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ORIGINAL ARTICLE

Spectrum of histiocytic neoplasms associated with diverse haematological malignancies bearing the same oncogenic mutation

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Abstract

Histiocytic disorders are a spectrum of rare diseases characterised by the accumulation of macrophage-, dendritic cell-, or monocyte-differentiated cells in various tissues and organs. The discovery of recurrent genetic alterations in many of these histiocytoses has led to their recognition as clonal neoplastic diseases. Moreover, the identification of the same somatic mutation in histiocytic lesions and peripheral blood and/or bone marrow cells from histiocytosis patients has provided evidence for systemic histiocytic neoplasms to originate from haematopoietic stem/progenitor cells (HSPCs). Here, we investigated associations between histiocytic disorders and additional haematological malignancies bearing the same genetic alteration(s) using the nationwide Dutch Pathology Registry. By searching on pathologist-assigned diagnostic terms for the various histiocytic disorders, we identified 4602 patients with a putative histopathological diagnosis of a histiocytic disorder between 1971 and 2019. Histiocytosis-affected tissue samples of 187 patients had been analysed for genetic alterations as part of routine molecular diagnostics, including from nine patients with an additional haematological malignancy. Among these patients, we discovered three cases with different histiocytic neoplasms and additional haematological malignancies bearing identical oncogenic mutations, including one patient with concomitant *KRAS* p.A59E mutated histiocytic sarcoma and chronic myelomonocytic leukaemia (CMML), one patient with synchronous *NRAS* p.G12V mutated indeterminate cell histiocytosis and CMML, and one patient with subsequent *NRAS* p.Q61R mutated Erdheim–Chester disease and acute myeloid leukaemia. These cases support the existence of a common haematopoietic cell-of-origin in at least a proportion of patients with a histiocytic neoplasm and additional haematological malignancy. In addition, they suggest that driver mutations in particular genes (e.g. *N/KRAS*) may specifically predispose to the development of an additional clonally related haematological malignancy or secondary histiocytic neoplasm. Finally, the putative existence of derailed multipotent HSPCs in these patients emphasises the importance of adequate (bone marrow) staging, molecular analysis and long-term follow-up of all histiocytosis patients.

Keywords: histiocytosis; malignant histiocytic disorders; histiocytic sarcoma; Langerhans cell sarcoma; indeterminate cell histiocytosis; Erdheim–Chester disease; non-Langerhans-cell histiocytosis; Langerhans-cell histiocytosis; leukaemia; lymphoma

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No conflicts of interest were declared.

Introduction

Histiocytic disorders are a spectrum of rare diseases characterised by the accumulation of macrophage-, dendritic cell-, or monocyte-differentiated cells in various tissues and organs [1]. Based on their clinical, radiographic and histopathological features, a wide variety of different subtypes of histiocytic diseases has been described [1]. Recurrent genetic alterations have been identified in many of these histiocytoses [2], including Langerhans cell histiocytosis (LCH) [3–5], Erdheim–Chester disease (ECD) [6], indeterminate cell histiocytosis (ICH) [7] and histiocytic sarcoma (HS) [8,9]. These genetic alterations primarily comprise somatic missense mutations, indels and fusions involving genes that encode proteins of the mitogen-activated protein kinase (MAPK) signalling pathway [10]. Consequently, many of the histiocytic disorders are now considered clonal neoplastic diseases [11,12], characterised by constitutive MAPK pathway activation [10].

The recent identification of the same somatic mutation in histiocytic lesions and peripheral blood and/or ($CD34^+$) bone marrow mononuclear cells from patients with disseminated histiocytoses has provided compelling evidence for systemic histiocytic neoplasms to originate from somatically mutated haematopoietic stem/progenitor cells (HSPCs) [13–16]. These cells are multipotent and have intrinsic proliferative and self-renewal potential. Therefore, patients with histiocytic neoplasms derived from (long-lived) somatically mutated HSPCs could be at increased risk of developing additional clonally related haematological neoplasms derived from these cells. Conversely, transformation of antecedent haematological malignancies to secondary histiocytic neoplasms may also occur [17,18]. In the 1990s, the LCH-Malignancy Study Group of the Histiocyte Society already reported recurrent associations between LCH and leukaemia or lymphoma [19,20], which were confirmed in later studies [21,22]. Likewise, a high prevalence of haematological malignancies was also observed in adults with ECD [23] or HS [24]. Some isolated case reports and small case series described associations between single histiocytic disorders and haematological malignancies harbouring the same genetic alteration(s) [15,23,25–32], supporting a common clonal origin of both diseases. Yet, the rarity of the histiocytic disorders, as well as the reality that adult patients are treated by a diverse range of medical specialists, and the fact that not all histiocytic neoplasms (e.g. ICH) are registered by national cancer registries, have thus far limited a comprehensive study of the occurrence of this phenomenon among a large cohort of patients with different types of histiocytic neoplasm. To address this issue, we requested data from the nationwide Dutch Pathology Registry (PALGA) [33], and retrieved the pathology reports of all patients diagnosed with a histologically confirmed and professionally PALGA-coded histiocytic disorder in the Netherlands between 1971 and 2019. In this population-based dataset, we searched for histiocytosis patients with an additional (histologically confirmed) haematological malignancy harbouring the same genetic alteration(s). Using this approach, we identified three cases with different histiocytic neoplasms and acute or chronic myeloid leukaemia bearing identical oncogenic mutations.

