

Erdheim-Chester disease associated with chronic myelomonocytic leukemia harboring the same clonal mutation

Extensive xanthelasma-like lesions of the eyelids is the most frequent cutaneous manifestation of Erdheim-Chester disease (ECD).¹ Recently, mutations activating the MAPK pathway have been found in ECD patients and it has been suggested in the revised classification of histiocytic disorders that all extracutaneous or disseminated juvenile xanthogranulomata with gain-of-function mutation of *BRAF*, *NRAS*, *KRAS*, or *MAP2K1*, should be

considered as ECD.² Chronic myelomonocytic leukemia (CMML) is a rare clonal hematopoietic disorder that presents features of both myeloproliferative neoplasm and myelodysplastic syndrome. Its diagnosis requires a persistent peripheral blood monocytosis $>1 \times 10^9/L$ after exclusion of a reactive monocytosis; myelodysplastic features and/or the presence of an acquired clonal cytogenetic or a mutational abnormality in hematopoietic cells.³ Specific skin lesions in CMML are rare with an estimated prevalence of 10%.⁴ Diffuse or periorbital xanthomatous lesions associated with CMML have been reported.^{5,6} Some authors suggested that xanthomatous lesions may be specific cutaneous involvement of

Table 1. Clinical, biological, histological and molecular features of included patient with extensive xanthomatous lesions of the eyelid associated with CMML.

	Case 1	Case 2	Case 3
Age at xanthelasma occurrence, years	55	54	39
Age at CMML diagnosis, years	64	58	48
Haematological features at CMML diagnosis:			
White blood cell count, n/mm ³	5700	11680	4280
Monocyte count, n/mm ³	1600	1280	1600
CD14 ⁺ /CD16 ⁺ monocyte fraction	NA	98%	92%
Blasts in peripheral blood	No	No	No
Cytopenia	No	No	Neutropenia 800/mm ³
Bone marrow blasts	4%	NA	2%
Bone marrow dysplasia	No	NA	Mild
Karyotype	Normal	NA	Normal
Molecular mutations	-KRAS c.35G>A, p.Gly12Asp (46%) -ASXL1 c.1927dupG, p.G642fs (42%)	-NRAS c.38G>A, Gly13Asp (41%)	-DNMT3A c.1867delT, p.Tyr623fs (32%) -KRAS c.35G>A, p.Gly12Asp (33%)
Dermatological features			
Histology	Dense histiocytic infiltrate with plurinucleate cells and Touton cells	Dense histiocytic infiltrate with plurinucleate cells and Touton cells	Dense histiocytic infiltrate with Touton cells
Percentage of tumoral foamy histiocytes	40%	30%	NA
Immunohistochemistry	CD68 ⁺ CD 163 ⁺ CD1a - PS 100 -	CD68 ⁺ CD 163 ⁺ CD1a - PS 100 -	CD 163 ⁺ CD1a - PS 100 - Langerin -
Molecular mutations	-KRAS c.35G>A, p.Gly12Asp (19%) -ASXL1 c.1927dupG, p.G642fs (15%)	-NRAS c.38G>A, p.Gly13Asp (16%)	-DNMT3A c.1867delT, p.Tyr623fs (15%) -KRAS c.35G>A p.Gly12Asp (24%)
Associated manifestations			
Clinical manifestations	Pericarditis, pleural effusion, inflammatory arthralgia	Ig A vasculitis psoriasis	pleural effusion, inflammatory arthralgia, hemolytic anemia, pseudo-erysipelas
Auto-antibodies	Anti-cardiolipin, Anti- 2-GP1	ANA 1/80	ANA 1/400, anti-RNP, Positive Coombs for IgG and C3
Treatments used and outcomes for xanthelasma	-Surgery: relapse at 1 year. -Prednisone 10 mg/d + HCQ 400 mg/d: failure (prescribed for associated manifestations)	-Surgery: relapse at 6 months -Methotrexate 20 mg/w : failure (prescribed for psoriasis)	-Surgery and laser: relapse. -Prednisone 10 mg/d, methotrexate 20 mg/w, nicobion: failure -anti-MEK trametinib 2 mg/d : cessation for toxicity

CMML: chronic myelomonocytic leukemia; FCI: Flow cytometry immunophenotyping; ANA: antinuclear autoantibodies; VAF: variant allele frequency.

