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MYELOID NEOPLASIA

High prevalence of myeloid neoplasms in adults with non–Langerhans cell histiocytosis

Matthias Papo,[1,*] Eli L. Diamond,[2,*] Fleur Cohen-Aubart,[1,*] Jean-François Emile,[3,4] Damien Roos-Weil,[5] Nishant Gupta,[6] Benjamin H. Durham,[7] Neval Ozkaya,[7] Ahmet Dogan,[7] Gary A. Ulaner,[8] Raajit Rampal,[9] Jean-Emmanuel Kahn,[10] Thomas Sené,[11] Frédéric Charlotte,[12] Baptiste Hervier,[13] Caroline Besnard,[1] Olivier A. Bernard,[5] Catherine Settegrana,[14] Nathalie Droin,[15] Zofia Hélias-Rodzewicz,[3,4] Zahir Amoura,[1] Omar Abdel-Wahab,[9,16,*] and Julien Haroche[1,*]

[1]Service de Médecine Interne 2, Institut E3M, Centre de Références des Maladies Auto-Immunes et Systémiques Rares, Centre de Référence des Histiocytoses, Hôpital de la Pitié-Salpêtrière, Assistance Publique–Hôpitaux de Paris (AP-HP), Université Pierre et Marie Curie, Paris, France; [2]Department of Neurology, Memorial Sloan Kettering Cancer Center, New York, NY; [3]EA4340, Versailles University, Paris-Saclay University, Boulogne, France; [4]Pathology Service, Hôpital universitaire Ambroise Paré, AP-HP, Boulogne, France; [5]UMR1170, INSERM and Institute Gustave Roussy, Villejuif, France; [6]Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Internal Medicine, University of Cincinnati, Cincinnati, OH; [7]Department of Pathology, [8]Department of Radiology, and [9]Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY; [10]Department of Internal Medicine, Foch Hospital, Suresnes, France; [11]Department of Internal Medicine, Croix Saint-Simon Hospital, Paris, France; [12]Department of Pathology, [13]Department of Internal Medicine and Immunology, and [14]Department of Biological Hematology, University Hospital La Pitié Salpêtrière, AP-HP, Paris, France; [15]Gustave Roussy, Paris-Saclay University, Genomic Platform UMS AMMICA, Villejuif, France; and [16]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

Key Points

- Some 10.1% of adults with non–Langerhans cell histiocytosis have a concomitant myeloid neoplasm with each often harboring distinct mutations.
- The presence of distinct kinase mutations in histiocytosis and myeloid neoplasms resulted in discordant responses to targeted therapy.

Erdheim-Chester disease (ECD) is a rare non–Langerhans cell histiocytosis that most commonly affects adults and is driven by a high frequency of mutations in *BRAF*, *MAP2K1*, and kinases promoting MAPK signaling. Because of the relative rarity of ECD, key clinical features of the disease may not be well defined. Across a multi-institutional cohort of 189 patients with ECD and ECD overlapping with Langerhans cell histiocytosis (so-called mixed histiocytosis [MH]), we identified an unexpected and heretofore undescribed frequent occurrence of myeloid neoplasms among patients with ECD and MH. Some 10.1% (19/189) of patients with ECD have an overlapping myeloid neoplasm, most commonly occurring as a myeloproliferative neoplasm (MPN), myelodysplastic syndrome (MDS), or mixed MDS/MPN overlap syndrome (including chronic myelomonocytic leukemia). Consistent with this, molecular analysis frequently detected hallmark driver mutations of myeloid neoplasms (such as *JAK2*V617F and *CALR* mutations) coexisting with those characteristic of histiocytosis (such as *BRAF*V600E and *MAP2K1* mutations). Histiocytosis patients diagnosed with a concomitant myeloid malignancy were significantly older at diagnosis and more commonly presented with MH than those without a myeloid malignancy. In some cases, the presence of distinct kinase mutations in the histiocytosis and myeloid neoplasm resulted in discordant and adverse responses to kinase-directed targeted therapies. These data highlight the clinical importance of evaluating adults with histiocytosis for a concomitant myeloid neoplasm. (*Blood*. 2017;130(8):1007-1013)