Materials and methods

In the Netherlands, all histo- and cytopathology reports generated at each of the (current) 43 pathology laboratories are digitally archived in the central network and registry of histo- and cytopathology – called PALGA [33]. Personal data are pseudonymised. This internationally unique archive was founded in 1971, achieved nationwide coverage in 1991, and contained more than 76 million pathology reports from over 12 million patients at the end of 2019 (Figure 1A). At sign-out, the reporting pathologist adds one or more diagnosis coding lines – consisting of a combination of diagnostic terms (referring to the localisation, acquisition technique and abnormality) – to each pathology report. These diagnostic terms are automatically linked with one or more classification codes. These codes were originally related to the Systematised Nomenclature of Medicine, 1982 version, published by the College of American Pathologists. Researchers may

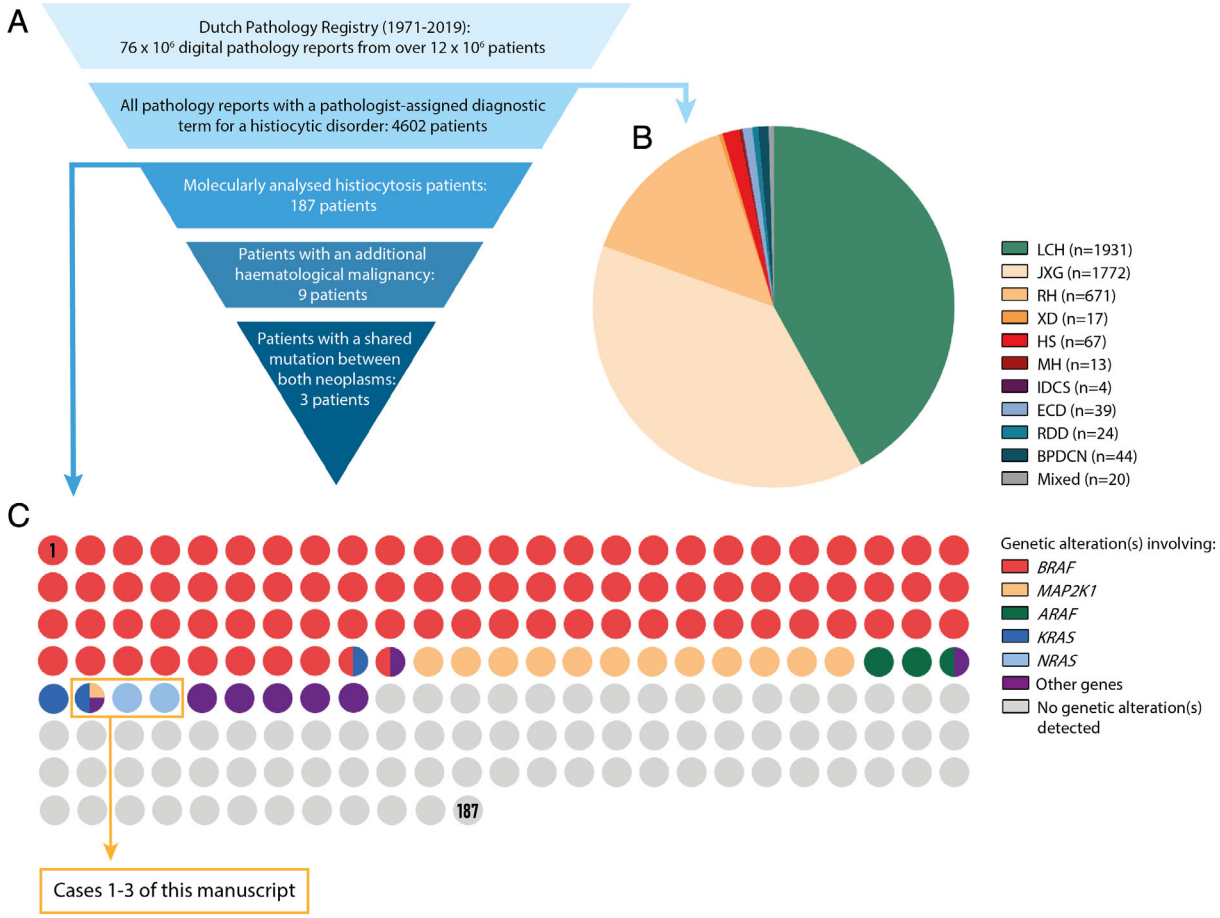


Figure 1. Study methodology. (A) Schematic representation of the funnel method used for patient identification. (B) Pie chart showing the number of identified patients with a pathology report registered in the Dutch Pathology Registry between 1971 and 2019 that contains a pathologist-assigned diagnostic term for a specific histiocytic disorder. (C) Dot matrix chart displaying the mutational status of the 187 identified histiocytosis patients whose tissue samples were successfully molecularly analysed. Every dot represents one patient. The colour of the dot depicts whether a genetic alteration involving a specific gene has been detected in the histiocytosis-affected tissue specimen(s) of the represented patient. Dots with multiple colours represent patients with multiple detected genetic alterations involving multiple genes. The three dots inside the orange box represent Cases 1–3, who are described in detail in the results section of this manuscript.

request pseudonymised data from the national PALGA database.

