Dramatic transient improvement of metastatic BRAF<sup>V600E</sup>-mutated Langerhans cell sarcoma under treatment with dabrafenib

**Running head:** Dabrafenib in Langerhans cell sarcoma

Samia Mourah<sup>1</sup>, PharmD, PhD; Gwenaël Lorilllon<sup>2</sup>, MD; Véronique Meignin<sup>3</sup>, MD, PhD; Laetitia Vercellino<sup>4</sup>, MD; Constance de Margerie-Mellon<sup>5</sup>, MD; Cécile Pages<sup>6</sup>, MD; Lauriane Goldwirt<sup>7</sup>, PharmD, PhD; Alexandre How-Kit<sup>8</sup>, PhD; Jorg Tost<sup>9</sup>, PhD; Céleste Lebbe<sup>6,10</sup>, MD, PhD; Abdellatif Tazi<sup>2,11</sup>, MD, PhD

<sup>1</sup>Assistance Publique-Hôpitaux de Paris, Laboratoire de Pharmacologie Biologique, Hôpital Saint-Louis; Univ Paris-Diderot, Sorbonne Paris Cité; INSERM U976, Paris, France

<sup>2</sup>Assistance Publique-Hôpitaux de Paris, Centre National de Référence de l’Histiocytose Langerhansienne, Service de Pneumologie, Hôpital Saint-Louis, Paris, France

<sup>3</sup>Assistance Publique- Hôpitaux de Paris, Service de Pathologie, Hôpital Saint-Louis, INSERM UMR_S1165, Paris, France

<sup>4</sup>Assistance Publique- Hôpitaux de Paris, Service de Médecine Nucléaire, Hôpital Saint-Louis, Paris, France

<sup>5</sup>Assistance Publique- Hôpitaux de Paris, Service de Radiologie, Hôpital Saint-Louis, Paris, France

<sup>6</sup>Assistance Publique- Hôpitaux de Paris, Département de Dermatologie, Hôpital Saint-Louis, Paris, France

<sup>7</sup>Assistance Publique- Hôpitaux de Paris, Laboratoire de Pharmacologie Biologique, Hôpital Saint-Louis, Paris, France
Corresponding author: Abdellatif Tazi, MD, PhD

Service de Pneumologie, Hôpital Saint-Louis

1 Avenue Claude Vellefaux, 75475, Paris cedex 10, France

Tel.: 33 1 42 49 96 18, Fax: 33 1 42 49 93 95

E-mail: abdellatif.tazi@sls.aphp.fr
Langerhans cell sarcoma (LCS) is a rare histiocytic neoplasm with overt malignant
cytological features and an aggressive clinical course.\textsuperscript{1} Disseminated LCS carries a poor
prognosis.\textsuperscript{1} We report a case of a metastatic BRAF\textsuperscript{V600E}-mutated LCS that dramatically
improved after administration of the BRAF inhibitor (BRAFi) dabrafenib.