Introduction

Erdheim-Chester disease (ECD) is a form of histiocytosis characterized by tissue infiltration with foamy histiocytes that are $CD68^{+}$, $CD163^{+}$, $CD1a^{-}$, and Langerin $(CD207)^{-}$. Prior to 2012, there was a long-standing debate as to whether ECD represented a clonal hematopoietic disorder vs an inflammatory disease related to aberrant immune activation. However, since 2010, a series of genomic studies have uncovered *BRAF*V600E mutations in 55% to 70% of ECD as well as Langerhans cell histiocytosis (LCH) patients, providing evidence that these diseases represent clonal disorders driven by activated MAPK signaling.[1,2] Subsequently, activating mutations in *MAP2K1*,[3-6] *ARAF*,[6,7] and fusions in kinases including *BRAF*[6,8] were found in the majority of *BRAF*V600-wild-type ECD and LCH patients. *PI3KCA*[9] and *N/KRAS* mutations are more frequent in ECD than in LCH. Despite the distinct clinical and histologic presentations of LCH and ECD, the previous studies identify a similar constellation of genomic alterations across both disorders. Moreover, nearly 20% of patients with ECD have a diagnosis of both ECD and LCH simultaneously (so-called mixed histiocytosis [MH]) where both lesions may contain the *BRAF*V600E mutation.[10] For these reasons, ECD and LCH have been grouped together in the

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*M.P., E.L.D., F.C.-A., O.A.-W., and J.H. contributed equally to this study.

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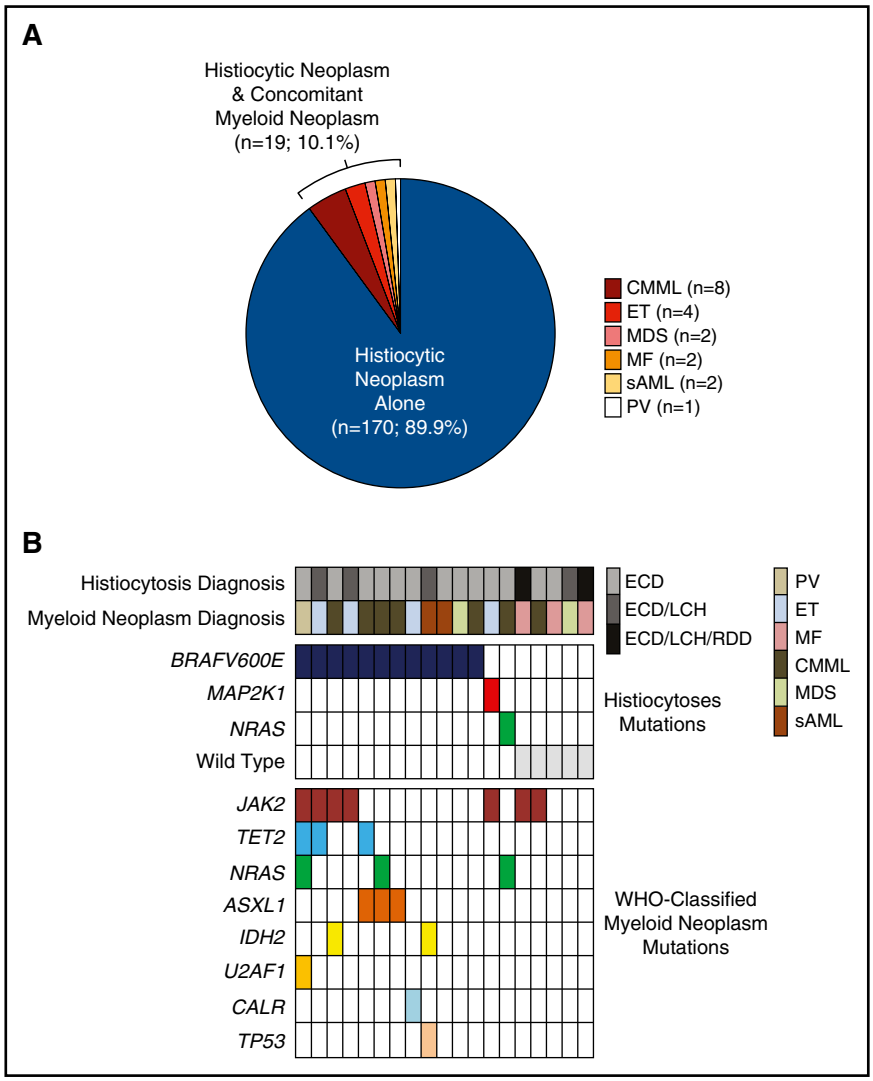