For this study, we retrieved all pathology reports with a pathologist-assigned diagnostic term for one of the various histiocytic disorders (see supplementary material, Table S1) from the entire PALGA database from its start in 1971 until (and including) 2019. Because HSs and malignant histiocytoses reported before the development and widespread use of immunohistochemistry and clonality testing have later been demonstrated to often comprise lymphomas [34–36], we decided to exclude pathology reports with a diagnostic term for HS, interdigitating dendritic cell sarcoma (IDCS) or malignant histiocytosis (MH) from 2001 or before. In 2001, the third edition of the World Health Organisation classification of tumours of haematopoietic and lymphoid tissues was published [37], which underscored the importance of excluding a lymphoma before making a diagnosis of a malignant histiocytic disorder. Furthermore, we manually reviewed the digital pathology reports of all patients with one or more pathology reports that (collectively) contained diagnostic terms of two or more histiocytic disorders, to evaluate whether these patients had mixed histiocytosis or whether one diagnosis (e.g. xanthogranuloma) had been changed into another (e.g. ECD) after pathology review and/or histopathological analysis of ensuing tissue samples. Using this

approach, we identified n = 4602 patients with a putative diagnosis of a histiocytic disorder (Figure 1B), including n = 1931 with LCH, n = 1772 with juvenile xanthogranuloma (JXG), n = 671 with reticulohistiocytoma (RH), n = 17 with xanthoma disseminatum (XD), n = 67 with HS, n = 13 with MH not otherwise specified, n = 4 with IDCS, n = 39 with ECD, n = 24 with Rosai–Dorfman disease (RDD), n = 44 with blastic plasmacytoid dendritic cell neoplasm (BPDCN) and n = 20 with mixed histiocytosis. Using the pseudonymised patient identification number provided with each pathology report, we also retrieved all additional pathology reports of these 4602 patients with a pathologist-assigned diagnostic term for any given malignancy.

In the final output file, we searched all pathology reports with a diagnostic term for a histiocytic disorder for free-text terms referring to molecular analysis. We manually reviewed the discovered pathology reports and identified n = 187 histiocytosis patients (n = 100 adults; n = 87 children) from whom tissue samples affected by their histiocytic disorder had been analysed for genetic alterations (Figure 1C), including n = 109 patients (n = 51 adults; n = 58 children) with a successfully detected genetic alteration. By manually reviewing all pathology reports of the 187 molecularly analysed patients, we discovered n = 9 patients with an additional (histologically confirmed) haematological malignancy (Table 1). Notably, these patients were all adults. Among them, four patients had presentation of the histiocytic neoplasm and additional haematological malignancy (all lymphomas) in the same tissue specimen (Cases 6–9); mutations were detected in the tissue specimen of Case 9. The resected subcutaneous tumour of this patient, who was known to have chronic lymphocytic leukaemia (CLL), consisted of a proliferation of spindle cells intermingled with many small lymphocytes and was classified as mixed IDCS and small lymphocytic lymphoma (SLL)/CLL. As microdissection of (relatively) pure spindle or small lymphocytic cell populations could not be performed, the existence of shared or unique genetic alterations in the IDCS or SLL/CLL of this patient remains elusive. In addition, no evidence for shared or unique genetic alterations was obtained in a patient with LCH and myelodysplastic syndrome (Case 5), since *BRAF* mutation analysis of the LCH specimen was negative. In the remaining four cases, a unique *BCL2* translocation was identified in the follicular lymphoma (FL) and high-grade B-cell lymphoma of a patient who also developed HS without a *BCL2* translocation (Case 4). These molecular findings contradict the transformation of FL into HS in this case, which has been reported previously in other patients [17,18,38,39]. Finally, shared genetic alterations were identified in the remaining three patients (Cases 1–3), who presented with different histiocytic neoplasms and acute or chronic myeloid leukaemia harbouring identical oncogenic *RAS* mutations. The molecular analyses had been performed at three University Medical Centres using targeted next-generation sequencing (NGS), and were executed as part of routine molecular diagnostics and according to conventional molecular diagnostic laboratory standards for variant allele detection and reporting. DNA samples were isolated from haematoxylin stained tissue slides, except for the acute myeloid leukaemia (AML) and germline DNA samples of Case 3, which were isolated from a 6 ml EDTA bone marrow aspirate and saliva, respectively. Detailed information regarding the applied NGS panels is provided in the supplementary material (Figure S1; Cases 1–3).

Via the 'PALGA intermediary procedure', the pathology laboratories where the histopathological analyses of the tissue samples of Cases 1–3 were originally performed were identified, while complying with EU privacy laws and regulations. This enabled us to retrieve the pseudonymised tissue slides and blocks for central pathology review and additional immunohistochemical investigations. In addition, the pathology laboratories could refer us to the treating physicians of the patients, who subsequently provided us with pseudonymised clinical data and images. This study was approved by the PALGA Scientific Council and Privacy Committee (LZV-2016-183) and the Institutional Review Board of the Leiden University Medical Center (B19.074).

Results

Case 1 is a 47-year-old male who presented with fatigue, weight loss (18 kg), night sweats and left upper abdominal pain. Complete blood count showed anaemia, thrombocytopenia and monocytosis (2.1×10^9/l monocytes). A PET-CT scan revealed fluorodeoxyglucose (FDG)-avid bone lesions and enlarged cervical lymph nodes, as well as extreme splenomegaly (29.5 cm) with diffuse moderately increased metabolism (Figure 2A,B). In addition, FDG-avid pre-auricular and scalp skin lesions were noted (Figure 2C,D). A bone marrow biopsy revealed diffuse fibrosis and extensive infiltration by atypical $CD163^+$ $CD68^+$ $CD56^+$ $CD1a^-$ histiocytes with a high (80%) Ki67 proliferation index (Figure 3A). A

Table 1. All identified histiocytosis patients with an additional haematological malignancy who were analysed for genetic alterations.