CMML,⁷ however this has never been confirmed. Furthermore, an association between CMML and ECD has been reported⁸ and the recent classification of histiocytoses added a subtype of ECD associated with another myeloproliferative disorder.² Mutations activating the MAPK pathway including *KRAS*, *NRAS* and *BRAF* have also been described in CMML patients.⁹ However, the clonal relationship between the two diseases has not been clearly demonstrated.⁸

We report herein three cases of xanthelasma-like lesions fulfilling clinical, histological and molecular criteria for ECD associated with CMML with a proven clonal relationship between the skin lesions and CMML cells based on molecular analyses.

Briefly, for next-generation targeted sequencing (NGS) of CMML cells, genomic DNA was extracted from the bone marrow (patient 1 and 3) or from peripheral blood mononuclear cells (patient 2). A panel of 41 genes frequently mutated in myeloid malignancies was designed including *ASXL1*, *ASXL2*, *ATM*, *BCOR*, *CALR*, *CBL*, *CEBPA*, *CSF3R*, *DDX41*, *DNMT3A*, *EZH2*, *FLT3*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF1*, *JAK2*, *KIT*, *KMT2A*, *KRAS*, *MPL*, *NF1*, *NPM1*, *NRAS*, *PHF6*, *PTPN11*, *RAD21*, *RUNX1*, *SETBP1*, *SF3B1*, *SH2B3*, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *TET2*, *TP53*, *U2AF1*, *WT1* and *ZRSR2*. Libraries were obtained from 112.5 or 200 ng of DNA according to the manufacturer's instructions, using HaloPlex Target Enrichment System® (Agilent technologies), and sequenced on a MiSeq® sequencer (Illumina). Read alignment, variant calling, and annotation were performed using Sophia DDM® software version 5.0.12 (Sophia genetics). The sensitivity was 1%. All variants were checked using Intergated Genomics Viewer (IGV) software v2.3.

For formalin-fixed and paraffin-embedded (FFPE) skin biopsies, NGS was not available routinely and therefore biopsies were sent to the Department of Pathology of

Ambrose Pare Hospital. Genomic DNA was extracted from FFPE fragments as previously described.¹⁰ A custom panel of 76 genes frequently mutated in cancers (hot spots) and in myeloid malignancies was used including *AKT1*, *ALK*, *ARAF*, *ASXL1*, *BRAF*, *CALR*, *CBL*, *CDK4*, *CDKN1B*, *CDKN2A*, *CEBPA*, *CSF1R*, *CSF3R*, *CTNNA1*, *DNMT3A*, *EGFR*, *ETV6*, *EZH2*, *FLT3*, *GATA2*, *GNA11*, *GNAQ*, *GNAS*, *HERC1*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *JAK3*, *KIT*, *KRAS*, *KTM2D*, *MAML3*, *MAMLD1*, *MAP2K1*, *MAP2K2*, *MAP2K3*, *MAP2K4*, *MAP2K6*, *MAP3K1*, *MAP3K8*, *MAP3K9*, *MAP3K10*, *MAP3K19*, *MAP4K4*, *MAPK1*, *MAPK11*, *MAPK9*, *MPL*, *NF1*, *NOTCH1*, *NOTCH2*, *NPM1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PPP6C*, *PTEN*, *PTPN11*, *RAC1*, *RAF1*, *RIT1*, *RUNX1*, *SETBP1*, *SF3B1*, *SRSF2*, *STAG2*, *STK19*, *SYNGAP1*, *TAOK1*, *TAOK2*, *TET2*, *TP53*, *U2AF1*, *WT1*, *ZRSR2* (the 30 mutual genes between both panels are highlighted in bold). DNA quality was evaluated with the Agilent NGS FFPE QC KiT (Agilent) and libraries were prepared from 25-150 ng of DNA depending of its quality using TruSeq® Custom Amplicon Low Input kit (Illumina), and were sequenced on a Miseq sequencer (Illumina). Read alignment, variant calling, and annotation were performed using GensearchNGS v.1.6.31 (PhenoSystems SA). The sensitivity was 2%. All variants were checked using IGV software v2.3. The study was conducted according to the French CNIL methodology reference and in accordance with the Declaration of Helsinki.

