvement (liver, spleen, hematology), 68.6% of patients (35 of 51) with multisystem LCH without risk organ involvement, 43.9% of patients (86 of 196) with single-system LCH, and 42.1% of patients (8 of 19) with lung-involved LCH (P < .001). BRAFV600E mutation was also associated with organ involvement that could lead to permanent, irreversible damage, such as neurologic (75%) and pituitary (72.9%) injuries. Compared with patients with wild-type BRAF, patients with BRAFV600E more commonly displayed resistance to combined vinblastine and corticosteroid therapy (21.9% v 3.3%; P = .001), showed a higher reactivation rate (5-year reactivation rate, 42.8% v 28.1%; P = .006), and had more permanent, long-term consequences from disease or treatment (27.9% v 12.6%; P = .001).

Conclusion
In children with LCH, BRAFV600E mutation was associated with high-risk features, permanent injury, and poor short-term response to chemotherapy. Further population-based studies should be undertaken to confirm our observations and to assess the impact of BRAF inhibitors for this subgroup of patients who may benefit from targeted therapy.

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INTRODUCTION

Langerhans cell histiocytosis (LCH) is the most common type of histiocytosis and is characterized by inflammatory lesions with abundant CD1a+/CD207+ histiocytes, which provoke the destruction of affected tissues. This disease most commonly affects children.1,2

Clinical behavior of LCH is remarkably heterogeneous; some cases are limited, indolent, and self-regressive, whereas others recur sequentially, are refractory to conventional therapy, and exhibit systemic and sometimes life-threatening multiorgan involvement. The severe clinical form of the disease principally affects young children (age < 2 years) and tends to involve risk organs (RO), including the hematopoietic system, spleen,
and/or the liver. Despite a low mortality rate, long term, irreversible adverse effects are common. In particular, endocrine dysfunction, secondary to pituitary involvement, and neurodegenerative disease are reported in approximately 20% and 5% of cases, respectively. Currently, the molecular mechanisms that underlie these different LCH subtypes remain poorly understood.

Since 2010, LCH has been known to harbor the \( \text{BRAF}^{V600E} \) activating mutation in 38% to 64% of all cases. Experiments conducted in a mouse model have suggested that this mutation may be mitogenic for dendritic cells. This hypothesis was corroborated when a BRAF inhibitor demonstrated efficacy in LCH; however, to date, the frequency of this mutation has only been assessed in small, unrepresentative patient samples.

The current study includes a cohort of children with LCH who were enrolled in the national French registry. We conducted careful analyses of this patient cohort to determine correlations between \( \text{BRAF}^{V600E} \) mutation status and clinical manifestations of LCH in their entirety.

### Patients and Sample Collection

Of 1,747 patients with childhood LCH (age < 18 years) who were included in the French LCH registry (from 1983 to 2015), 399 patients had biopsy samples available and were contacted to participate in this study (Fig 1). Some biopsies were unavailable as a result of the destruction of samples after 10 years of banking, and some samples had damaged DNA because of preservation with Bouin’s fixation. This study was approved by the Ethics Committee, Ile de France III (#2011-A00447–34) and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. Comprehensive descriptions of the experimental plan, sample size, and study organization are given in the Appendix (online only).

Demographic data, type of treatment, clinical characteristics, and extent of disease were recorded according to classifications established by the Histiocyte Society. Patients were classified according to the Histiocyte Society LCH IV guidelines, which consider the number of organs—systems—involved, lung involvement, and risk organ (RO) involvement.

### Identification of the \( \text{BRAF}^{V600E} \) Mutation

Child patients with parents who were included in the French cohort and who had an available biopsy sample were contacted. Signed informed consent was obtained for 399 patients. For the majority of patients (76%), \( \text{BRAF} \) status was obtained in an ISO 15189-certified laboratory. For 306 patients, \( \text{BRAF}^{V600E} \) status was successfully determined with sequencing analyses. In brief, macro dissection was performed to obtain an infiltrate of CD207+ histiocyte cells, which comprised > 20% of the cell population. \( \text{BRAF}^{V600E} \) mutation was detected by performance of pyrosequencing with PyroMark Q24 (n = 261; Qiagen, Valencia, CA) or by performance of real-time polymerase chain reaction (n = 16; LightCycler 480; Roche, Basel, Switzerland). When histiocytes were a minor component of the infiltrate of CD207+ cells, multiplex pyrosequencing analyses were conducted.
infiltrate, that is, < 20% of the cell population, and sequencing produced a negative result, or when histiocyte infiltration dipped beneath 10%, we performed a droplet digital polymerase chain reaction assay (n = 29) with a Raindrop system (Raindance Technologies, Billerica, CA). In nine patients, immunohistochemistry was performed by staining histiocytes with the mouse monoclonal antibody VEL. Sixteen patients had been previously reported by Satoh et al. We failed to determine BRAF status in 83 patients (20.8%) either because of a small sample with a low percentage of CD1a+ histiocytes (n = 29) or a failure in DNA amplification (n = 54). Finally, the patient with BRAFV600E mutation previously reported was excluded from the present analysis.