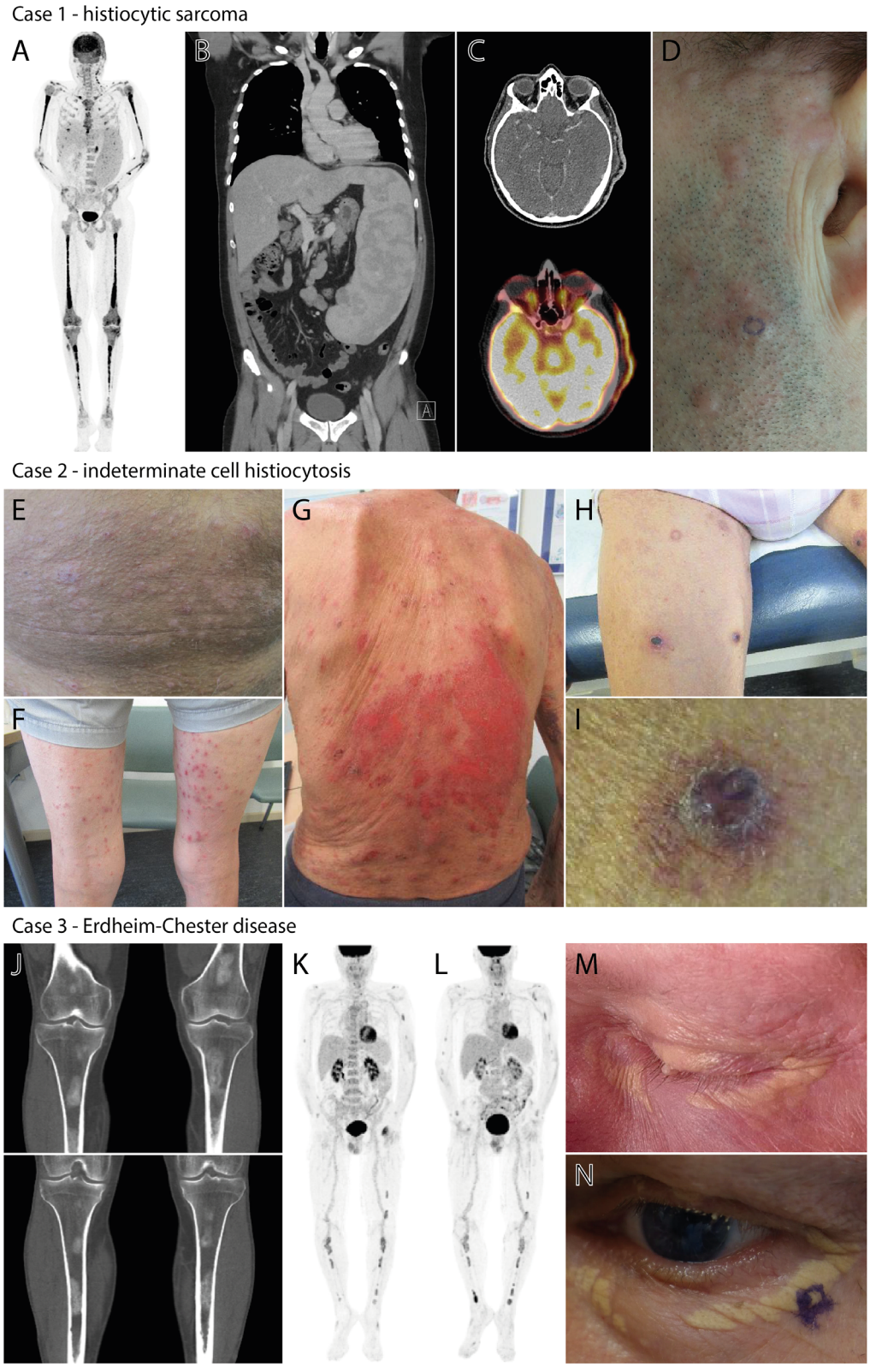
Case	Age* (years)	Histiocytic disorder	Additional haematological malignancy†	Histiocytic disorder specimen(s)	Additional haematological malignancy specimen(s)	Interval	Genetic alteration(s) detected	Shared or unique genetic alteration(s) detected
1	47	HS	CMML	Bone marrow; skin	Bone marrow	CMML diagnosed 1 month after HS	Using NGS, mutations were detected in *KRAS* (p.A59E, VAF 34%) *MAP2K1* (p.F53L, VAF 3.8%) and *RAF1* (p.S257L, VAF 7.9%) in the HS bone marrow specimen, and in *KRAS* (p.A59E, VAF 42%), *MAP2K1* (p.F53L, VAF 2%) and *RAF1* (p.S257L, VAF 0.7%) in the mixed CMML/HS bone marrow specimen	**Yes:** a **shared** *KRAS* mutation, and (presumably) unique *MAP2K1* and *RAF1* mutations (HS)
2	68	ICH	CMML	Skin	Bone marrow	Diagnosed in the same month	Using NGS, a mutation was detected in *NRAS* (p.G12V) in both the ICH (VAF 20%) and CMML (VAF 42%) specimen	**Yes:** a **shared** *NRAS* mutation
3	61	ECD	MM and AML	Bone marrow; left femur; left tibia; skin	Bone marrow (MM); bone marrow (AML)	ECD and MM synchronous; AML diagnosed 2 years after ECD/MM	Using NGS, a mutation was detected in *NRAS* (p.Q61R) in the mixed MM/ECD bone marrow specimen (VAF not reliable), the ECD left tibia and skin specimens (VAF 37% in both samples), and the mixed AML/ECD bone marrow specimen (VAF 44%)	**Yes:** a **shared** *NRAS* mutation (ECD and AML)‡
4	59	HS	FL and HGBL	Soft tissue right shoulder	Lymph nodes and bone marrow (FL); mediastinal tumour and bone marrow (HGBL)	HS diagnosed 4 months after FL; HGBL diagnosed 8 months after FL	No *BCL2* translocation (HS); *BCL2* translocation (FL); *BCL2*, *BCL6* and *MYC* translocations (HGBL)	Yes: a unique *BCL2* translocation (FL and HGBL)
5	81	LCH	MDS	Skin; inguinal lymph node	Bone marrow	LCH diagnosed 4.5 years after MDS	No mutations detected in *BRAF* exon 15 using HRM (LCH)	No
6	44	LCH	FL	Right inguinal lymph node (same specimen)	Right inguinal lymph node	Synchronous	No *BCL2* translocation	No
7	40	LCH	HL	Right cervical lymph node (same specimen)	Right cervical lymph node	Synchronous	No mutations detected using NGS (incl. *BRAF* exon 15, *MAP2K1* exon 2–3, *NRAS* exon 2–4 and *KRAS* exon 2–4)	No
8	45	LCH	HL	Right inguinal lymph node (same specimen); left cervical lymph node (same specimen)	Right inguinal lymph node; left cervical lymph node (relapse)	Synchronous	No mutations detected in both specimens using NGS (incl. *BRAF* exon 15, *NRAS* exon 2–4 and *KRAS* exon 2–4)	No
9	83	IDCS	SLL/CLL	Soft tissue right upper arm (same specimen)	Soft tissue right upper arm	Synchronous	No *BCL2*, *BCL6* or *MYC* translocation(s); using NGS, mutations were detected in *MET* (p.E1017K, VUS, VAF 8%), *TP53* (p.R248W, VAF 35%) and *SF3B1* (p.K700E, VAF 29%)	No

