Clinical phenotype of adult-onset systemic histiocytosis harboring *BRAF* in-frame deletions

L-group histiocytoses (Erdheim-Chester disease [ECD] and Langerhans-cell histiocytosis [LCH]) are multi-system diseases characterized by histiocyte infiltration in several organs.[1-3] In these diseases, histiocytes frequently display activating somatic mutations of intracellular signaling pathway protein kinases (mostly the MAPKinase pathway).[1-3] Many of these mutations seem to be associated with a specific phenotype: *BRAF*^{V600E}-mutated ECD patients more frequently have cardiac and retroperitoneal manifestations,[4] *MAP2K1*-mutated ECD patients may exhibit overt Rosai-Dorfman disease (RDD) component,[5] and *ALK*-mutated patients have a high prevalence of neurological manifestations.[6] Therefore, we suspect that each specific mutation or mutation type could be associated with a specific clinical phenotype of histiocytosis. Our objective was to describe characteristics of patients with histiocytosis and in-frame deletion within exon 12 of *BRAF* (*BRAF*$^{\Delta\beta3\text{-}\alpha C}$).

Inclusion criteria were: 1) diagnosis of histiocytosis confirmed by central review according to the most recently published guidelines;[3,7] 2) presence of *BRAF*$^{\Delta\beta3\text{-}\alpha C}$; and 3) clinical data available. Patients were retrieved from the files of the pathology laboratory in Ambroise-Paré Hospital (Boulogne, France). Clinical, biological, and morphological data were retrieved, as well as treatment received and follow-up.

DNA was extracted as previously described.[8] Since 2020, DNA extraction has been automated on a Maxwell® RSC Instrument (Promega, France), with extraction performed according to the supplier's recommendations. For formalin-fixed paraffin-embedded (FFPE) and frozen biological materials, the Maxwell® RSC DNA FFPE Kit and Maxwell® RSC Tissue DNA Kit were used, respectively. The DNA-Seq next-generation sequencing (NGS) panel included almost 60 genes covering hot spots or all exons previously reported to be mutated in histiocytoses and genes involved in the MAP-kinase pathway and myeloid neoplasia. The sequencing data were analyzed depending on the applied technique. Mutations detected by DNA sequencing were interpreted according to standards and guidelines previously described.[9]

We identified patients with *BRAF*$^{\Delta\beta3\text{-}\alpha C}$ and contacted the centers for clinical, morphological, and biological data.

This study was conducted in accordance with the Declaration of Helsinki. Patients provided written informed consent (*clinicaltrials.gov 04437381*: Molecular Targets for the Treatment of Histiocytosis HISTIO-TARGET).

In 429 patients in the L-group histiocytosis cohort, 189 had a *BRAF*^{V600E} mutation (46.2%), 25 had a *BRAF*$^{\Delta\beta3\text{-}\alpha C}$ (5.8%), and 9 another *BRAF* mutation (2.1%). Among patients with *BRAF*$^{\Delta\beta3\text{-}\alpha C}$, data were available for 20 of them. Patient and mutation characteristics are described in Table 1 and Figure 1. Most patients (N=18) had LCH, and 2 had an ECD, one pure and the other mixed with LCH. Median age at diagnosis was 50.5 years (interquartile range [IQR], 34-78). The most frequent manifestations were hepatic (N=9, 45%) and vulvar (8/11 female patients, 73%). Other localizations were cystic interstitial lung disease (N=7), lytic bone lesions (N=8), classical cutaneous manifestations (N=8), diabetes insipidus (N=8), panhypopituitarism (N=3), pachymeningitis (N=2), long bone osteosclerosis (N=1), perirenal infiltration (N=1), salivary gland infiltration (N=1), and digestive track infiltration (N=1). Among patients with LCH, 2 patients had a single-system pulmonary disease, one patient a single-system liver disease, and one patient a single-system multifocal bone disease; all other patients had multi-system manifestations.