Figure 1. Distribution of myeloid neoplasms in patients with concomitant non-LCH and genetic analysis of both disorders. (A) Pie chart demonstrating proportion of non-LCH patients with concomitant myeloid neoplasm and types of myeloid neoplasms diagnosed. ET, essential thrombocytosis; MDS, myelodysplastic syndrome; MF, primary myelofibrosis; PV, polycythemia vera; sAML, secondary acute myeloid leukemia transformed from antecedent hematological malignancy. (B) Genetic analysis of non-LCH and concomitant myeloid neoplasm. Each patient is noted by a column. Patients had clinical diagnosis of ECD or an overlap of ECD plus LCH or ECD/LCH plus Rosai Dorfman disease (RDD) based on tissue biopsy and clinical evaluation in addition to a form of WHO-classified myeloid. Mutations identified in histiocytosis tissue lesion biopsy alone in each patient are noted in the middle boxes, and those mutations detected in PB or BM mononuclear cells are noted in bottom boxes.

same class of conditions in a recent revision of histiocytosis classifications.[11]

Although there have been great molecular advances in ECD and LCH over the last 7 years, our knowledge of the natural history of ECD and MH and their associated clinical and prognostic features remains incomplete. Nevertheless, despite great recent progress in the understanding of histiocytosis, the cellular origins of LCH, ECD, and MH are not completely understood.

In hopes of clarifying the clinical and molecular features of ECD and MH and facilitating translational research in these disorders, we assembled ECD patient data across 2 institutions in Europe and the United States with a specific research focus on ECD. Using these data, we have uncovered an unexpected high frequency of concomitant clinically diagnosed myeloid neoplasms in patients diagnosed with ECD and MH. Here we describe the clinical, pathological, and molecular features of the hematological disorders associated with ECD. Diagnosis of a concurrent myeloid neoplasm and histiocytosis had important therapeutic consequences for patients. These findings have clear clinical relevance in addition to potentially enlightening our understanding of the etiology of ECD and its disease classification.

Methods

Study design

We performed an international 2-center retrospective study of patients with biopsy-proven ECD who were referred at least once to the Internal Medicine Department of Pitié-Salpêtrière Hospital (Paris, France) or Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY) between November 1981 and November 2016. A total of 189 cases of ECD were reviewed (39 from the MSKCC and 150 from the Pitié-Salpêtrière Hospital; 12 of which were also studied by whole exome sequencing previously[6]). Written informed consent was obtained from patients according to Helsinki convention, and this study received approval from the Ethics Committee Ile-de-France III and the Institutional Review Board at MSKCC.

Patients

ECD was diagnosed according to published criteria: (1) tissue biopsy demonstrating typical pathological signs of ECD (infiltration with foamy histiocytes and $CD68^{+}CD1a^{-}$ on immunohistochemical staining); (2) symmetric skeletal uptake on ^{99m}Tc bone scintigraphy or 18-fluorodeoxyglucose positron emission tomographic (^{18}F-FDG PET) or magnetic resonance imaging scan with the involvement of at least 1 other organ typically affected in ECD (xanthelasma, perinephric infiltration, "coated aorta," pericardial infiltration,

Table 1. Comparison of clinical and biological characteristics of ECD patients with or without concomitant myeloid neoplasm