Abbreviations: M, male; F, female; CMML, chronic myelomonocytic leukaemia; MM, multiple myeloma; HGBL, high-grade B-cell lymphoma; MDS, myelodysplastic syndrome; HL, Hodgkin lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; NGS, next-generation sequencing; HRM, high resolution melt analysis; VUS, variant of unknown significance.

*Age at diagnosis of the first presenting disorder.

†Excluding histiocytosis patients with an additional histiocytic malignancy, such as Langerhans cell sarcoma (LCS), as the histiocytic neoplasms are a spectrum of diseases, with regularly mixed histiocytosis (e.g. LCH/ECD or LCH/LCS).

‡As the VAF of the *NRAS* p.Q61R mutation in the mixed MM/ECD bone marrow specimen was unreliable due to poor DNA quality resulting in low number of reads, it could not be established whether the multiple myeloma also harboured the *NRAS* p.Q61R mutation.



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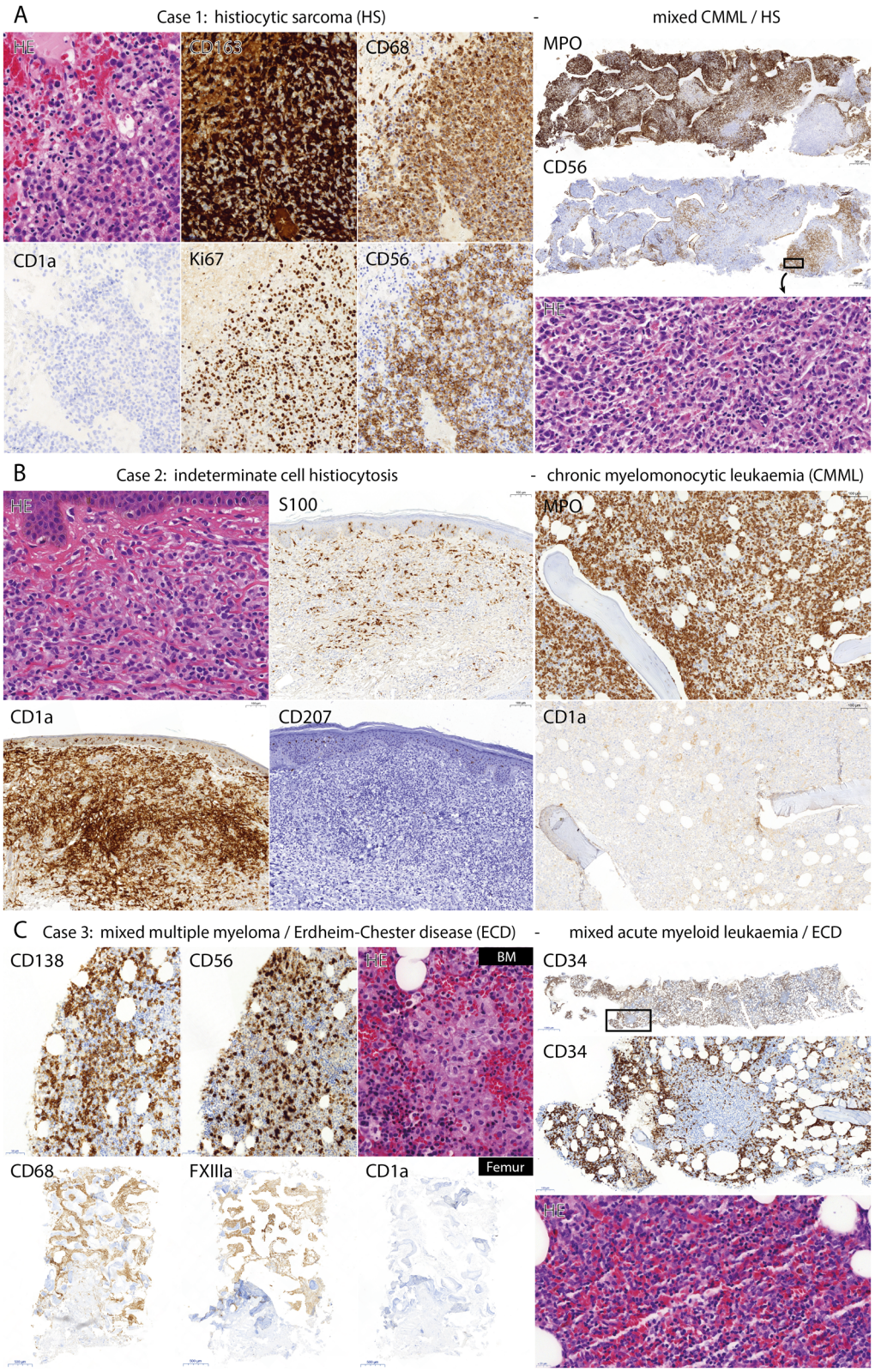
diagnosis of HS was made, and the patient was referred to a University Medical Centre for treatment of this rare disease. Using NGS, *KRAS* p.A59E (variant allele frequency [VAF] 34%), *MAP2K1* p.F53L (VAF 3.8%) and *RAF1* p.S257L (VAF 7.9%) mutations were detected in the bone marrow biopsy (Table 2). A pre-auricular skin biopsy (Figure 2D) and second bone marrow biopsy were taken before start of chemotherapy. While the skin biopsy showed a diffuse dermal proliferation of $CD56^+$ histiocytic cells, confirming HS involvement, the second bone marrow biopsy revealed myelodysplastic/myeloproliferative neoplasia characterised by left-shifted myelopoiesis, dysplastic features of the erythroid, neutrophilic and megakaryocytic lineages, and MF2-MF3 fibrosis. CD34 staining showed no increased blast count, whereas CD117 staining revealed 5–10% clustered myeloid precursors and myeloperoxidase (MPO) stained the majority of cells in the bone marrow (Figure 3A). A diagnosis of concomitant chronic myelomonocytic leukaemia (CMML)-0 was made. A few scattered clusters of atypical ($CD163^+$ $CD14^+$) $CD56^+$ MPO^- histiocytes were also observed in the second bone marrow biopsy (Figure 3A), indicative of a small focus of HS. Using NGS, the same *KRAS* p.A59E mutation (VAF 42%) was detected in this CMML/HS-affected second bone marrow biopsy (Table 2). The high VAF provides evidence for the presence of the *KRAS* mutation in the CMML. Interestingly, the *RAF1* p.S257L and *MAP2K1* p.F53L mutations were only detected at very low frequencies (VAF 0.7 and 2%, respectively), indicating that these mutations were probably HS-specific. The patient was treated with high-dose Cytarabine and Mitoxantrone chemotherapy (Cytarabine twice daily 1000 mg/m^2, day 1–6; Mitoxantrone 10 mg/m^2, day 5–7; one cycle completed), but developed a neutropenic enterocolitis and died due to septic/hyper-inflammatory shock 62 days after HS diagnosis.

