To the editor:

The *BRAF*-V600E mutation in circulating cell-free DNA is a promising biomarker of high-risk adult Langerhans cell histiocytosis

We read with great interest the recent review article on Langerhans cell histiocytosis (LCH) by Delprat and Aricò.[1] As they mentioned, LCH is a rare disorder characterized by local accumulation of dysplastic Langerhans cells and a wide range of organ involvement. Although the precise pathophysiology remains unknown, recent findings suggest that LCH is likely to be a clonally expanding myeloid neoplasm. One of the strongest lines of evidence is a report by Badalian-Very et al that the oncogenic *BRAF*-V600E mutation was detected in LCH lesions from a majority of patients.[2] Furthermore, Berres et al found that patients with active, high-risk LCH carried the *BRAF*-V600E mutation in circulating $CD11c^+/CD14^+$ cell fractions as well as in bone marrow $CD34^+$ progenitor cells.[3] In patients with various solid tumors, circulating cell-free DNA (cfDNA) in peripheral blood contains cancer-derived genomic DNA and has been used in a noninvasive diagnostic procedure, the so-called "liquid biopsy." In a recent report, *BRAF*-V600E was detected successfully in cfDNA from patients with colorectal cancer, with 100% sensitivity and specificity.[4] LCH can involve organs and tissues not readily accessible for biopsy, and the specimens are sometimes not available for genetic analyses after pathologic procedures. Thus, we evaluated the *BRAF* mutation in cfDNA as a potential biomarker of LCH using an allele-specific quantitative polymerase chain reaction (ASQ-PCR).

We cloned normal and mutant *BRAF* alleles that included exon 15 and its neighboring sequences into pCR2.1 to prepare a standard curve. cfDNA was prepared from the plasma of adult LCH patients by using the QIAamp DNA Blood Mini Kit (Qiagen) and was subjected to genotyping for the *BRAF* alleles by ASQ-PCR that was specifically designed to detect *BRAF*-V600E by using a 3′-phosphate-modified oligonucleotide blocker, according to Thierry et al.[4] Each assay reaction was performed in triplicate. The mutant *BRAF* load was estimated from the standard curve in each assay and was expressed as the mean percentage of mutant alleles relative to the total number of alleles by using the StepOnePlus Real-Time PCR System (Life Technologies).

Plasma cfDNA was prepared from 8 adult patients with LCH (listed in Table 1) as well as 8 normal participants. DNA from lesion tissues was not available for all patients. The mean quantity of cfDNA recovered from patients with LCH vs normal participants was 316.5 pg/mL (median, 290.4 pg/mL) vs 92.0 pg/mL (median, 91.8 pg/mL). Three high-risk patients with active multiple lesions were positive for *BRAF*-V600E but 8 normal participants were not. In these patients, the mean ratio of mutant *BRAF* alleles to total alleles was 3.25% (median, 2.59%). Immunohistochemical analyses that used a *BRAF*-V600E–specific antibody (Spring Bioscience) in biopsy specimens from 2 patients revealed that patient 3 (unique patient number 3 [UPN 3]) was positive for *BRAF*-V600E but UPN 7 was negative, which may be explained by the lower sensitivity of the detection method and/or the possibility that some but not all lesions are positive for *BRAF*-V600E in patients with multisystem LCH. Next, we compared the sensitivity of ASQ-PCR for *BRAF*-V600E between cfDNA and cellular DNA in the same blood sample. Naturally, much more DNA was recovered from mononuclear cells than from the same blood volume of plasma, but the ratio of mutant to total alleles was more than 10-fold higher in the cfDNA, suggesting that LCH-derived genomes are significantly enriched in cfDNA compared with cellular DNA and that cfDNA is adequate for liquid biopsies in LCH with *BRAF*-V600E.

Next, in UPN 7, we observed the mutant *BRAF* load during the course of initial chemotherapy. The ratio of mutant to total alleles was estimated as 1.00% prior to chemotherapy and was unmeasurable after chemotherapy. These data were compatible with the improved findings of computed tomography and positron emission tomography performed at the same time. Based on these results, ASQ-PCR for *BRAF*-V600E in cfDNA may contribute to planning risk-based treatment as well as monitoring treatment efficacy in LCH, especially in a group with active, high-risk LCH. Several *BRAF*-targeted inhibitors have been approved or are in clinical trials for various cancers with *BRAF* mutations, and one of those inhibitors, vemurafenib, is also active against LCH with *BRAF*-V600E.[5]

Despite an obviously very small cohort, we demonstrated the feasibility of *BRAF*-V600E in cfDNA as a biomarker of active, high-risk LCH. The utility of *BRAF*-V600E in cfDNA should be validated in a larger cohort of LCH patients.