Variables	Myeloid neoplasm (n = 19)	Others (n = 170)	*P*
MH, %	6 (31.5)	18 (10.6)	.02
Male sex, %	17 (89.4)	115 (68.8)	.06
Age, y	68 (60-73)	56.5 (46-66)	.0005
*BRAF*V600E mutation in ECD or MH, %	12 (63.2)	89 (52.3)	.37
*JAK2*V617F mutation in MPN/MDS, %	7 (36.8)	0 (0)	<.0001
Elevated CRP, %	15 (78.9)	130 (76.4)	1.00
Bone scintigraphy or PET uptake, %	14 (73.7)	155 (91.1)	.018
Number of sites	3 (2-4)	3 (2-5)	.21
Coated aorta, %	8 (42.1)	66 (38.8)	.78
Pericardial infiltration, %	6 (31.5)	42 (24.7)	.51
Right auricular pseudotumor, %	6 (31.5)	57 (33.5)	.86
Xanthelasmas, %	2 (10.5)	41 (24.1)	.25
Exophthalmos, %	4 (21.0)	36 (21.1)	1.00
CNS infiltration, %	2 (10.5)	68 (40.0)	.01
Pituitary infiltration, %	2 (10.5)	35 (20.6)	.37
Perirenal infiltration, %	14 (73.6)	94 (55.9)	.12
IFN treatment, %	13 (68.4)	110 (64.7)	.74
BRAF inhibitor treatment, %	7 (36.8)	30 (17.6)	.045
Death, %	8 (42.1)	33 (19.4)	.002
Survival, mo	82 (40-99)	364 (129-364)	.001

CNS, central nervous system; CRP, C-reactive protein; IFN, interferon-α.

right atrial pseudotumor, or brain or dural infiltration). Patients with MH were included as well. Bone marrow (BM) aspirations and biopsies were performed according to standard of care when a complete blood count abnormality (anemia, thrombocytopenia, polycythemia, thrombocytosis, or monocytosis) was noted and not explained by nonmalignant causes such as iron, vitamin B12, or folic acid deficiencies or inflammatory syndrome. Hematological malignancies (myeloid neoplasms, lymphoma, and myeloma) were diagnosed according to the 2016 revision to the World Health Organization (WHO) classification of hematological malignancies.[12,13] For each patient, clinical parameters (age at diagnosis, sex, and main organ involvement), laboratory parameters (blood count, electrolytes, liver function tests, and C-reactive protein), and outcome (treatments and death) were obtained from medical records.

Molecular analyses

For patients from Pitié-Salpêtrière Hospital, *BRAF*V600E mutational status was evaluated as previously described.[2] *JAK2*V617F mutational status was evaluated in BM aspirate, peripheral blood (PB), or tissue biopsy using the TaqMan method with an Applied 7500 automaton. For patients with available material, targeted deep sequencing of the exonic regions of 24 genes (*ASXL1*, *ATRX*, *BCOR*, *BCORL1*, *CBL*, *CEBPA*, *CSF3R*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *GATA2*, *JAK2*, *KDM6A*, *KIT*, *NRAS*, *RUNX1*, *SETBP1*, *SF3B1*, *STAG2*, *TET2*, *TP53*, *WT1*, and *ZRSR2*) was performed on flow-sorted $CD14^+$ cells (for patients with chronic myelomonocytic leukemia [CMML]) or $CD14^-CD15^+CD16^+$ polymorphonuclear cells (for patients with classic myeloproliferative neoplasms [MPNs]) as well as T cells ($CD3^+CD19^-CD14^-CD34^-$) for germ-line control. This specific gene panel was used as it includes the most frequently mutated genes in myeloid neoplasms. Targeted regions were polymerase chain reaction amplified using an AmpliSeq (Life Technologies), and polymerase chain reaction products sequenced with a MiSeq (Illumina, San Diego, CA) instrument with a mean depth of 1000X (range 590-1800). We used a variant allele frequency minimal threshold of 10% to detect mutation.

For patients from MSKCC, genomic analysis of histiocytosis tissue biopsy and PB mononuclear cells was performed using the MSKCC IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) assay as previously described.[14]

Statistical analysis

Continuous variables were expressed as median and range, and categorical variables as numbers and percentages. Differences between groups of patients were tested with the Mann-Whitney *U* test for continuous data, and by Fischer exact test or the χ^2 test for categorical data. Survival analyses were performed with Kaplan-Meier curves and log-rank test. We used SAS version 9.0 (SAS Institute) and GraphPad Prism 5 for analyses.