Case 2 is a 68-year-old male who presented with pruritic skin lesions on the trunk and extremities that had existed for 3 months (Figure 2E,F). A skin biopsy from the lower right abdomen (Figure 2E) revealed a dermal proliferation of CD1a and (partly) S100 positive histiocytes (Figure 3B) with low (5%) MIB-1 expression. Notably, eosinophilic granulocytes were not observed. A PET-CT scan showed no FDG-avid lesions (images not shown), and a diagnosis of cutaneous LCH was made clinically. However, additional immunohistochemical investigations performed during central pathology review revealed overt CD207 (and MPO) negativity of the dermal histiocytes (Figure 3B), which led to an altered histopathological diagnosis of ICH. Using NGS, a *NRAS* p.G12V mutation (VAF 20%) was detected in the skin biopsy (Table 2). Complete blood count revealed a mild anaemia, thrombocytopenia and monocytosis, which retrospectively had been present for 7 months (1.9×10^9/l monocytes). A bone marrow biopsy showed no infiltrating ($CD1a^+$) histiocytes, but revealed a hypercellular bone marrow with substantial expansion of myelopoiesis, as illustrated by abundant MPO^+ cells (Figure 3B). A diagnosis of concomitant CMML-0 was made. The same *NRAS* p.G12V mutation (VAF 42%) was detected in the bone marrow biopsy (Table 2), while additional NGS performed on DNA isolated from unaffected tissue of the patient excluded a germline *NRAS* mutation. Due to stable, asymptomatic CMML but increasing pruritic ICH skin lesions (Figure 2G), topical corticosteroids and UV-B phototherapy for the ICH and an active monitoring strategy for the CMML were initiated. Despite initial adequate response of the skin lesions, they recurred as typical purple-red papules (Figure 2H,I). A skin biopsy of one of these papules (Figure 2I) again showed a dermal infiltrate of $CD1a^+$ $CD207^-$ MPO^- histiocytes (lacking eosinophilic infiltration), but now with high (50–60%) MIB-1 expression. Renewed staging by PET-CT (images not shown) showed FDG-avid thickened skin at the right lower leg and novel splenomegaly (16 cm). In addition, thickening of the right proximal-mid ureter wall was observed. Soon thereafter, the patient was diagnosed with an invasive urothelial carcinoma (2 years after ICH diagnosis), and died 9 days after