Case reports: three women between 52 and 62 years of age presented with a long-term history of extensive periorbital and circumferential xanthomatous lesions (Figure 1 A). Clinical, hematological, histological and molecular features are presented in Table 1. All of them had a history of CMML-0 based on the results of blood samples, bone marrow (BM) examination and/or flow cytometry immunophenotyping. Circulating monocytes were measured to be between $1.0-1.6 \times 10^9/L$ and no specific treat-

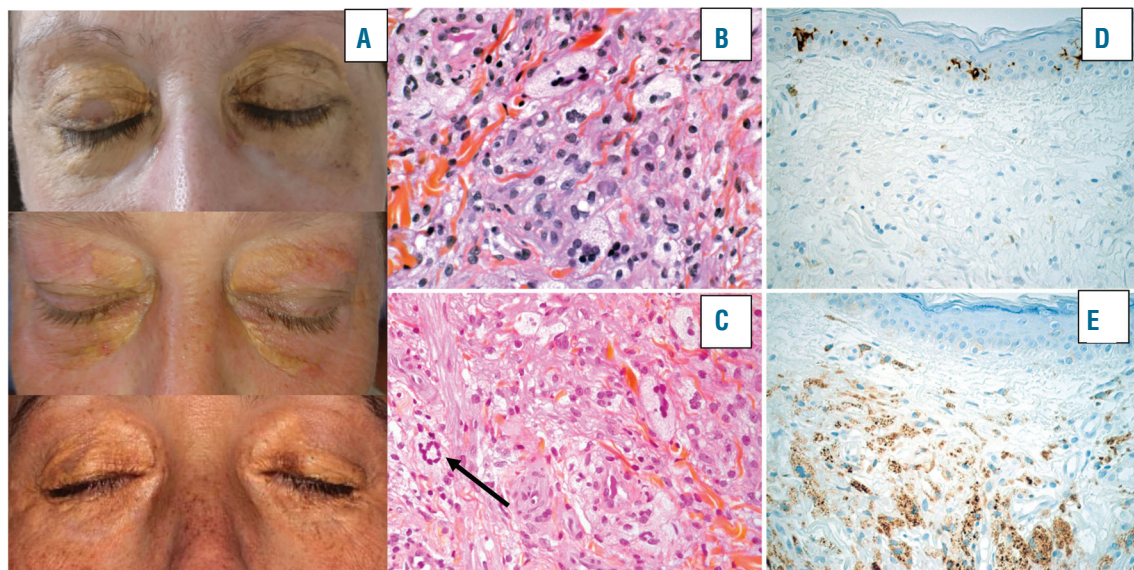


Figure 1. Clinicopathological features of cases. A. Clinical features of xanthomatous lesions of the eyelids. Extensive, multiple yellowish papules/plaques with color variations from yellowish to gold located on the inner and outer canthus of the eyelids with extension to the cheek and temporal area. B, C, D, E. Biopsies of the eyelids. (B, C) Dense dermal infiltrate comprised of macrophages with foamy cytoplasm and small central nucleus, some multinucleated cells and Touton cells (black arrow). (D) Immunostaining for CD1a was negative and diffusely positive for CD68 (E). (B and C, Hematoxylin-eosin stain; D and E, Immunoperoxidase stain; original magnification 400x).

