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To the editor:

Dramatic transient improvement of metastatic BRAF^{V600E}-mutated Langerhans cell sarcoma under treatment with dabrafenib

Langerhans cell sarcoma (LCS) is a rare histiocytic neoplasm with overt malignant cytological features and an aggressive clinical course.[1] Disseminated LCS carries a poor prognosis.[1] We report a case of a metastatic BRAF^{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. Histologic examination of a lymph node biopsy indicated LCH recurrence, although some atypical cells were described. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) 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 computed tomography (CT) scan. These lesions were highly hypermetabolic on fluorodeoxyglucose (^{18}-FDG) positron emission tomography (PET)-CT (Figure 1). A new lymph node biopsy revealed massive infiltration by very large cells with irregular folded nuclei and necrotic areas. The mitotic rate was >50 per 10 high-power fields. The tumor cells expressed CD1a and langerin

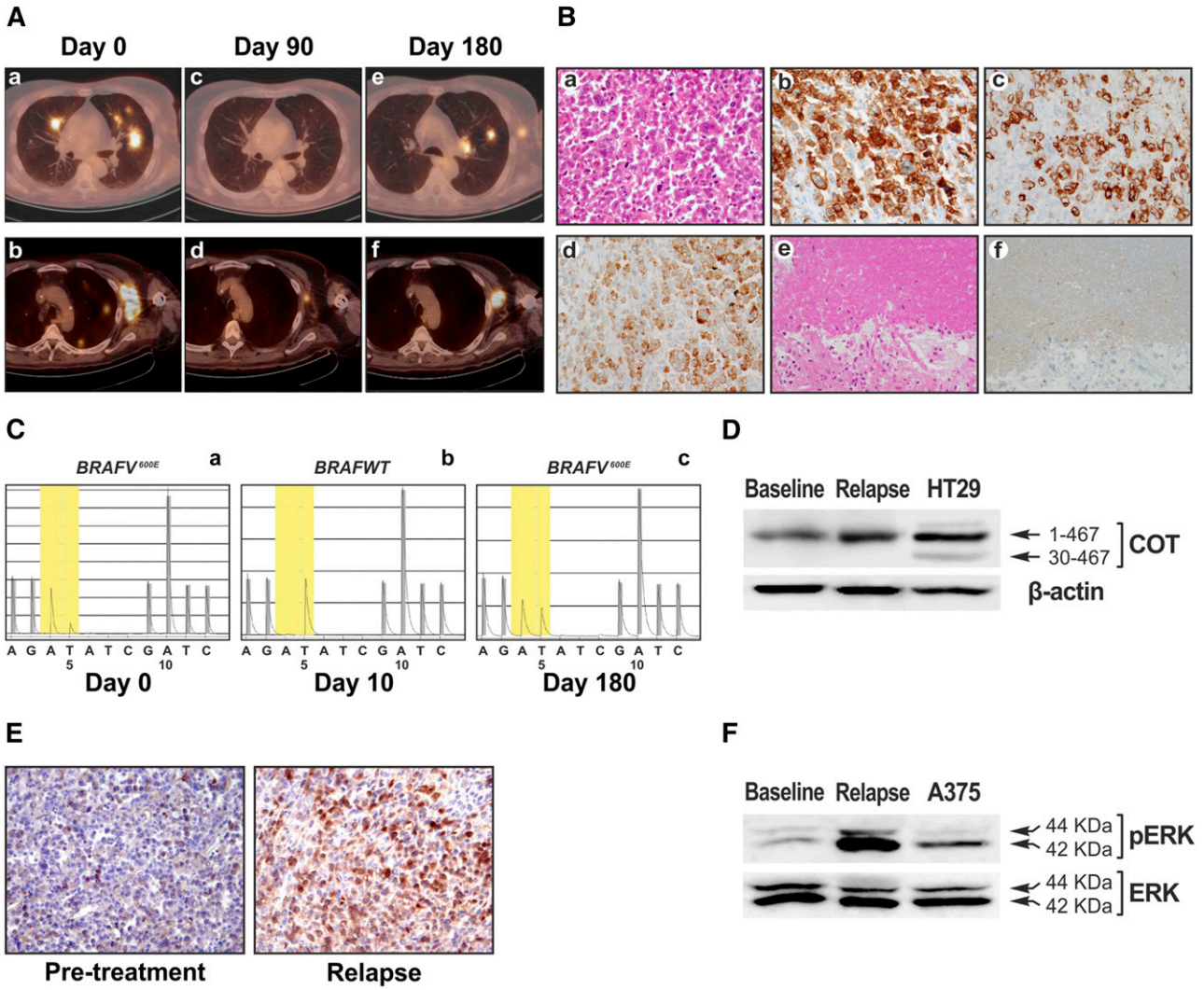

Figure 1. BRAFV600 genotyping. (A) ^{18}-FDG PET-CT before the onset of treatment. (a) Fused PET-CT axial view of pulmonary nodules (standardized maximal uptake [SUVmax] = 12). (b) Left axillary mass (SUVmax = 11.9). ^{18}-FDG PET-CT after 3 months of dabrafenib treatment. (c) Fused PET-CT showing no 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 (ie, >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 6 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-and-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 LCS. (d) Anti-BRAF^{V600E} (clone VE1; Spring Biosciences) immunostaining is positive in tumor cells. (e) 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, ×400 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^{V600} 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 pretreatment 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. β-actin served as loading control. Extracellular signal-regulated kinase (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 pretreatment tumor cells. Original magnification, ×400. (F) Western blot analysis for phosphorylated ERK (pERK) and ERK (endogenous total ERK) in the pretreatment and relapsing tumors is shown. A375 *BRAF*^{V600E} melanoma cells were used as a positive control.

(Figure 1), features characteristic of LCS.[1,2] A review of previous biopsies showed the presence of small areas of similar tumor cells. All tissue specimens harbored the BRAF^{V600E} mutation, as determined by immunohistochemistry and molecular genotyping (Figure 1). After informed consent, dabrafenib (150 mg twice daily) 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^{V600E} mutation (Figure 1). Serial ^{18}-FDG PET-CT scans after 3 months of treatment revealed marked improvement of the lesions, with residual small axillary adenopathy (Figure 1). The