Figure 2. Clinical and radiological features of the patients described in this study. (A,B) Images of the PET-CT scan made at diagnosis, showing FDG-avid bone lesions and enlarged cervical lymph nodes, as well as extreme splenomegaly (29.5 cm) with diffuse moderately increased metabolism. (C,D) PET-CT and clinical images of the pre-auricular skin lesions. The localisation of the skin biopsy confirming the involvement of HS is encircled in panel (D). (E,F) Skin lesions on the abdomen and lower extremities at diagnosis. (G) Evidently progressed skin lesions at 7 months after initial diagnosis. (H–I) Recurrent skin lesions after treatment with topical corticosteroids and UV-B phototherapy, with an altered phenotype of typical purple-red papules. The localisation of the biopsy of one of these papules on the left upper leg is shown in panel (I). (J) Images of the CT-scan performed at diagnosis, showing bilateral sclerotic femur and tibia lesions. (K) PET-CT scan showing FDG uptake of ECD-associated bone lesions after chemotherapy for the patient's multiple myeloma. (L) PET-CT scan showing slightly increased FDG uptake of existing ECD bone lesions at diagnosis of acute myeloid leukaemia. (M) Peri-orbital xanthelasma-like lesions before myeloma-directed chemotherapy. (N) Peri-orbital xanthelasma-like lesions after myeloma-directed chemotherapy and autologous haematopoietic stem cell transplantation. The localisation of the skin biopsy confirming involvement of ECD is encircled.



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laparoscopic nephro-ureterectomy due to acute respiratory failure after developing a hospital-acquired pneumonia.

Case 3 is a 61-year-old male who was referred due to fatigue with underlying mild anaemia. A diagnosis of multiple myeloma (MM) International Staging System (ISS) stage 2 was made based on a kappa to lambda free light chain (FLC) ratio of 122.8 in the peripheral blood and 10–20% $CD138^+$ $CD56^+$ kappa-monoclonal plasma cells in a bone marrow biopsy (Figure 3C). Notably, some clusters of histiocytic cells were also observed in the bone marrow biopsy (Figure 3C). A CT scan showed no osteolytic lesions in the context of MM, but bilateral sclerotic femur and tibia lesions were clearly observed (Figure 2J). A PET-CT scan revealed FDG-avid lesions in both femurs, both tibiae, both fibulae, the right ilium and both humeri. Biopsies from sclerotic lesions in the left femur and left tibia showed no signs of MM, but both specimens displayed widespread proliferation of $CD68^+$ $FXIIIa^+$ $CD1a^-$ ($S100^-$) histiocytic cells (Figure 3C), supportive of a diagnosis of concomitant ECD. Of note, the patient also had bilateral xanthelasma-like lesions (Figure 2M), which were now suspected also to be ECD manifestations. NGS performed on DNA isolated from the mixed MM/ECD bone marrow and ECD left tibia biopsies revealed a *NRAS* p.Q61R mutation in both samples (Table 2), which was confirmed in the MM/ECD bone marrow biopsy by Sanger sequencing (see supplementary material, Case 3). Myeloma treatment consisted of four courses of Bortezomib (1.3 mg/m^2; day 1,4,8,11), Thalidomide (100 mg/day; day 1–21 of every 4 weeks) and Dexamethasone (40 mg/day; day 1, 2, 4, 5, 8, 9, 11, 12) and the patient achieved a very good partial response [40] (FLC kappa 27.70 mg/l; FLC ratio 1.45). However, PET-CT still showed unchanged FDG uptake and sclerosis of the ECD bone lesions (Figure 2K). The patient underwent autologous haematopoietic stem cell transplantation after high dose Melphalan conditioning (200 mg/m^2; day −2), and received Lenalidomide as post-remission myeloma-directed therapy. The bilateral xanthelasma-like lesions persisted, and a skin biopsy (Figure 2N) confirmed ECD involvement with again the same *NRAS* p.Q61R mutation (VAF 37%; Table 2). At a routine follow-up consultation, 13 months after autologous haematopoietic stem cell transplantation, 3% of leukocytes in the peripheral blood appeared to be leukaemic blasts. The patient underwent bone marrow examination and a diagnosis of AML-M5 (CD34, CD13, HLA-DR and MPO positive; CD19, CD10, CD3, CD4, CD5 and CD8 negative) was made based on the presence of 30% of $CD34^+$ leukaemic blasts in a bone marrow biopsy (Figure 3C). As in the earlier collected MM/ECD-affected bone marrow biopsy, a few clusters of histiocytic cells were present in this AML-affected bone marrow biopsy (Figure 3C), indicative of a small focus of ECD. A PET-CT scan showed no extramedullary AML lesions, but revealed slightly increased FDG uptake of existing ECD lesions (Figure 2L). While NGS revealed no recurrent cytogenetic or molecular abnormalities associated with AML in the AML/ECD-affected bone marrow sample (see