Statistical Analyses

Differences between groups were tested with the Mann-Whitney U test for quantitative variables and with Fisher’s exact test for qualitative variables. For statistical analysis, threshold significance was .01, and for univariable analyses of LCH presentation according to BRAF status—because multitests were performed—P < .002 was considered statistically significant (Bonferroni correction). Multivariable binary logistic regression analyses were performed to calculate odds ratios (ORs) and 95% CIs to identify significant features associated with BRAF mutational status. End points for survival analyses were any type of reactivation. Survival analyses included the interval between diagnosis and an event (reactivation or death) or the last examination. Survival rates were estimated by using the Kaplan-Meier method, and subgroups were compared with the log-rank test. All statistical analyses were performed with STATA 13 software (STATA, College Station, TX; Computing Resource Center, Santa Monica, CA). Cutoff date for these analyses was October 30, 2015. Eight patients received targeted therapy with a BRAF inhibitor, and date of last follow-up was censored on the day the first dose was administered.

RESULTS

BRAF somatic status was obtained for 315 children who were diagnosed with LCH. This cohort comprised 167 (53%) boys and 148 (47%) girls. Patient clinical characteristics (Table 1) were comparable between the study cohort and 1,431 children who were not investigated for BRAF status but were included in the LCH registry from 1983 to 2015; however, these groups had different follow-up durations. The study cohort had shorter a follow-up period (range, 0 to 17.9 years). The 315 patients were classified as follows: 196 (62.2%) patients with SS LCH, 51 (16.2%) with MS RO− LCH, 19 (6.0%) with Lung+ LCH, and 49 (15.6%) with MS RO+ LCH. BRAF was mutated in 172 patients (54.6%) with LCH.

BRAF Status and Clinical Extent of Disease

BRAF status of patients with LCH was related to patient characteristics, disease features, and extent of disease (Fig 2A). At diagnosis, patients with mutant BRAF LCH were typically younger than patients with wild-type BRAF (median age, 2.5 and 3.7 years, respectively; P = .01). Among patients with mutant BRAF, multisystem disease was over-represented, particularly disease with RO involvement. BRAFV600E mutation was found in 87.8% of patients with MS RO+ LCH, 68.6% of patients with MS RO− LCH, 43.9% of patients with SS RO− LCH, and 42.1% of patients with Lung+ LCH (P < .001). Among patients with LCH that involved ROs, BRAFV600E mutation was identified in 88.9% of patients with spleen involvement (P < .001), 89.2% of patients with liver involvement (P < .001), and 89.7% of patients with hematologic system involvement (P < .001). BRAFV600E mutation was apparent in 75% of patients with LCHs that involved the CNS (P = .05) and 72.9% of patients with LCH with pituitary gland involvement (P = .007). BRAF status was not correlated with sex or with involvement of lymph nodes, thymus, lung, or bone. In addition, BRAFV600E mutation was not significantly correlated with localized or multifocal bone involvement (52.6% and 53.4%, respectively; P = .81; Fig 2B). In contrast, skin involvement was associated with BRAFV600E (77.0%; P < .001; Fig 2A); however, few infants presented with features of localized, solitary skin SS LCH (n = 6), a rare presentation formerly called Langerhans cell histiocytoma.

Mean DAS, measured at the maximum extent of LCH disease, was higher in patients with mutant BRAF than in those with wild-type BRAF (means, 3.6 and 1.4, respectively; P < .001). Among patients with BRAFV600E, DAS values were high (DAS > 6) in 18.6%, intermediate (DAS, 3 to 6) in 14.5%, and low (DAS < 3) in 60.8%. The 315 patients were classified as follows: 196 (62.2%) patients with SS LCH, 51 (16.2%) with MS RO− LCH, 19 (6.0%) with Lung+ LCH, and 49 (15.6%) with MS RO+ LCH. BRAF was mutated in 172 patients (54.6%) with LCH.