Results

Frequent occurrence of myeloid neoplasms in patients with ECD

We reviewed 189 cases of ECD, including MH. Associated hematological disorders (excluding another histiocytosis) were observed in 23 patients (12.2%). Apart from patients with lymphoproliferative or autoimmune disorders (2 patients had lymphoma, 2 had immune thrombocytopenic purpura, 1 had myeloma, and 1 developed an acute lymphoblastic leukemia [ALL] in the context of a primary myelofibrosis), hematological diseases associated with ECD were myeloid neoplasms (10.1%) (Figure 1; supplemental Table 1 and supplemental Figure 1, available on the *Blood* Web site). This frequency was higher in the United States (15.3%) than the French (8.6%) cohort ($P = .22$). Among these 19 patients, 8 had CMML; 4, ET; 2, MDSs; 2, primary myelofibrosis; 2, AML (1 secondary to MDS and 1 to PV); and 1, PV. One patient also developed an ALL in the course of his MPN. Seven patients were diagnosed with myeloid neoplasm before the ECD diagnosis (median 4 years between the 2 diagnoses, range 1-22 years), 6 were diagnosed simultaneously, and 6 were diagnosed with myeloid neoplasm after a diagnosis of histiocytosis (median 1 year, range 1-4 years).

ECD patients with a myeloid neoplasm were also more likely to have a diagnosis of an overlap histiocytosis (ECD associated with LCH [n = 6] and Rosai Dorfman disease [n = 2]) than those patients with ECD and no concomitant myeloid neoplasm ($P = .02$; Table 1). In addition, patients with ECD plus a concomitant myeloid neoplasm were significantly older at ECD diagnosis (68 vs 56.5 years; $P = .0005$) and had a lower survival (82 vs 364 months; $P = .001$) than ECD patients without a myeloid neoplasm (supplemental Figure 2). Deaths were mainly because of cardiac insufficiency or infections, but 2 patients died of hematological disease (patient #1 died of ALL, and patient #11 died of an AML secondary to MDS).