patient was in complete response according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria and in partial metabolic response according to European Organisation for Research and Treatment of Cancer (EORTC) criteria. The treatment was well tolerated except for diarrhea (which resolved after the dabrafenib dose was reduced to 100 mg twice daily) and palmoplantar hyperkeratosis. Average values of dabrafenib plasma trough and maximal concentrations, which were monitored monthly, reached 16 ng/mL (15-27 ng/mL) and 604 ng/mL (507-786 ng/mL), respectively, consistent with previous pharmacokinetic studies.[3]

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 1). The axillary lymph

node biopsy confirmed the recurrence of $BRAF^{V600E}$-mutated LCS (Figure 1). 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 pretreatment and relapsing tumors, which contained 95% and 90% tumor cells, respectively[4-6] (supplemental Methods and supplemental Table 1, see supplemental Data available on the *Blood* Web site). No $NRAS^{G12/G13/Q61}$, $KRAS^{G12/G13}$, or *MAP2K1* (exons 2 and 3) mutations or *BRAF* splicing variants were detected in the recurrent lesion.[4-6] The MAP3K8/COT messenger RNA level, normalized to the number of tumor cells, was threefold (3.3 ± 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 mitogen-activated protein kinase (MAPK) pathway (Figure 1). Indeed, in melanoma, MAP3K8/COT overexpression drives resistance to BRAFi through MAPK pathway reactivation which may be overcome by the addition of a MAPK kinase 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 had an Eastern Cooperative Oncology Group (ECOG) performance 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 $BRAF^{V600E}$ mutation was identified in a variable proportion of dendritic cell neoplasms, including LCS, suggesting that these patients may benefit from BRAFi.[1,7,8] The dramatic response of the present case to dabrafenib further supports the key role of the $BRAF^{V600E}$ mutation in histiocytic disorders,[9,10] although secondary resistance to BRAFi treatment may occur in malignant forms.[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.[11,12] A similar strategy might be more appropriate in progressive $BRAF^{V600E}$-mutated histiocytic malignancies.

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Contribution: S.M. and A.T. designed the research, analyzed and interpreted the data, and wrote the manuscript; G.L. managed sample acquisition and collected clinical data; V.M. analyzed and interpreted histologic data; L.V. analyzed and interpreted ^{18}-FDG PET-CT findings; C.d. M.-M. analyzed and interpreted radiologic results; C.P. analyzed and interpreted dermatologic clinical findings; L.G. analyzed and interpreted pharmacologic data; A.H.-K. and J.T. analyzed BRAF, NRAS, and KRAS genotyping and interpreted the data; and C.L. provided expertise in the melanoma field and intellectual content.