A 58-year-old man was referred in August 2014 with a diagnosis of progressive Langerhans
cell histiocytosis (LCH). He was treated in July 2013 by surgery and radiotherapy for left
humerus LCH diagnosed by bone biopsy. In February 2014, enlargement of the left axillary,
pectoral and supraclavicular lymph nodes was observed. Histological examination of a lymph
node biopsy indicated LCH recurrence, although some atypical cells were described. CHOP
chemotherapy was initiated but was discontinued after 3 cycles because of disease
progression. The patient’s condition deteriorated. The lymph nodes increased in size and
multiple lung nodules were observed on lung CT. These lesions were highly hypermetabolic
on \textsuperscript{18}FDG PET-CT (Figure). A new lymph node biopsy revealed massive infiltration by very
large cells with irregular, folded nuclei and necrotic areas. The mitotic rate was greater than
50 per 10 high-power fields. The tumor cells expressed CD1a and Langerin (Figure), features
characteristic of LCS.\textsuperscript{1,2} A review of previous biopsies showed the presence of small areas of
similar tumor cells. All tissue specimens harbored the BRAF\textsuperscript{V600E} mutation, as determined by
immunohistochemistry and molecular genotyping (Figure). After informed consent,
dabrafenib (150 mg b.i.d.) was initiated. Within a week, the patient improved, and the lymph
nodes dramatically decreased in size. A lymph node biopsy performed on treatment day 10
showed massive necrosis of tumor cells and the absence of the BRAF\textsuperscript{V600E} mutation (Figure).
Serial \textsuperscript{18}FDG PET-CT scans after 3 months of treatment revealed marked improvement of
the lesions, with residual small axillary adenopathy (Figure). The patient was in complete
response according to RECIST 1.1 criteria and in partial metabolic response according to
EORTC criteria. The treatment was well tolerated except for diarrhea (which resolved after
the dabrafenib dose was reduced to 100 mg b.i.d.) and palmoplantar hyperkeratosis. Average values of dabrafenib plasma trough and maximal concentrations, which were monitored monthly, reached 16 ng/mL (15 to 27 ng/mL) and 604 ng/mL (507 to 786 ng/mL) respectively, consistent with previous pharmacokinetic studies. At 6 months, $^{18}$FDG PET-CT revealed an increase in size of the left axillary lymph node, the enlargement of mediastinal lymph nodes and a left pulmonary nodule (Figure). The axillary lymph node biopsy confirmed the recurrence of $\text{BRAF}^{V600E}$-mutated LCS (Figure). To determine the mechanisms by which dabrafenib resistance was acquired, genomic and transcriptomic analyses of the main previously described factors involved in BRAFi resistance were performed on the pre-treatment and relapsing tumors, which contained 95% and 90% tumor cells respectively ($^{4-6}$) (see online supplement). No $\text{NRAS}^{G12/G13/Q61}$, $\text{KRAS}^{G12/G13}$ or $\text{MAP2K1}$ (exons 2 and 3) mutations or $\text{BRAF}$ splicing variants were detected in the recurrent lesion ($^{4-6}$). The MAP3K8/COT mRNA level, normalized to the number of tumor cells, was 3-fold ($3.3 \pm 0.2$) increased in the relapsing specimen as compared with the initial tumor. This increase, which was also observed at the protein level, may explain the observed intense activation of the MAPK pathway (Figure). Indeed in melanoma, MAP3K8/COT overexpression drives resistance to BRAFi through MAPK pathway reactivation which may be overcome by the addition of a MEK inhibitor (MEKi) ($^{4}$). The patient was thereafter switched to combined vemurafenib and cobimetinib treatment. Within 3 days, the patient clinically improved. At 10 weeks of treatment, he was with an ECOG performans status of 1 and the different tumor localizations had decreased in size.

LCS is a rare malignant histiocytic disorder that affects the lymph nodes as well as extranodal sites ($^{1}$). LCS is differentiated from LCH based on cytological criteria, a high mitotic index and more aggressive behavior ($^{1,2}$). Various chemotherapy regimens, primarily CHOP, are used with limited response rates and high mortality ($^{1}$). Recently, the $\text{BRAF}^{V600E}$ mutation was identified...
in a variable proportion of dendritic cell neoplasms, including LCS, suggesting that these patients may benefit from BRAFi.\textsuperscript{1,7,8} The dramatic response of the present case to dabrafenib further supports the key role of the $\text{BRAF}^{V600E}$ mutation in histiocytic disorders\textsuperscript{9,10}, although secondary resistance to BRAFi treatment may occur in malignant forms.\textsuperscript{8}

In melanoma, a first-line therapeutic strategy with the combination of BRAFi and MEKi has been demonstrated to prevent or delay the onset of resistance observed with treatment with BRAFi alone, in addition to increasing progression-free survival.\textsuperscript{11,12} A similar strategy might be more appropriate in progressive $\text{BRAF}^{V600E}$-mutated histiocytic malignancies.
Acknowledgements

The authors thank Aurélie Sadoux, Coralie Reger de Moura (Laboratoire de Pharmacologie Biologique, Hôpital Saint-Louis, Paris, France) and Maeva Valluci (Centre National de Référence de l’Histiocytose Langerhansienne, Service de Pneumologie, Hôpital Saint-Louis, Paris, France) for technical support and Elisabeth Savariau (Institut Universitaire d’Hématologie, Service d’Infographie, Hôpital Saint-Louis, Paris, France) for her assistance with the figures.

Authorship Contributions

SM and AT designed the research, analyzed and interpreted the data and wrote the manuscript. GL managed sample acquisition and collected clinical data. VM analyzed and interpreted histological data. LV analyzed and interpreted 18FDG PET-CT findings. CdeM analyzed and interpreted radiological results. CP analyzed and interpreted dermatological clinical findings. LG analyzed and interpreted pharmacological data. AHK and JT analyzed BRAF, NRAS and KRAS genotyping and interpreted the data. CL provided her expertise in melanoma field and intellectual content.