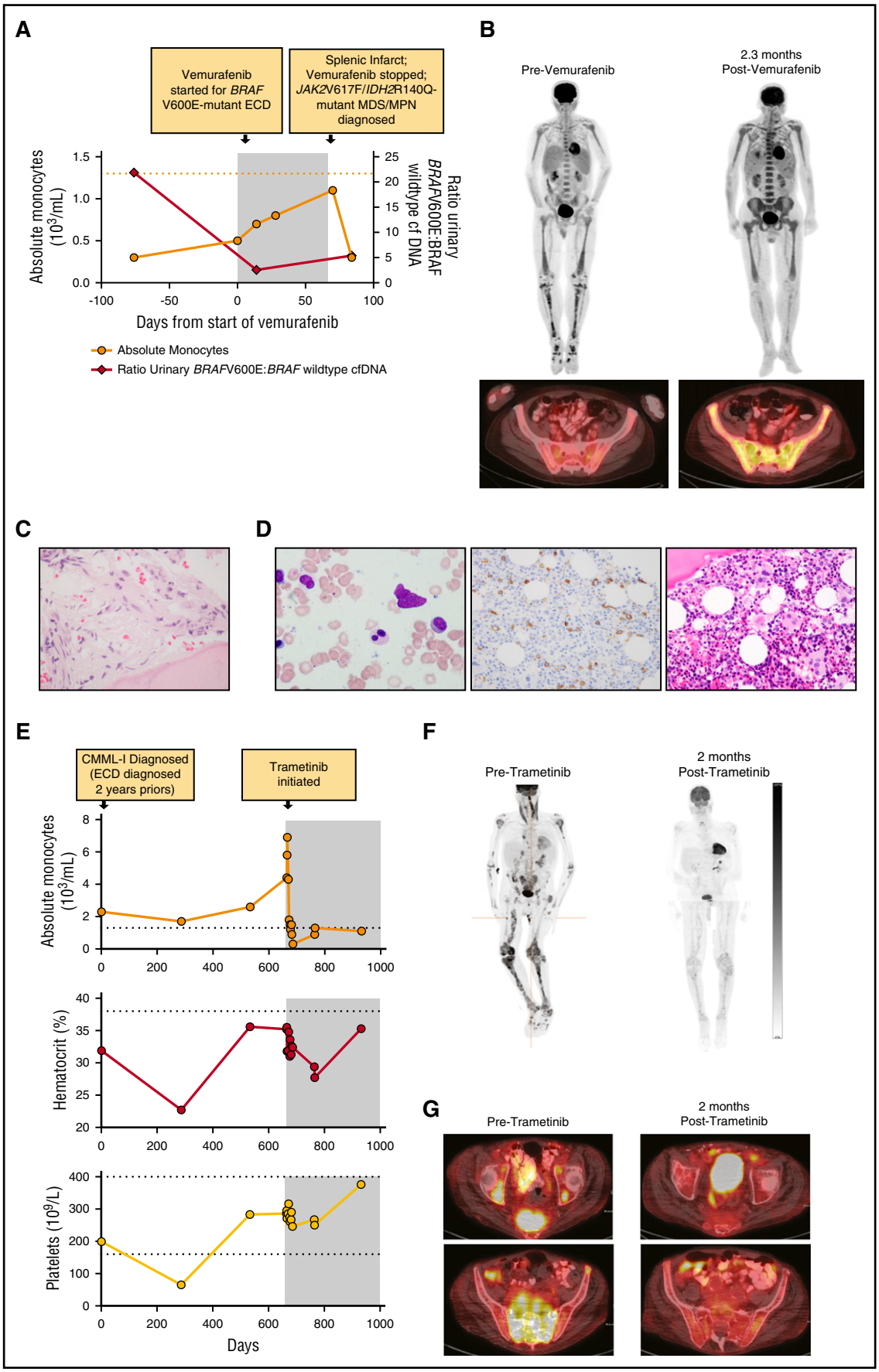

Figure 2.

Molecular analysis of patients with ECD and concomitant myeloid neoplasm

Given the unexpected high frequency of myeloid neoplasms associated with histiocytosis, we next sought to examine the molecular features of histiocytosis lesions and the coexisting myeloid neoplasms in these individuals. Many of these patients harbored kinase alterations characteristic of both ECD and myeloid neoplasms. Among the 19 patients with concomitant myeloid neoplasm and ECD, 12 (63.2%) harbored the *BRAF*V600E mutation in ECD tissue biopsy material, and 7 (36.8%) were positive for *JAK2*V617F in PB and/or BM. Interestingly, 4 patients (23.5%) had both *BRAF*V600E and *JAK2*V617F mutations simultaneously (Figure 2). Similarly, 1 patient with ET had a *CALR* mutation as well as the *BRAF*V600E mutation, and another had a *MAP2K1* mutation in the histiocytic disease associated with a *JAK2*V167F mutated ET. Unlike *BRAF*V600E or *MAP2K1* mutations, which were detected only in histiocytosis lesions, mutations in *NRAS* could be found in both histiocytosis and myeloid neoplasms, as exemplified by a patient who had the same *NRAS* mutation in ECD lesions from perirenal tissue as well as BM and PB following a diagnosis of CMML. In addition to harboring mutations in *JAK2* and *CALR*, patients with myeloid neoplasm-associated histiocytosis also carried additional mutations in transcriptional regulatory genes common in myeloid neoplasms but rare in ECD,[6] such as mutations in *TET2*, *ASXL1*, *IDH2*, *U2AF1*, and *TP53* (Figure 1).