Table 1. Characteristics of Patients in the Studied Cohort (n = 315) Compared With Patients Not Investigated but Included in the French LCH Registry From 1983 to 2015 (n = 1,431)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients Studied for BRAF (n = 315)</th>
<th>Patients Not Studied for BRAF (n = 1,431)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>.23</td>
</tr>
<tr>
<td>Male</td>
<td>53.0</td>
<td>56.8</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>47.0</td>
<td>43.2</td>
<td></td>
</tr>
<tr>
<td>Median age at diagnosis, years</td>
<td>3.2</td>
<td>3.3</td>
<td>.90</td>
</tr>
<tr>
<td>HS classification</td>
<td></td>
<td></td>
<td>.35</td>
</tr>
<tr>
<td>SS LCH</td>
<td>62.2</td>
<td>61.8</td>
<td></td>
</tr>
<tr>
<td>MS RO− LCH</td>
<td>16.2</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Lung+ LCH</td>
<td>6.0</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>MS RO+ LCH</td>
<td>15.6</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>81.6</td>
<td>83.2</td>
<td>.52</td>
</tr>
<tr>
<td>Skin</td>
<td>35.9</td>
<td>36.4</td>
<td>.90</td>
</tr>
<tr>
<td>Pharytary</td>
<td>15.2</td>
<td>14.4</td>
<td>.72</td>
</tr>
<tr>
<td>CNS</td>
<td>7.0</td>
<td>4.5</td>
<td>.09</td>
</tr>
<tr>
<td>Liver</td>
<td>11.8</td>
<td>10.3</td>
<td>.48</td>
</tr>
<tr>
<td>Hematologic</td>
<td>12.4</td>
<td>10.4</td>
<td>.31</td>
</tr>
<tr>
<td>Spleen</td>
<td>11.4</td>
<td>8.8</td>
<td>.16</td>
</tr>
<tr>
<td>Lung</td>
<td>11.8</td>
<td>10.3</td>
<td>.42</td>
</tr>
<tr>
<td>Lymph node</td>
<td>8.6</td>
<td>11.3</td>
<td>.16</td>
</tr>
<tr>
<td>Median follow-up, years</td>
<td>3.2</td>
<td>4.4</td>
<td>.003</td>
</tr>
<tr>
<td>5-year relapse</td>
<td>36.2</td>
<td>38.2</td>
<td>.97</td>
</tr>
<tr>
<td>Death</td>
<td>2.2</td>
<td>4.3</td>
<td>.11</td>
</tr>
<tr>
<td>Permanent consequence</td>
<td>21.0</td>
<td>17.8</td>
<td>.20</td>
</tr>
</tbody>
</table>

NOTE: Data are given as percentages unless otherwise noted. Abbreviations: HS, Histioocyte Society; LCH, Langerhans cell histiocytosis; Lung +, lung involvement; MS, multiple system; RO+, risk organ involvement; RO−, no risk organ involvement; SS, single system.
66.9% of patients. Among patients with wild-type BRAF 1.4%, 11.2%, and 87.4% of patients, had high, intermediate, and low DAS, respectively (Fig 2).

We constructed a logistic regression model with BRAFV600E as the dependent variable and patient age and RO involvement (ROs grouped together) as independent binary covariates. In this model (Table 2), BRAFV600E probability was associated only with ROs (OR, 6.35; 95% CI, 2.03 to 19.85; *P* = .001) and skin (OR, 3.65; 95% CI, 1.81 to 7.35; **P** < .002).

**BRAF Status and Biologic Parameters**

Hemoglobin level, platelet and leukocyte counts, fibrinogen, C-reactive protein, erythrocyte sedimentation rate, and albuminemia were recorded at diagnosis and at reactivation when applicable. In addition, any occurrence of hemophagocytic syndrome, according to the HLH-2004 criteria, was recorded. Among patients with LCH, those with BRAFV600E had a lower median hemoglobin level at diagnosis than did those with wild-type BRAF (10.1 g/dL vs 11.8 g/dL, respectively; *P* = .001).
Significant hypoalbuminemia, which was defined as albuminemia < 30 g/L, occurred more frequently in patients with \( \text{BRAF}^{V600E} \) than in those with wild-type \( \text{BRAF} \) (31.0% and 9.1%, respectively; \( P = .002 \)). Inflammatory biologic syndrome at diagnosis, defined as erythrocyte sedimentation rate > 40 mm and/or fibrinogen > 5 g/L and/or C-reactive protein > 30 mg/L, occurred more frequently in patients with \( \text{BRAF}^{V600E} \) than in those with wild-type \( \text{BRAF} \) (20.4% and 11.2%, respectively; \( P = .03 \)). Hemophagocytic syndrome (\( n = 9 \)) was only reported in patients with \( \text{BRAF}^{V600E} \).