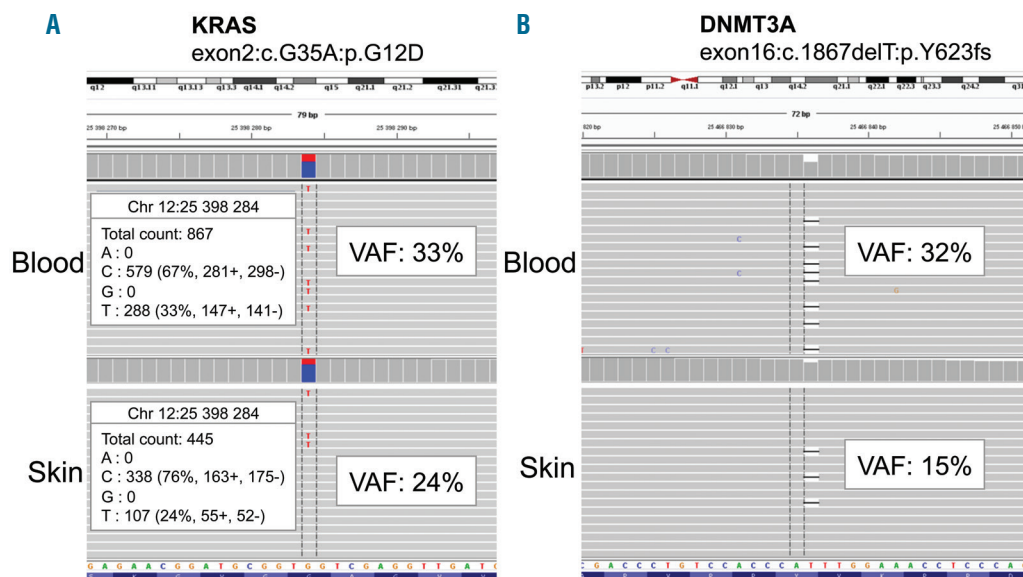


Figure 2. Next generation sequencing data from patient number 3 on DNA obtained from a peripheral blood sample (up) and the eyelid skin lesion (down), showing the same two identical mutations in both samples. (A) *KRAS* c.35G>A, p.Gly12Asp mutation, (B) *DNMT3A* c.1867delT, p.Tyr623fs mutation. Data are shown as screenshots from the Integrated Genomics Viewer visualization tool (<http://software.broadinstitute.org/software/igv>). For base substitution, the read counts for each base are indicated. VAF, variant allelic frequency.

ment for CMML was required. NGS studies performed on DNA obtained from the BM aspirate or blood samples identified the following mutations *KRAS* c.35G>A p.(Gly12Asp) (variant allele frequency (VAF): 0.46) and *ASXL1* 31022441-chr20c.1926-1927insG (VAF: 0.42) for patient 1; *NRAS* mutation c38G>A, p.(Gly13Asp) (VAF: 0.41) for patient 2 and *KRAS* (exon2:c.G35A:p.G12D) (VAF 0.33) and *DNMT3A* (exon 16:c.1867delT:p.Y623fs) (VAF: 0.32) for patient n°3. Skin biopsies of periorbital lesions of the eyelid revealed a similar pattern with a dense mononuclear infiltrate of the dermis, extending to the dermo-hypodermic junction. The infiltrate consisted of macrophages with foamy cytoplasm and a small central nucleus, and some multinucleated cells and Touton cells. Immunohistochemical studies showed that these histiocytes were negative for CD1a and S100, positive for CD163 and CD68 (Figure 1B-E). These findings were similar to those observed in ECD.¹ Given the history of CMML together with histological findings suggestive of ECD, NGS analyses were also performed on DNA obtained from FFPE tissue sections of the eyelids to investigate the relationship between the histiocytes comprising the skin lesions and the CMML clones. These studies identified the same mutations as those found in the CMML cells from the BM aspirate or blood samples (Table 1 and Figure 2). Positron emission tomography and computed tomography did not show bone lesions, perinephric fat or periaortic infiltration suggestive of systemic involvement of ECD. However, these cases were classified as ECD based on the identification of a clonal mutation of the MAPK pathway in cutaneous lesions with clinical and pathological findings of adult-xanthogranuloma as suggested by the revised classification of histiocytoses.²

We report three cases of extensive xanthelasma-like lesions of the eyelids clinically and histologically similar to those observed in adult-xanthogranuloma or ECD¹ fulfilling criteria for both ECD and CMML.^{2,5} We identified

the same mutations in the skin ECD lesions and the CMML cells, demonstrating a clonal relationship between both diseases. Of note, although all patients had monocytosis at the time of the skin biopsy, a contamination by blood CMML monocytes is unlikely. Indeed, the VAF ranged from 15 to 24%, corresponding to 30 to 48% of cells with a heterozygous mutation consistent with the percentage of tumoral histiocytes in skin biopsies (Table 1). Moreover, the three patients had mild monocytosis, and only <5% monocytes were present within areas of the dermis selected for molecular analysis.

Recently, Papo *et al.* identified that 19/189 of ECD were associated with myeloid neoplasms including eight CMML. In the only patient who had NGS targeted sequencing in both ECD and CMML cells, the same *NRAS* mutations were identified in perirenal tissue suggesting that the association is not restricted to ECD with limited skin involvement as in our cases.⁸ Moreover, recently, Ghobadi *et al.* identified the same *BRAF-V600E* mutation in the blasts from BM aspirate of an acute myeloid leukemia (AML) M5 and in ECD cells from a lung biopsy with bone, peri-aortic infiltration, lung, neurological and retroperitoneal involvement.¹¹ Moreover, using whole exome sequencing, they confirmed that the ECD and AML had multiple shared mutations and arose from the same cell of origin with additional mutations mostly for AML cells.¹¹ Some authors suggested that cutaneous involvement of CMML may be clonal.¹² Using fluorescence *in situ* hybridization, Vitte *et al.* found the same karyotype in cutaneous lesions and blood of 4/8 CMML patients.¹²