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To the editor:

Ferric chloride thrombosis model: unraveling the vascular effects of a highly corrosive oxidant

The topical application of ferric chloride ($FeCl_3$) to the vasculature is one of the most commonly used experimental approaches to induce thrombosis. The method was first described by Kurz and colleagues[1] and has subsequently been proven to be a highly effective and reliable approach to elucidate the role of platelet receptors, ligands, and activation pathways in promoting thrombosis.[2-7] It has also shed new light on the role of coagulation proteases in regulating thrombin generation and thrombus growth[8-11] and has been used in the context of thrombolysis,[12-17] although the model appears to have significant limitations in this regard. Despite its widespread use, the precise mechanism by which $FeCl_3$ induces thrombosis remains controversial. A recent study in *Blood* by Ciciliano and colleagues has provided further insight into the molecular mechanisms underlying $FeCl_3$-induced thrombosis, suggesting an important role for charge-dependent aggregation effects of $FeCl_3$ on blood cells and plasma proteins.[18]

It has long been assumed that the major effects of $FeCl_3$ are limited to the vasculature. $FeCl_3$ ions have been localized to the endothelium with uptake of iron through endothelial pinocytic processes. Several studies have described ferric ion-rich membrane-enclosed bodies which transmigrate into the endothelium, followed by exocytosis into the vessel lumen.[19,20] It was assumed that this accumulation of iron and generation of reactive oxygen species produce endothelial toxicity and denudation, leading to the exposure of subendothelial elements that promote thrombus formation.[1,21] However, a number of studies have revealed minor endothelial denudation and collagen exposure following $FeCl_3$ treatment,[20,22,23] raising the possibility that $FeCl_3$ has effects beyond the vessel wall.

The first demonstration that red blood cells (RBCs) may play an important role in promoting $FeCl_3$-induced thrombosis was derived from our in vitro studies using isolated blood cell components perfused through mouse aortae ex vivo.[23] Surprisingly, endothelial cells exposed to $FeCl_3$ alone exhibited minor levels of injury. However, in the presence of whole blood or isolated RBCs, $FeCl_3$-induced red cell hemolysis and hemoglobin oxidation promoted extensive vascular injury and thrombosis.[23] Elegant electron microscopy studies by Barr and colleagues confirmed extensive red cell accumulation on the endothelium following $FeCl_3$ exposure in vivo, with platelets rapidly recruited to accumulated red cell-derived structures.[22] Eckly and colleagues revealed surface expression of tissue factor on the ferric ion-rich spherical bodies, which they attributed as the primary mediator of platelet adhesion and fibrin formation.[20]

The plot thickens further. Ciciliano and colleagues used microfluidic devices coated with endothelial cells to dissect the effects of $FeCl_3$ on individual blood cell and plasma components.[18] These studies demonstrated concentration-dependent effects of $FeCl_3$ on protein and blood cell aggregation, independent of effects on the endothelium. This aggregation effect was principally attributed to colloidal chemistry, whereby cells and proteins adhere and aggregate as a result of their charge. The authors have proposed that this physiochemical effect of $FeCl_3$ on blood cells is the primary instigator driving blood cell adhesion to the endothelium. They argue that this mechanism then facilitates the "secondary" phase of $FeCl_3$ injury, with red cell aggregates and damaged endothelium providing a reactive surface for the accumulation of platelets and initiation of blood coagulation, necessary for stable thrombus formation.[19-21] However, it remains to be determined to what extent oxidative damage vs physicochemical effects predominate to induce RBC aggregation and thrombosis.[23] In this context, aluminum chloride ($AlCl_3$), which carries a similar charge to Fe^{3+} and was used as an additional means of evidence to support the role of colloidal chemistry in cellular and protein aggregation, is also well known to cause oxidative damage, including lipid peroxidation and RBC hemolysis.[24] This is in contrast to chromium chloride ($CrCl_3$), which was also used in this study as a negative control; however, this molecule has antioxidant properties.[25] Whether the effects of $AlCl_3$ can be offset by antioxidants, as was demonstrated for $FeCl_3$ in our own ex vivo studies using isolated aorta,[23] will be important to determine. It is also interesting to note that the oxidative effects of $FeCl_3$ we observed in isolated aorta were initiated with concentrations of $FeCl_3$ lower than that observed to induce macroscopic precipitation of plasma proteins.[23]

Collectively, the studies highlighted above unequivocally demonstrate that the effects of $FeCl_3$ on vascular injury and