**DISCUSSION**

In this population-based study of 315 patients, those with a \( \text{BRAF}^{V600E} \) mutation had characteristics of high-risk LCH, including an increased proportion of patients with RO involvement. \( \text{BRAF}^{V600E} \) mutation was also associated with reduced sensitivity to standard LCH chemotherapy and increased rates of disease reactivation and irreversible PC.

Identification of \( \text{BRAF}^{V600E} \) in more than one half of patients with LCH changed our understanding of LCH pathobiology, however, previously, the clinical relevance of \( \text{BRAF}^{V600E} \) remained obscure. Previous studies focused on the discovery of the mutation or its pathophysiology, but they failed to represent the full spectrum of pathology observed in this disease. Those studies concluded that \( \text{BRAF}^{V600E} \) mutation occurred more frequently in patients with \( \text{BRAF}^{V600E} \) than among those with wild-type \( \text{BRAF} \) (27.9% and 12.6%, respectively; \( P = .001 \); Table 3). Two of the main causes of PC, diabetes insipidus and neurodegenerative disease, occurred at higher rates in patients with \( \text{BRAF}^{V600E} \) than in those with wild-type \( \text{BRAF} \) (diabetes insipidus: 19.8% vs 8.4%, \( P = .006 \); neurodegenerative disease: 6.4% vs 1.4%; \( P = .04 \), respectively; Fig 3B).

The 5-year mortality rate was low for both groups (\( \text{BRAF}^{V600E} \), 3.9%; wild-type \( \text{BRAF} \), 0.8%; \( P = .16 \); Appendix).

### Table 2. Logistic Regression Analyses of Associations Between \( \text{BRAF} \) Status and Independent Clinical Binary Covariates

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis &lt; 3 years</td>
<td>150</td>
<td>1.01 (0.57 to 1.81)</td>
<td>.96</td>
</tr>
<tr>
<td>Female sex</td>
<td>148</td>
<td>1.06 (0.65 to 1.75)</td>
<td>.80</td>
</tr>
<tr>
<td>Involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>262</td>
<td>1.52 (0.70 to 3.31)</td>
<td>.29</td>
</tr>
<tr>
<td>Skin</td>
<td>113</td>
<td>3.65 (1.81 to 7.39)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>RO</td>
<td>49</td>
<td>6.35 (2.03 to 19.85)</td>
<td>.001</td>
</tr>
<tr>
<td>Pituitary</td>
<td>48</td>
<td>1.60 (0.63 to 4.08)</td>
<td>.32</td>
</tr>
<tr>
<td>Lung</td>
<td>37</td>
<td>0.67 (0.26 to 1.54)</td>
<td>.31</td>
</tr>
<tr>
<td>Lymph node</td>
<td>27</td>
<td>0.33 (0.11 to 1.01)</td>
<td>.05</td>
</tr>
<tr>
<td>CNS</td>
<td>24</td>
<td>1.30 (0.36 to 4.73)</td>
<td>.69</td>
</tr>
</tbody>
</table>

**NOTE.** Dependent variable was the \( \text{BRAF} \) status, and the independent covariates were patient age, sex, and potential involvement of different organs. Abbreviations: OR, odds ratio; RO, risk organ.

### Table 3. Therapeutic Response and Outcome According to \( \text{BRAF} \) Status and First-Line Therapy

<table>
<thead>
<tr>
<th>Outcome</th>
<th>All Patients</th>
<th></th>
<th></th>
<th>No Systemic Chemotherapy</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{BRAF} )</td>
<td>WT</td>
<td>( P )</td>
<td>( \text{BRAF} )</td>
<td>WT</td>
<td>( P )</td>
</tr>
<tr>
<td>All presentation at diagnosis</td>
<td>172</td>
<td>143</td>
<td>-</td>
<td>96</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>Responders, No. (%)</td>
<td>32 (19.6)</td>
<td>5 (3.5)</td>
<td>&lt; .001</td>
<td>29 (30.2)</td>
<td>4 (6.7)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>5-year cumulative incidence of reactivations, % ± SE</td>
<td>42.8 ± 4.4</td>
<td>28.1 ± 4.5</td>
<td>.006</td>
<td>44.7 ± 5.7</td>
<td>37.8 ± 7.1</td>
<td>.14</td>
</tr>
<tr>
<td>Reactivation in ROs, No. (%)</td>
<td>12 (7.0)</td>
<td>1 (0.7)</td>
<td>.008</td>
<td>8 (8.3)</td>
<td>0 (0.0)</td>
<td>.02</td>
</tr>
<tr>
<td>Patients with PC, No. (%)</td>
<td>48 (27.9)</td>
<td>18 (12.6)</td>
<td>.001</td>
<td>38 (39.6)</td>
<td>12 (20.0)</td>
<td>.01</td>
</tr>
</tbody>
</table>