Hepatic manifestation was sclerosing cholangitis in all patients and 5/6 patients had histiocytic infiltration in liver biopsy. All patients with sclerosing cholangitis had biological cholestasis, elevated aminotransferases, and hyperbilirubinemia. No patient had cirrhosis. Hepatic magnetic resonance imaging (MRI), when performed, always showed cholangitis (6/6). Positron emission tomography scan showed liver abnormalities in 4/7 patients (heterogenous liver uptake or uptake in biliary ducts). Six patients had additional mutations in tissue biopsy, including *DNMT3A* (N=4), *TET2* (N=2), *ASXL1* (N=1), and *PGDFRA* (N=1). Among 5 patients who had a bone marrow aspiration, 4 of them had additional mutations, including *DNMT3A* (N=2), *TET2* (N=2), *STAG1* (N=1), *PPM1D* (N=1), and *RAD21* (N=1).

First-line treatments included vinblastine (N=6), cytarabine (N=1), methotrexate (N=1), cladribine (N=3), lenalinomide (N=1), and cobimetinib (N=1), with various responses depending on the clinical manifestation. Nine patients did not receive any specific treatment for histiocytosis. Four patients with cholangitis received ursodeoxychlic acid without significant improvement. Patients with cholangitis also received vinblastine (N=3 with one disease progression, one stable disease, and one partial remission), cladribine (N=1 with stable disease), cytarabine (N=1 with stable disease), and lenalimode (N=1 with stable disease). Two patients received cobimetinib that resulted in partial remission in both patients (PERCIST criteria) at six months, while liver function tests and bili-MRI remained stable in one patient (Figure 2). One patient had a liver transplant, with no further relapse. After a median follow-up of 47 (IQR, 13-315) months, one patient had died from coronary heart disease.

Table 1. Characteristics of L-group histiocytosis patients with *BRAF* in-frame deletion.

Patient	Gender	Age at diagnosis in years	*BRAF* mutation	Histiocytosis	Clinical manifestations	Treatment & outcome	Follow-up duration in months
#1	M	78	c.1459_1473del	LCH, ECD	Sclerosing cholangitis, cystic interstitial lung disease, lytic bone lesions	None (death from coronary heart disease)	14
#2	M	74	c.1457_1471del	LCH	Sclerosing cholangitis	UDCA (SD)	11
#3	M	66	c.1457_1471del	LCH	Cystic interstitial lung disease	None	29
#4	F	59	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, lytic bone lesions, diabetes insipidus	Vinblastine (disease progression), cladribine (disease progression)	98
#5	F	65	c.1457_1471del	LCH	Sclerosing cholangitis, vulvar manifestations, cutaneous manifestations, diabetes insipidus	Liver transplantation (remission)	55
#6	M	56	c.1471_1476del	ECD	Long bone osteosclerosis, pachymeningitis, perirenal infiltration	Cobimetinib (PR)	120
#7	F	61	c.1457_1471del	LCH	Sclerosing cholangitis, vulvar manifestations, lytic bone lesions, diabetes insipidus	UDCA (SD), vinblastine (disease progression), cobimetinib (PR)	43
#8	F	33	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, cystic interstitial lung disease, lytic bone lesions, diabetes insipidus, panhypopituitarism	Methotrexate (SD)	315
#9	F	56	c.1457_1471del	LCH	Sclerosing cholangitis, cystic interstitial lung disease	None	0
#10	F	45	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, lytic bone lesions, diabetes insipidus, pachymeningitis	None	89
#11	M	41	c.1457_1471del	LCH	Sclerosing cholangitis, cutaneous manifestations, salivary gland infiltration	Cytarabine (skin improvement, cholangitis stability), cladribine (skin improvement, cholangitis stability), lenalinomide (skin improvement, cholangitis stability)	44
#12	M	34	c.1459_1473del	LCH	Cystic interstitial lung disease	None	3
#13	F	21	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, diabetes insipidus, panhypopituitarism	Cladribine (CR)	148
#14	M	23	c.1457_1471del	LCH	Sclerosing cholangitis, digestive track infiltration	UDCA (SD), vinblastine (PR)	62
#15	M	17	c.1458_1472del	LCH	Lytic bone lesions	None	42
#16	F	39	c.1458_1472del	LCH	Cystic interstitial lung disease	Vinblastine (PR)	2
#17	F	58	c.1457_1471del	LCH	Vulvar manifestations, panhypopituitarism	None	10
#18	F	38	c.1457_1471del	LCH	Sclerosing cholangitis, cystic interstitial lung disease	None	50
#19	F	73	c.1457_1471del	LCH	Sclerosing cholangitis, cystic interstitial lung disease, vulvar manifestations, cutaneous manifestations, diabetes insipidus	UDCA (SD), vinblastine (SD)	77
#20	M	11	c.1457_1471del	LCH	Cystic interstitial lung disease, perianal manifestations, diabetes insipidus, lytic bone lesions	Vinblastine (remission and relapse)	146