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Table 1. Characteristics of patients with adult LCH

UPN	Age, years	Gender	Organ involvement	Risk	Activity	Treatment	*BRAF*-V600E immunohistostaining	*BRAF*-V600E (%)*
1	56	F	Multi	High	Inactive	Completed	N/A	0
2	38	F	Single	High	Inactive	Completed	N/A	0
3	65	F	Multi	High	Active	Interrupted	Positive	2.59 ± 0.21
4	48	M	Single	High	Inactive	During	N/A	0
5	41	F	Single	High	Inactive	During	N/A	0
6	28	M	Multi	High	Inactive	During	N/A	0
7	29	M	Multi	High	Active	Not started	Negative	1.00 ± 0.28
8	47	F	Multi	High	Active	Interrupted	N/A	6.16 ± 0.33

F, female; M, male; N/A, not available; UPN, unique patient number.
*Mean ± standard error.

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Acknowledgments: The authors thank Safia El Messaoudi and Alain R. Thierry (Institut de Recherche en Cancérologie de Montpellier, Montpellier, France) for technical advice in ASQ-PCR. We also appreciate Masanori Ohta for immunohistochemical analysis and the medical staff in the Department of Hematology/Oncology, Research Hospital, Institute of Medical Science, The University of Tokyo, for collecting samples from patients.

This work was supported by grants from the Japan LCH Study Group.

Contribution: M.K. designed and performed the experiment, analyzed data, and wrote the paper; and A.T. designed and supervised the experiment.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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To the editor:

Calreticulin mutation does not modify the IPSET score for predicting the risk of thrombosis among 1150 patients with essential thrombocythemia

An international prognostic score for the risk of thrombosis (IPSET-thrombosis) in essential thrombocythemia (ET) was developed.[1] Risk factors included the following: age >60 years (1 point), cardiovascular (CV) risk factors (1 point), previous thrombosis (2 points), and the presence of *JAK2*V617F mutation (2 points). Low-, intermediate-, and high-risk categories were identified by scores 0 to 1, 2, and ≥3, respectively. Mutations in the exon 9 of calreticulin (*CALR*) gene were recently identified in a large proportion of patients with *JAK2*V617F-negative ET and associated with a reduced thrombotic risk as compared with *JAK2*V617F-positive patients.[2-5] However, the utility of incorporating *CALR* mutation status into current risk stratification for thrombosis in ET is not yet tested. Answering this question was the purpose of the present study.

Under the auspices of the Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative, 4 Italian centers convened to create a database of 1150 patients previously diagnosed with and treated for ET. The study was approved by each Institutional Review Board. Patients' eligibility criteria included diagnosis

Table 1. Patients' characteristics at diagnosis

	Total	*CALR*+ (A)	*JAK2*V617F+ (B)	*MPL*W515+ (C)	*CALR*, *JAK2*, *MPL* wild type (D)	*P* A vs B	*P* A vs C	*P* A vs D
Number of patients, (%)	1150*	164 (14)	736 (64)	44 (4)	198 (17)			
Gender M/F, n (%)	403/739 (35/65)	84/80 (51/49)	266/470 (36/64)	13/31 (30/70)	40/158 (20/80)	<.0001	.010	<.0001
Age, years, median (5th-95th percentile)	57.6 (27-82)	53.5 (27-81)	60.8 (28-83)	59.7 (27-87)	47.8 (21-78)	.001	.396	.245
Hemoglobin, g/dL, median (5th-95th percentile)	14.1 (11.8-16.3)	13.7 (11.6-16.1)	14.5 (11.9-16.4)	13.4 (11.6-16.0)	13.6 (11.7-15.8)	<.0001	.681	.099
Hematocrit, %, median (5th-95th percentile)	43.0 (36.0-48.8)	42.1 (35.6-47.6)	43.7 (37.2-49.3)	41.8 (35.0-48.5)	41.0 (35.1-47.0)	.002	.880	.133
White blood cell count, $\times 10^9$/L, median (5th-95th percentile)	8.7 (5.4-14.7)	7.8 (5.2-12.0)	9.0 (5.7-15.1)	7.9 (4.8-14.0)	8.4 (5.3-14.0)	<.0001	.725	.034
Platelet count, $\times 10^9$/L, median (5th-95th percentile)	718 (486-1313)	842 (551-1769)	704 (490-1234)	834 (544-1700)	647 (464-1318)	<.0001	.971	<.0001
CV risk factors, n (%)	568 (50)	71 (43)	386 (52)	27 (61)	84 (42)	.034	.033	.868
Smoke, n (%)	98 (9)	7 (4)	66 (9)	5 (11)	20 (10)	.046	.073	.035
Diabetes, n (%)	107 (9)	11 (7)	77 (10)	5 (11)	14 (7)	.143	.303	.892
Hypertension, n (%)	459 (40)	59 (36)	314 (43)	21	65	.116	.497	.175
Previous major thrombosis, n (%)	167 (15)	13 (8)	122 (17)	9 (20)	23 (12)	.005	.016	.243
IPSET score, n (%)						<.0001	<.0001	.124
Low risk, n (%)	263 (23)	110 (67)	0 (0)	17 (39)	136 (69)			
Intermediate risk, n (%)	316 (28)	48 (29)	206 (28)	16 (36)	46 (23)			
High risk, n (%)	563 (49)	6 (4)	530 (72)	11 (25)	16 (8)			

*Eight patients with double positivity for *JAK2*V617F and *MPL*W515 were excluded from further analysis