**NOTE.** Dashes indicate not applicable. Abbreviations: \( \text{BRAF} \), \( \text{BRAF}^{V600E} \) mutated Langerhans cell histiocytosis cases; PC, permanent consequence; RO, risk organ; SE, standard error; WT, wild-type \( \text{BRAF} \).
frequently in younger patients than in adults—on the basis of an adult/child cohort (n = 61) and that it was associated with an elevated reactivation rate, but not with RO involvement (n = 100).7,23 Our study enrolled children with a wide spectrum of disease phenotypes; in fact, we aimed to represent the full diversity of pathologies observed in this disease. This inclusion increased the accuracy of correlations with \( \text{BRAF}^{V600E} \) expression. Our data showed that \( \text{BRAF}^{V600E} \) mutation was correlated with the most aggressive forms of LCH, that is, those prevalent in patients who are diagnosed at a young age. These aggressive disease phenotypes included multisystem disease, skin involvement, spleen, liver, and hematologic dysfunction, and localizations associated with PC, such as pituitary and CNS disorders.1 Moreover, two infrequent variants of LCH segregated strictly in terms of \( \text{BRAF} \) status. The localized LCH variant of the self-healing Hashimoto Prizker form was always found in patients with wild-type \( \text{BRAF} \). In contrast, the multisystemic LCH disease with hemophagocytic syndrome–associated RO+ was always found in patients with \( \text{BRAF}^{V600E} \). Apart from these two infrequent LCH variants, our results suggest that factors other than \( \text{BRAF} \) were likely to be involved in establishing the full LCH clinical phenotype.

One limitation of this study is that we did not investigate genotypes more fully among patients with LCH who carried wild-type \( \text{BRAF} \); however, although other somatic mutations are interesting, previous correlation studies have only identified rare or largely nonrecurrent mutations in \( \text{BRAF} \), \( \text{ARAF} \), \( \text{ERBB3} \), \( \text{PI3KCA} \), and \( \text{MAP3K1} \). An exception to this observation was the discovery of recurrent mutations in exon 2 and exon 3 of \( \text{MAP2K1} \). Among patients with wild-type \( \text{BRAF} \), these mutations—mostly deletions—affected 17% (n = 7) to 27.5% (n = 11) of patients with LCH. In the current study, \( \text{MAP2K1} \) deletions were detected in six patients with wild-type \( \text{BRAF} \); these mutations were identified by Sanger sequencing of DNA from fresh frozen samples. We also detected one \( \text{PIK3CA} \) mutation, which was reported previously.26 These cases were categorized as benign bone SS LCH (data not shown). Previous studies have shown that most LCH cases were associated with constitutive activation of the MAPK pathway. On the basis of the clinical differences found in the current study between patients with mutated and wild-type \( \text{BRAF} \), we hypothesized that in LCH, \( \text{BRAF}^{V600E} \) mutation has
a stronger oncogenic potential than it has by other molecular alterations in the MAPK pathway that occurred in the presence of wild-type BRAF.

Association of BRAFV600E with a more aggressive, and sometimes resistant LCH disease, suggests an avenue for development of new therapeutic agents. LCH is an extremely heterogeneous disease, and some forms may be curable without drugs or with only minimal therapy. At the other end of the spectrum, more severe disease forms can be treated with effective second-line therapies, but these are reported to be highly toxic.3 This toxicity is relevant, given that incidence of long-term adverse effects (PC) remains substantial for patients with LCH; more than one quarter of patients with BRAFV600E developed PC with LCH. Thus, anti-BRAF therapies represent a promising new line of inquiry.29 Initial reports on anti-BRAF therapies have indicated efficacy,11,12 but more data are needed to validate a tailored regimen that is tolerable for children, in particular, infants, who are most susceptible to high-risk LCH. Because all nine cases of LCH associated with hemophagocytic activation syndrome occurred in patients with BRAFV600E in our study, this association should be investigated further. Indeed, this subgroup of patients might benefit most from the addition of a targeted therapy.

In terms of improved diagnosis, BRAFV600E mutation can now be identified and quantified in plasma or serum-free cell DNA.30,31 Future studies should be able to validate these assays and assess their value for prediction of prognosis and treatment response.