M: male; F: female; LCH: Langherans cell histiocytosis; ECD: Erdheim-Chester disease; UDCA: ursodeoxycholic acid; SD: stable disease; PR: partial remission; CR: complete remission.

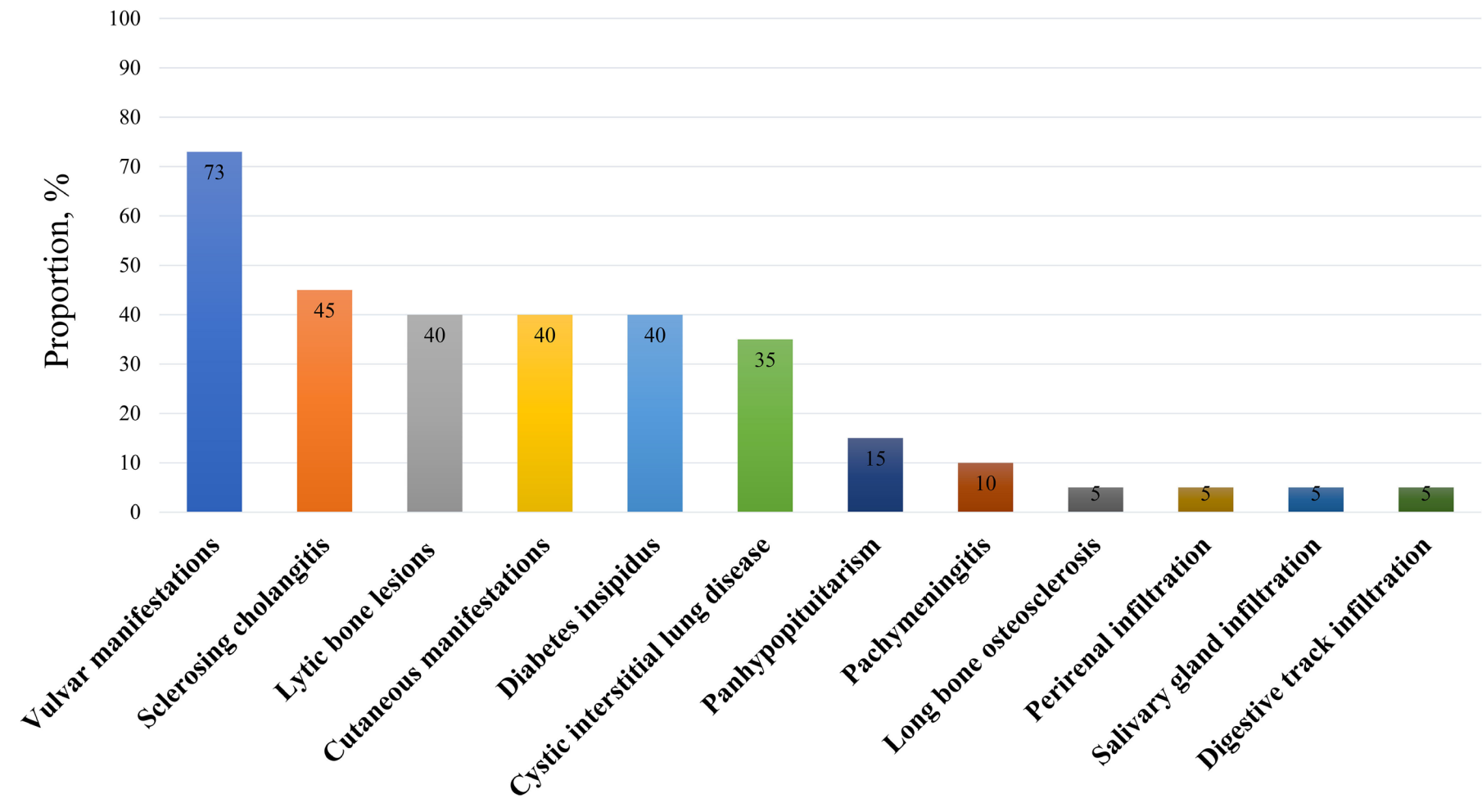

Figure 1. Proportion of clinical manifestations in patients with histiocytosis and *BRAF* in-frame deletions.