Effect of targeted therapies on coexisting ECD and myeloid neoplasm

Although BRAF inhibition has resulted in remarkable clinical responses for patients with *BRAF*V600E-mutant histiocytosis,[15-18] there is a well-described risk of paradoxical activation of cytokine signaling in cells bearing kinase mutations other than *BRAF*V600E upon exposure to RAF inhibitors.[19-22] BRAF inhibitors (vemurafenib [n = 6] or dabrafenib [n = 1]) were given to 7/19 patients with *BRAF*V600E-mutant ECD and coexisting myeloid neoplasm here. In 3 cases, vemurafenib treatment resulted in an increase in blood counts, which led to treatment discontinuation. For example, patient #14 was treated with vemurafenib for *BRAF*V600E-mutant ECD with a partial metabolic response at 2.3 months and substantial reduction in *BRAF*V600E mutant allele burden as measured by analysis of urinary cell-free DNA[23] (Figure 2A-B). However, vemurafenib was discontinued at this time point because of a significant increase in monocyte count in conjunction with development of abdominal pain because of a new splenic infarct. Although PET scan at this point revealed resolution of FDG-avid femur lesions characteristic of ECD, there was a paradoxical increased in FDG avidity in all other major bones of the body (Figure 2A-C). This constellation of findings prompted a BM aspirate and biopsy that led to a diagnosis of *JAK2*V617F/*IDH2*R140Q-mutant MDS/MPN overlap disorder (Figure 2D). The patient's monocytosis returned to normal levels following vemurafenib discontinuation further suggesting that paradoxical stimulation of *JAK2*-mutant cells in response to RAF inhibition was the basis for the patient's adverse response to vemurafenib (Figure 2).

In contrast to the previous cases where distinct activating kinase mutations complicated use of a single kinase inhibitor, in other instances where the histiocytosis and myeloid neoplasm shared the same kinase mutation, use of targeted therapeutics resulted in beneficial response across both conditions. For example, patient #13 had *NRAS*-mutated ECD associated with a CMML-1 with the same *NRAS* mutation. MEK inhibition with cobimetinib in this patient led to a complete metabolic response by PET scan after 2 months in addition to improving monocyte and platelet counts (Figure 2E-G).

Discussion

This study identifies a high prevalence (10.1%) of myeloid neoplasms among adults with non-LCH. This frequency of myeloid neoplasms is much higher than that encountered general population which ranges between 0.7 and 17.1 per 100 000 people in Europe, depending on the subtype.[24] Although prior case reports and case series[25-29] have noted additional hematological malignancies in patients with histiocytosis of the L group,[11] the high frequency of myeloid neoplasms in a large population of adult histiocytosis patients has not previously been recognized, likely because of the relative rarity of ECD. Nonetheless, knowledge of the presence of an associated myeloid neoplasm in ECD patients has important implications for clinical management of adult histiocytosis patients as well as the classification and biological understanding of these disorders. The difference in prevalence of myeloid neoplasms in histiocytosis patients between MSKCC (15.3%) and Pitié-Salpêtrière Hospital (8.6%) could be attributable to an institution bias, because Pitié-Salpêtrière Hospital is not a referral center for neoplastic diseases. Although this might result in a skewed estimation of the prevalence of myeloid neoplasms among histiocytosis patients, the prevalence at either center remains high.

Histiocytosis patients with an associated myeloid neoplasm seemed to be significantly older than those without a myeloid neoplasm. One hypothesis for this observation could be the well-described association between aging and clonal hematopoiesis with an increased frequency of myeloid neoplasms because of acquisition of somatic mutations in genes commonly mutated in myeloid neoplasms.[30-32] Moreover, the lower survival rate observed in histiocytosis patients with coexisting myeloid neoplasms might be attributable to the older age of those patients and not to a more severe disease.

From a clinical standpoint, the data presented here suggest that patients with ECD should be carefully evaluated at diagnosis for a coexisting myeloid neoplasm. We propose that any histiocytosis patient with a complete blood count abnormality that cannot be explained by a nonmalignant cause should undergo a BM aspiration and