Patients with BRAF mutations who develop life-threatening histiocytoses, such as Erdheim-Chester disease and/or LCH, have been shown to manifest significant clinical responses to targeted therapy. Here, we show that children with BRAFV600E experienced more severe LCH disease, had higher rates of sequelae, and showed a diminished response to vinblastine-steroid chemotherapy than did children with wild-type BRAF. Our data argue that clinical trials should assess the benefits of BRAF inhibitor treatment in the early stages of disease progression.

REFERENCES


AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Sébastien Héritier, Jean-François Emile, Jean Donadieu
Financial support: Jean-François Emile, Jean Donadieu
Administrative support: Sébastien Héritier, Jean-François Emile, Mohamed-Azz Barkaouei, Jean Miron, Zoﬁa Hélias-Rodziewicz, Jean Donadieu
Provision of study materials or patients: Caroline Thomas, Sylvie Fraytag, Sabah Boujdema, Florence Renaud, Anne Moreau, Michel Peuchmaur, Catherine Chassagne-Clement, Frédérique Dijoud, Valérie Rigau, Despina Mosiou, Anne Lambilliotte, Françoise Mazingue, Kamila Kebaili, Eric Jeziorski, Geneviève Plat, Nathalie Aladji, Alina Ferster, Hélène Pacquement, Claire Galambrun, Guy Leverger, Ludovic Mansuy, Catherine Paillard, Anne Deville, Corinne Armani-Alla, Anne Lutun, Marion Gillibert-Vert, Jean-Louis Stephan, Fleur Cohen-Aubart, Julien Haroche, Isabelle Pellan, Frédéric Millot, Brigitte Lescouer, Virginie Gandemner, Christine Bodemer, Jean Donadieu
Collection and assembly of data: Sébastien Héritier, Mohamed-Azz Barkaouei, Caroline Thomas, Sylvie Fraytag, Sabah Boujdema, Florence Renaud, Anne Moreau, Michel Peuchmaur, Catherine Chassagne-Clement, Frédérique Dijoud, Valérie Rigau, Jean Miron, Isabelle Pellan, Brigitte Lescouer, Roger Lacave, Zoﬁa Hélias-Rodziewicz, Valérie Taly, Frédéric Geissmann
Data analysis and interpretation: Sébastien Héritier, Jean-François Emile, Despina Mosiou, Anne Lambilliotte, Françoise Mazingue, Kamila Kebaili, Eric Jeziorski, Geneviève Plat, Nathalie Aladji, Alina Ferster, Hélène Pacquement, Claire Galambrun, Laurence Brugères, Guy Leverger, Ludovic Mansuy, Catherine Paillard, Anne Deville, Corinne Armani-Alla, Anne Lutun, Marion Gillibert-Vert, Jean-Louis Stephan, Fleur Cohen-Aubart, Julien Haroche, Frédéric Millot, Virginie Gandemner, Christine Bodemer, Valérie Taly, Frédéric Geissmann, Jean Donadieu
Manuscript writing: All authors
Final approval of manuscript: All authors
Affiliations