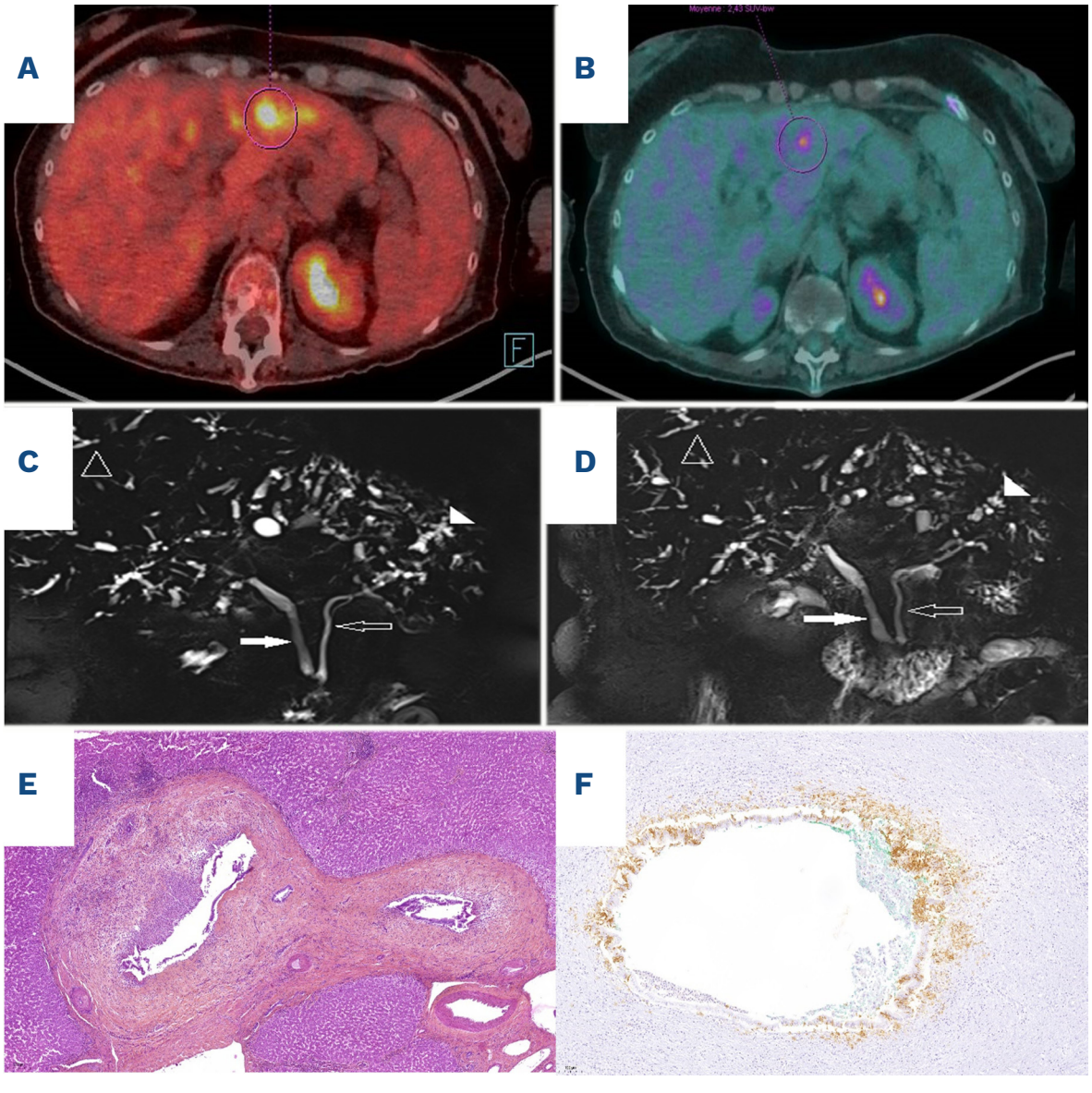

Figure 2. Sclerosing cholangitis in Langerhans cell histiocytosis patients with *BRAF* in-frame deletion. (A) Intense and diffuse hypermetabolism of the intrahepatic biliary ducts (Standardized Uptake Value [SUV] max 9.5) on FDG positron emission tomography-computed tomography (PET-CT) before cobimetinib onset. (B) Partial regression of intense and diffuse hypermetabolism of the intrahepatic biliary ducts (SUVmax 5.9) on FDG PET-CT six months after cobimetinib onset. (C, D) Magnetic resonance imaging cholangiopancreatography performed at onset (C) and after six months (D) of cobimetinib treatment. The main bile duct (white arrow) is normal, as is the main pancreatic duct ("empty arrow"). Numerous peripheral bile ducts appear multifocally dilated and suspended in the right (empty arrowhead) or left (arrowhead) liver. The successive examinations showed no change in the number, distribution or dilatation of intrahepatic bile ducts. (E, F) Large portal tract with destructive infiltration of biliary duct by numerous mononucleated histiocytes (x50) (E) expressing CD1a (F).

Clinical manifestations of L-group histiocytosis may vary from single-organ benign disease to multi-organ, life-threatening neoplasm.[2,3] To date, the cause of the variety of clinical manifestations in these diseases is unknown, and the type of mutation involved could play a role in the clinical phenotype.[4-6]

Our study is the first to describe the clinical phenotype of histiocytosis patients with a *BRAF*$^{\Delta\beta3\text{-}\alpha C}$, and showed a high frequency of sclerosing cholangitis and vulvar manifestations, which are typical LCH manifestations, although they are rarely observed. In previous published cohorts of adults, liver manifestations are described in 10-15% of LCH cases,[10] and a study of 14 pediatric patients with LCH and liver involvement showed a 100% prevalence of *BRAF*^{V600E} mutation.[11] Vulvar manifestations have only been described in some case series.[12] They can present as erythematous plaques, eczema, ulcer or polypoid appearance, which are non-specific, and sometimes it can mimic many other diseases, such as squamous cell carcinoma, malignant melanoma, herpes or some inflammatory reaction.[12]