Figure 2. Effect of targeted therapies on non-LCH and concomitant myeloid neoplasm. (A-E) Effect of vemurafenib on a 75-year-old patient with *BRAF*V600E-mutant ECD and concomitant *JAK2*V617F/*IDH2*R140Q-mutant MDS/MPN. (A) Absolute monocytes (orange line; left y-axis) and urinary *BRAF*V600E cell-free DNA quantitation (red line; right y-axis) pre- and postvemurafenib therapy (shaded area represents period of vemurafenib treatment). (B) ^{18}F-FDG PET scan pre- (left) and postvemurafenib (right) with corresponding fused computed tomography/^{18}F-FDG PET below. (C) Hematoxylin and eosin–stained biopsies of femoral bone revealing characteristic xanthogranulomatous lesion of ECD within a fibrotic background (histiocytes were CD68$^+$ by immunohistochemistry [not shown]). Original magnification ×400. (D) Evidence of myeloid neoplasm because of the presence of dysplastic myeloid cells (hypogranulation and pseudo–Pelger-Huet cell) in BM aspirate (left; original magnification ×400), increased number of CD34$^+$ cells (middle; original magnification ×100), and hematoxylin and eosin stain revealing hypercellular marrow with dysplastic megakaryocytes (right; original magnification ×200). (E-G) Effect of MEK inhibitor therapy on monocytosis and PB counts on the 66-year-old patient with *NRAS*Q61R-mutant ECD and CMML described in Figure 1. ^{18}F-FDG PET (F) and fused computed tomography/^{18}F-FDG PET (G) pre- and 2 months posttrametinib treatment in this same patient.

biopsy with histomorphologic, flow cytometric, and genetic analysis according to standard of care for myeloid malignancies. Evaluation of a concomitant myeloid neoplasm in a patient with non-LCH will be particularly important before initiation of targeted therapies for refractory ECD. As illustrated here, the myeloid neoplasm may be diagnosed only after initiation of therapy for ECD, and knowledge of both neoplasms would have influenced therapeutic decision making. Because of the paradoxical activation of RAS signaling mediated by RAF inhibitors, use of RAF inhibitors may uncover or enhance growth of a malignancy driven by a mutation other than *BRAF*V600E[19-22] (including mutations in *NRAS*, *KRAS*, or *JAK2*) as illustrated here.

In the recently revised 2016 WHO classification, ECD and LCH are classified with lymphoid neoplasms.[12] This is largely based on several case reports describing individual patients with lymphoid neoplasms and a clonally related secondary malignant histiocytosis.[33-37] However, the high frequency of myeloid neoplasms in patients with ECD or MH suggests a nonfortuitous biological association of both conditions. Although it is well established that myeloid neoplasms such as classic MPNs, MDS, and AML originate from hematopoietic stem cells,[38-41] the cell(s) of origin of LCH and non-LCH conditions are far less clearly delineated. A common nonconstitutional trisomy 21 was recently reported in an adult patient with both LCH and mixed phenotype T/myeloid acute leukemia,[42] suggesting a common origin of both tumors. Recent data suggest that the *BRAF*V600E mutation is detectable in $CD34^+$ hematopoietic progenitors in at least a portion of pediatric LCH patients.[43] Moreover, blood monocytes harboring the same mutations as pathological histiocytes have been reported in LCH[43] and non-LCH.[44] At the same time, the discovery of patients with *BRAF*V600E/*JAK2* wild-type ECD lesions with a concomitant *BRAF* wild-type/*JAK2*V617F mutant clonal hematopoietic disorder suggests that the 2 mutations must arise at distinct points of hematopoietic development in many patients. Further efforts to functionally characterize the precise cellular origin of both conditions are therefore warranted.

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Authorship

Contribution: M.P., E.L.D., J.-F.E., D.R.-W., B.H.D., N.O., A.D., O.A.B., N.D., and Z.H.-R. performed the experiments and analyzed data; M.P., F.C.-A., E.L.D., N.G., C.S., G.A.U., R.R., J.-E.K., T.S., F.C., C.B., B.H., Z.A., and J.-F.E. collected patient material and clinical data; and M.P., E.L.D., J.-F.E., O.A.-W., and J.H. wrote the manuscript.

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Correspondence: Omar Abdel-Wahab, 1275 York Ave, New York, NY 10065; e-mail: abdelwao@mskcc.org; and Julien Haroche, Service de Médecine Interne 2, Institut E3M, Hôpital de la Pitié-Salpêtrière, 47-83 Boulevard de l'Hôpital, 75013 Paris, France; e-mail: julien.haroche@aphp.fr.

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