Sébastien Héritier, Mohamed-Aziz Barkaoui, Jean Miron, and Jean Donadieu, French Reference Center for Langerhans Cell Histiocytosis, Trousseau Hospital; Sébastien Héritier, Sahab Boudjemaa, Guy Leverger, and Jean Donadieu, Trousseau Hospital, Assistance Publique–Hôpitaux de Paris; Sylvie Fraïtak, Despina Moshous, and Christine Bodemer, Necker Hospital, Assistance Publique–Hôpitaux de Paris; Michel Peuchmaur and Brigitte Lescoeur, Robert Debré Hospital, Assistance Publique–Hôpitaux de Paris; Michel Peuchmaur, Université Paris Diderot, Sorbonne Paris Cité; Hélène Pacquement, Institut Curie Medical Center; Guy Leverger, Université Pierre et Marie Curie; Fleur Cohen-Aubart and Julien Haroche, Pitié-Salpêtrière Hospital, Assistance Publique–Hôpitaux de Paris; Roger Lacave, Tenon Hospital, Assistance Publique–Hôpitaux de Paris; Valérie Taly, Institut National de la Santé et de la Recherche Médicale, Unités Mixte de Recherche S1147, Centre National de la Recherche Scientifique SNC 5014, Université Paris Sorbonne Cité, Paris; Sébastien Héritier, Jean-François Emile, Zofia Hélias-Rodzewicz, and Jean Donadieu, Université de Versailles Saint-Quentin-en-Yvelines, Université Paris-Saclay; Jean-François Emile, Ambroise Paré Hospital, Assistance Publique–Hôpitaux de Paris, Boulouge-Billancourt; Caroline Thomas and Anne Moreau, Centre Hospitalo-Universitaire de Nantes, Nantes; Florence Renaud, Centre Hospitalier Régional Universitaire, Université de Lille; Anne Lambilliotte and Françoise Mazingue, Centre Hospitalo-Universitaire de Lille, Lille; Catherine Chassagne-Clément, Centre Léon Bérard; Frédérique Dijoud, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon; Kamila Kebaili, Institut d’Hématologie-Onco-Pathologie, Lyon; Valérie Rigau, Hôpital Armand Trousseau, Assistance Publique–Hôpitaux de Paris; Geneviève Plat, Centre Hospitalo-Universitaire de Toulouse, Toulouse; Nathalie Aladjidi, Centre Hospitalo-Universitaire de Bordeaux, Bordeaux; Claire Galambrun, Hôpital de la Timone, Marseille; Laurence Brugières, Institut Gustave Roussy, Villejuif; Ludovic Mansuy, Brabois-Enfants Hospital, Centre Hospitalo-Universitaire de Nancy, Vandoeuvre-lès-Nancy; Catherine Paillard, Centre Hospitalo-Universitaire de Strasbourg, Strasbourg; Anne Deville, Centre Hospitalo-Universitaire de Nice, Nice; Corinne Armani-Alla, Centre Hospitalo-Universitaire de Grenoble, Grenoble; Anne Autun, Centre Hospitalo-Universitaire d’Amiens, Amiens; Marion Gillibert-Yvert, Centre Hospitalo-Universitaire de Tours, Tours; Jean-Lois Stephan, Centre Hospitalo-Universitaire de Saint Etienne, Saint Etienne; Isabelle Pellerin, Centre Hospitalo-Universitaire de Angers, Angers; Frédéric Millot, Centre Hospitalo-Universitaire de Poitiers, Poitiers; Virginie Gandelmer, Centre Hospitalo-Universitaire de Rennes, Rennes, France; Alina Ferster, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium; and Frédéric Geissmann, Memorial Sloan Kettering Cancer Center, New York, NY.
AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy

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Sébastien Héritier
No relationship to disclose

Jean-François Emile
Honoraria: Roche

Mohamed-Aziz Barkaoui
No relationship to disclose

Caroline Thomas
No relationship to disclose

Sylvie Fraitag
No relationship to disclose

Sabah Boudjemaa
No relationship to disclose

Florence Renaud
No relationship to disclose

Anne Moreau
No relationship to disclose

Michel Peuchmaur
No relationship to disclose

Catherine Chassagne-Clément
No relationship to disclose

Frédérique Dijoud
No relationship to disclose

Valérie Rigau
No relationship to disclose

Despina Moshous
No relationship to disclose

Anne Lambilliotte
No relationship to disclose

François Mazingue
No relationship to disclose

Kamila Kebaili
No relationship to disclose

Jean Miron
No relationship to disclose

Eric Jezierski
No relationship to disclose

Geneviève Plat
No relationship to disclose

Nathalie Aladjidi
No relationship to disclose

Alina Ferster
Travel, Accommodations, Expenses: Jazz Pharmaceuticals, Bayer

Hélène Pacquement
No relationship to disclose

Claire Galambrun
No relationship to disclose

Laurence Brugières
Consulting or Advisory Role: Millennium Pharmaceuticals
Research Funding: Novartis, Chugai

Guy Leverger
No relationship to disclose

Ludovic Mansuy
No relationship to disclose

Catherine Paillard
No relationship to disclose

Anne Deville
No relationship to disclose

Corinne Armari-Alla
No relationship to disclose

Anne Lutun
No relationship to disclose

Marion Gillibert-Yvert
No relationship to disclose

Jean-Louis Stephan
No relationship to disclose

Fleur Cohen-Aubart
No relationship to disclose

Julien Haroche
No relationship to disclose

Isabelle Pellier
No relationship to disclose

Frédéric Millot
No relationship to disclose

Brigitte Lescoeur
No relationship to disclose

Virginie Gandemer
No relationship to disclose

Christine Bodemer
No relationship to disclose

Roger Lacave
No relationship to disclose

Zofia Hélia-Rodzewicz
No relationship to disclose

Valérie Taly
Honoraria: Raindance Technologies, Boehringer Ingelheim
Consulting or Advisory Role: Raindance Technologies
Frédéric Geissmann
Consulting or Advisory Role: Merck Sharp & Dohme
Jean Donadieu
No relationship to disclose
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Appendix

Comprehensive Description of Experimental Plan: Sample Size Estimation and Study Organization