Disclosure of Conflicts of Interest

Samia Mourah declares a consulting role for Roche and Novartis.

Gwenaël Lorillon has no conflicts of interest to declare.

Véronique Meignin has no conflicts of interest to declare.

Laetitia Vercellino has no conflicts of interest to declare.

Constance de Margerie-Mellon declares travel accommodation by Guerbet.

Cécile Pages has no conflicts of interest to declare.
Lauriane Goldwirt declares travel accommodation by Janssen.

Alexandre How-Kit has no conflicts of interest to declare.

Jorg Tost has no conflicts of interest to declare.

Céleste Lebbe declares honoraria from Roche, advisory roles for Roche, GSK, Novartis, BMS, MSD, and Amgen, and travel accommodation by Roche.

Abdellatif Tazi has no conflicts of interest to declare.
References


Figure Legend

**Figure A.** $^{18}$-FDG PET-CT before the onset of treatment. a) Fused PET-CT axial view of pulmonary nodules (standardized maximal uptake, SUVmax=12). b) Left axillary mass (SUVmax=11.9). $^{18}$-FDG PET-CT after three months of dabrafenib treatment. c) Fused PET-CT showing no significant residual uptake in the lung. d) Presence of a residual small axillary lymph node with decreased uptake (SUVmax=4.5). The patient was classified as partial metabolic response according to the EORTC criteria (i.e., more than 25% reduction of the SUVmax in the target lesions), with a decrease in SUVmax of 83% in the target lesions. $^{18}$-FDG PET-CT after six months of dabrafenib treatment at the time of LCS relapse. e) Fused PET-CT showing a hypermetabolic pulmonary nodule (SUVmax=6.3) and mediastinal lymph nodes (SUVmax=8.7); f) axillary nodal relapse (SUVmax=9.6).

**B.** Light optic microscopy of the left axillary lymph node biopsy before and during treatment. a) Hematoxylin & eosin (HE) staining showing a very pleomorphic proliferation composed of large tumor cells with folded nuclei, and numerous mitotic cells. b) Tumor cells stained with an anti-CD1a antibody (clone O10, Dako); c) most of the cells are Langerin (CD207)-positive (clone 12D6, Novocastra). These features are characteristic of Langerhans cell sarcoma. d) Anti-BRAF$^{V600E}$ (clone VE1, Spring Biosciences) immunostaining is positive in tumor cells. c) HE staining of a lymph node biopsy at day 10 of dabrafenib treatment, showing massive necrosis of tumor cells. f) No cells stained positive with the anti-BRAF$^{V600E}$ mutation-specific antibody. Original magnification: 400x in all sections.

**C.** Pyrosequencing of DNA extracted from lymph node biopsies. a) Before treatment, the BRAF$^{V600E}$ mutation is present (mutated allele=78%); b) on day 10 of dabrafenib treatment, only wild-type BRAF$^{V600E}$ is observed (mutated allele=1.4%); and c) at the time of LCS recurrence, demonstrating the resurgence of the BRAF$^{V600E}$ mutation (mutated allele=52%).

**D.** Expression of MAP3K8/COT protein in the pre-treatment and relapsing tumor.
HT-29 \(BRAF^{V600E}\) colorectal cells that express both the long (1-467) and short (30-467) forms of COT are shown as a positive control. \(\beta\)-actin served as loading control.

ERK expression and activation in relapsed LCS. E. Tumor cells are intensively stained with an anti-phospho-ERK-1/2 antibody (clone MAPK-YT, Sigma) compared with pre-treatment tumor cells. Original magnification: 400x. F. Western blot analysis for phosphorylated ERK (pERK) and ERK (endogenous total ERK) in the pre-treatment and relapsing tumors is shown. A375 \(BRAF^{V600E}\) melanoma cells were used as a positive control.
Dramatic transient improvement of metastatic BRAFV600E-mutated Langerhans cell sarcoma under treatment with dabrafenib

Samia Mourah, Gwenaël Lorillon, Véronique Meignin, Laetitia Vercellino, Constance de Margerie-Mellon, Cécile Pages, Lauriane Goldwirt, Alexandre How-Kit, Jorg Tost, Céleste Lebbe and Abdellatif Tazi