Additionally, two recent studies highlighted that the cell of origin of one third of patients with systemic histiocytosis resides in CD34⁺ hematopoietic progenitor cells prior to committed monocytes/macrophages.^{13,14} Two main biologic mechanisms might explain the link between CMML and ECD. The first one would be a differentiation from a clonal CMML cell into foamy

macrophages in the skin through an additional genetic hit or a transforming event. The second would be a mutant common progenitor cell giving rise to the two distinct neoplasms *via* divergent differentiation. We identified the same mutations in CMML cells and skin lesions without additional mutations, which may favor the first hypothesis. Moreover, Milne *et al.* showed that monocytes could differentiate into foamy macrophages *in vitro* with macrophage colony-stimulating factor and human serum.¹⁴ However, contrary to the case of Ghobadi *et al.*¹¹ exome sequencing has not been performed in our cases and therefore, further studies are needed to confirm this hypothesis.

Overall, the high prevalence of myeloid neoplasms including CMML in adults with non-Langerhans cell histiocytosis⁸ as well as in patients with Langerhans cell histiocytosis may be explained by a common bone marrow precursor.^{13,15} We report herein three cases of disseminated xanthelasma-like lesions of the eyelids fulfilling criteria for ECD² associated with CMML harboring the same mutations in skin biopsies and CMML cells. This study provides further evidence for a common progenitor cell between CMML and ECD in some cases.

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References

- Chasset F, Barete S, Charlotte F, et al. Cutaneous manifestations of Erdheim-Chester disease (ECD): Clinical, pathological, and molecular features in a monocentric series of 40 patients. *J Am Acad Dermatol.* 2016;74(3):513-520.
- Emile J-F, Ablu O, Fraitag S, et al. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. *Blood.* 2016;127(22):2672-2681.
- Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391-2405.
- Mathew RA, Bennett JM, Liu JJ, et al. Cutaneous manifestations in CMML: Indication of disease acceleration or transformation to AML and review of the literature. *Leuk Res.* 2012;36(1):72-80.
- Oka M, Okamura A, Kawano S, Fukumoto T, Sakaguchi M, Nishigori C. Diffuse plane normolipemic xanthoma associated with chronic myelomonocytic leukemia-1. *Eur J Dermatol.* 2014;24(1):112-113.
- Edwards LR, Kidd LL, Gru AA, Wilson BB. Image Gallery: Necrobiotic xanthogranuloma in association with chronic myelomonocytic leukaemia. *Br J Dermatol.* 2017;176(2):e16.
- Miralles ES, Escibano L, Bellas C, Núñez M, Ledo A. Cutaneous xanthomatous tumours as an expression of chronic myelomonocytic leukaemia? *Clin Exp Dermatol.* 1996;21(2):145-147.
- Papo M, Diamond EL, Cohen-Aubart F, et al. High prevalence of myeloid neoplasms in adults with non-Langerhans cell histiocytosis. *Blood.* 2017;130(8):1007-1013.
- Zhang L, Singh RR, Patel KP, et al. BRAF kinase domain mutations are present in a subset of chronic myelomonocytic leukemia with wild-type RAS. *Am J Hematol.* 2014;89(5):499-504.
- Colomba E, Hélias-Rodzewicz Z, Von Deimling A, et al. Detection of BRAF p.V600E mutations in melanomas: comparison of four methods argues for sequential use of immunohistochemistry and pyrosequencing. *J Mol Diagn.* 2013;15(1):94-100.
- Ghobadi A, Miller CA, Li T, et al. Shared cell of origin in a patient with Erdheim Chester disease and acute myeloid leukemia. *Haematologica.* 2019 Mar 28 [Epub ahead of print].
- Vitte F, Fabiani B, Bénét C, et al. Specific skin lesions in chronic myelomonocytic leukemia: a spectrum of myelomonocytic and dendritic cell proliferations: a study of 42 cases. *Am J Surg Pathol.* 2012;36(9):1302-1316.
- Durham BH, Roos-Weil D, Baillou C, et al. Functional evidence for derivation of systemic histiocytic neoplasms from hematopoietic stem/progenitor cells. *Blood.* 2017;130(2):176-180.
- Milne F, Bigley V, Bacon CM, et al. Hematopoietic origin of Langerhans cell histiocytosis and Erdheim-Chester disease in adults. *Blood.* 2017;130(2):167-175.
- Kiavash K, Malone JC. Langerhans cell histiocytosis associated with underlying hematolymphoid disorders in adults: report of 2 cases and review of the literature. *Am J Dermatopathol.* 2018;40(8):588-593.