BRAF$^{\Delta\beta3\text{-}\alpha C}$ were described in pancreatic, lung, ovarian, thyroid cancers, and melanoma;[13] they also occur in histiocytoses. These oncogenic deletions are predicted to shorten the $\beta3$ / αC-helix loop, which could favor dimer formation. They are resistant to the BRAF monomer inhibitors, such as vemurafenib, but sensitive *in vitro* to BRAF dimer inhibitors and MEK inhibitors.[14] So far, only 2 patients with histiocytosis harboring *BRAF*$^{\Delta\beta3\text{-}\alpha C}$ have been reported with targeted therapy, and both had complete remission with either trametinib[15] or cobimetinib.[16] Two patients of our series were treated with MEK inhibitors, with partial remission on PERCIST criteria, but no significant improvement in liver function tests or MRI cholangiopancreatography (Figure 2). Based on the low response rate of standard chemotherapy in liver locations, those patients may require first-line treatment with MEK-inhibitors.

To conclude, *BRAF*-deletion mutations in histiocytoses seem to be associated with a specific LCH pattern with high prevalence of hepatic and vulvar involvements. These manifestations should be carefully screened in these patients. These results also comfirm the hypothesis that each specific mutation in histiocytosis correlates with a specific clinical phenotype.

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Disclosures

No conflicts of interest to disclose.

Contributions

MP, JH and JFE are responsible for study conception and design. MP, JR, MDS, ZHR, VP, SB, VLS, SD, RL, QM, DT, TA, MC, VK, ER, OL, FCA, ZA, JH and JFE are responsible for acquisition of data and performing experiments. MP, JH and JFE are responsible for data analysis, and drafting and writing of the manuscript. All authors contributed to reviewing the manuscript and approved the final version for publication.

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Data-sharing statement

All data relevant to the study are included in the article or uploaded in the *Online Supplementary Appendix*.

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