At the start of the study, in September 2011, we estimated the size of the sample of patients with Langerhans cell histiocytosis (LCH) that we would need to test for \( \text{BRAF}^{V600E} \) mutation. We estimated a sample size sufficient to observe at least a 20% difference between high-risk LCH and low-risk LCH groups, with a 5% type I error and a 20% type II error. When we considered that approximately 20% of all patients would have high-risk LCH, including lung involvement, and that 80% of patients would have low-risk LCH, we calculated that at least 313 patients must be enrolled to achieve sufficient statistical power according to the Casagrande and Pike method for unequal sized groups (Fleiss et al: Biometrics 36:343-346, 1980). All enrolled patients had been included in the French LCH registry; therefore, this study was nested within the French LCH registry. To minimize potential bias in selection of patients according to extent of disease, we asked the participating centers to propose the study to all patients that were observed locally, regardless of the extent of disease.

Survival Data

Death occurred in five patients with mutant \( \text{BRAF} \) and two patients with wild-type \( \text{BRAF} \). Both patients with wild-type \( \text{BRAF} \) had multiple system with lung involvement LCH. Of those, one patient showed severe lung involvement at diagnosis and a mechanical complication that proved lethal at 4 months after diagnosis. The other patient had achieved long-term complete remission, and the fatality was unrelated to the disease. All five patients with \( \text{BRAF}^{V600E} \) who died had multiple system with involvement of risk organs LCH. Of those, two patients died after undergoing an allogeneic bone marrow transplantation, and three patients died of sepsis, secondary to the combined cladribine-cytarabine regimen. This low mortality rate could be explained by the efficacy of second-line therapy with the combined cladribine-cytarabine regimen. Treatment may overcome the refractory situation, but it had high toxicity.\(^3\)

| Table A1. Subgroup Analysis of Therapeutic Response and Outcome According to \( \text{BRAF} \) Status, First-Line Therapy, and RO Involvement |
|---------|---------------|---------------|---------------|
| Outcome | \( \text{BRAF} \) | WT | \( P \) | \( \text{BRAF} \) | WT | \( P \) | \( \text{BRAF} \) | WT | \( P \) |
| SS, MS RO–, and Lung+ LCH at diagnosis |
| Sample size, No. | 139 | 138 | — | 64 | 55 | .37 | 73 | 81 | — |
| Responders, No. (%) | — | — | — | 60 (93.7) | 54 (98.2) | — | — | — | — |
| Patients with second-line therapy, No. (%) | 11 (7.9) | 4 (2.9) | .11 | 8 (12.5) | 3 (5.6) | .22 | 3 (4.1) | 1 (1.2) | .35 |
| 5-year cumulative incidence of reactivations, % ± SE | 40.1 ± 4.7 | 27.6 ± 4.6 | .009 | 41.0 ± 6.7 | 37.6 ± 7.2 | .13 | 38.3 ± 6.7 | 17.5 ± 5.2 | .04 |
| Reactivation in ROs, No. (%) | 10 (7.2) | 1 (0.7) | .01 | 6 (9.4) | 0 (0) | .03 | 4 (5.5) | 1 (1.2) | .19 |
| Patients with PC, No. (%) | 33 (23.7) | 17 (12.3) | .02 | 24 (37.5) | 11 (20) | .04 | 8 (11) | 6 (7.4) | .58 |
| MS RO+ LCH at diagnosis |
| Sample size, No. | 33 | 5 | — | 32 | 5 | — | 0 | 0 | — |
| Responders, No. (%) | — | — | — | 15 (46.8) | 4 (80) | .34 | — | — | — |
| Patients with second-line therapy, No. (%) | 21 (63.6) | 1 (20) | .14 | 21 (65.6) | 1 (20) | .14 | — | — | — |
| 5-year cumulative incidence of reactivations, % ± SE | 52.3 ± 10.6 | 40.0 ± 21.9 | .89 | 52.3 ± 10.6 | 40 ± 21.9 | .89 | — | — | — |
| Reactivation in ROs, No. (%) | 2 (6.1) | 0 (0) | 1 | 2 (6.2) | 0 (0) | 1 | — | — | — |
| Patients with PC, No. (%) | 15 (45.5) | 1 (20) | .37 | 14 (43.7) | 1 (20) | .63 | — | — | — |

NOTE. Dashes indicate not applicable.

Abbreviations: \( \text{BRAF} \), \( \text{BRAF}^{V600E} \) mutated LCH cases; LCH, Langerhans cell histiocytosis; Lung+, lung involvement; MS, multiple system; PC, permanent consequence; RO+, risk organ involvement; RO–, no risk organ involvement; SE, standard error; SS, single system; VLB, vinblastine; WT, wild type \( \text{BRAF} \) LCH cases.