Figure 3. Histopathological features of tissue samples taken from the patients described in this study. (A) Case 1. Photomicrographs of the H&E (×40) and CD163, CD68, CD1a, Ki67 and CD56 (×20) stained first bone marrow biopsy (left), and the MPO, CD56 (×2) and H&E (×40) stained second bone marrow biopsy (right). The first bone marrow biopsy revealed extensive infiltration by atypical $CD163^+$ $CD68^+$ $CD56^+$ $CD1a^-$ histiocytes with a high (80%) Ki67 proliferation index, consistent with a diagnosis of HS. The second bone marrow biopsy showed myelodysplastic/myeloproliferative neoplasia, as illustrated by abundant MPO^+ cells, supporting a diagnosis of CMML. A few scattered clusters of $CD56^+$ MPO^- atypical histiocytic cells were also observed in the second bone marrow biopsy (depicted in the H&E panel), indicative of a small focus of HS. (B) Case 2. Photomicrographs of the H&E (×40), and S100, CD1a and CD207 (×10) stained skin biopsy (left), and the MPO and CD1a (×10) stained bone marrow biopsy (right). The skin biopsy revealed a dermal proliferation of CD1a and (partly) S100 positive histiocytes with overt CD207 negativity, consistent with a diagnosis of ICH. The bone marrow biopsy showed no infiltrating ($CD1a^+$) histiocytes, but displayed a hypercellular bone marrow with substantial expansion of myelopoiesis, as illustrated by abundant MPO^+ cells, supporting a diagnosis of synchronous CMML. (C) Case 3. Photomicrographs of the CD138, CD56 (×20) and H&E (×40) stained first bone marrow (BM) biopsy, and the CD68, FXIIIa and CD1a (×2) stained left femur biopsy (both left), as well as the CD34 (×1 and ×10) and H&E (×40) stained bone marrow biopsy taken after 3% of leukocytes in the peripheral blood that appeared as leukemic blasts were detected at a routine follow-up consultation (right). In the first bone marrow biopsy (left), 10–20% $CD138^+$ $CD56^+$ plasma cells were observed, supporting a diagnosis of MM. In addition, some clusters of histiocytic cells were recognised (depicted in the H&E panel), indicative of a small focus of the soon thereafter diagnosed ECD. The left femur biopsy showed widespread $CD68^+$ $FXIIIa^+$ $CD1a^-$ foamy histiocytes, supporting the clinical and radiological diagnosis of ECD. The bone marrow biopsy taken after blasts were detected in the peripheral blood at a routine follow-up consultation (right) revealed 30% of $CD34^+$ leukaemic blasts, consistent with a diagnosis of acute myeloid leukaemia (AML). Like in the first (MM/ECD-affected) bone marrow biopsy, a few clusters of histiocytic cells were present in this AML-affected bone marrow biopsy (depicted in the H&E panel), again indicative of a small focus of ECD.

Table 2. Mutations detected in the histiocytic neoplasms and additional myeloid leukaemias of the three patients presented in this study.

Case	Material	Diagnosis	Day	Method	Mutation(s)	VAF
1	First bone marrow biopsy	HS	0	NGS CHPv2.0	*KRAS* p.A59E	30%
1	First bone marrow biopsy	HS	0	NGS PATHv2D	*KRAS* p.A59E	34%
					MAP2K1 p.F53L	3.8%
					RAF1 p.S257L	7.9%
1	Second bone marrow biopsy	CMML/HS	38	NGS CHPv2.0	*KRAS* p.A59E	40%
1	Second bone marrow biopsy	CMML/HS	38	NGS PATHv2D	*KRAS* p.A59E	42%
					MAP2K1 p.F53L	2%
					RAF1 p.S257L	0.7%
2	Skin biopsy	ICH	0	NGS OPv3.0	*NRAS* p.G12V	20%
2	Bone marrow biopsy	CMML	21	NGS OPv3.0	*NRAS* p.G12V	42%
3	First bone marrow biopsy	MM/ECD	0	1. NGS DPv5.0	*NRAS* p.Q61R	1. 69%*
				2. NGS DPv5.0		2. 18%*
				3. Sanger sequencing		3. N/A
3	Left tibia biopsy	ECD	175	NGS DPv5.1	*NRAS* p.Q61R	37%
3	Skin biopsy	ECD	479	NGS DPv5.1	*NRAS* p.Q61R	37%
3	Bone marrow aspirate	AML/ECD	836	NGS Illumina TruSight Myeloid	*NRAS* p.Q61R	44%

Abbreviation: N/A, not available.
*VAF is unreliable due to poor DNA quality resulting in low number of reads.

supplementary material, Case 3), the same *NRAS* p.Q61R mutation (VAF 44%) as was previously detected in the ECD lesions was detected in this bone marrow specimen (Table 2). Importantly, the high VAF provides evidence for the presence of the *NRAS* mutation in AML blasts. Moreover, the *NRAS* p.Q61R mutation was not detected by NGS in the patient's germline DNA. The patient was treated for his AML with 7 + 3 chemotherapy of Cytarabine (200 mg/m^2; day 1–7) and Idarubicine (12 mg/m^2; day 1–3), and reached complete remission after one course of induction chemotherapy. Before the second course, he presented however with altered vision of the left eye, which was found to be caused by an invasive *Aspergillus fumigatus* infection in the left sphenoid sinus that was unresponsive to extensive anti-fungal treatment and quickly led to cranial nerve II, III, IV, V_1 and VI palsies. Eventually, the patient went home with palliative care and died shortly after.

Discussion

In this retrospective population-based study, we report three patients with different histiocytic neoplasms and additional haematological malignancies bearing identical oncogenic mutations. All patients were diagnosed and treated in different non-academic as well as tertiary referral hospitals across the Netherlands, emphasising the benefit of our study design based on the nationwide Dutch Pathology Registry. These patients add to the growing list of reported patients with a histiocytic neoplasm and additional haematological cancer bearing the same genetic alteration(s) (Table 3; see supplementary material, Tables S2 and S3). Of note, all three patients had typical histiocytic disease presentations, including bilateral osteosclerotic lesions of the long bones in the patient with ECD (Case 3). This is in contrast to three recently reported ECD-CMML patients with only xanthelasma-like lesions [30], which are not specific for ECD [31].

As routine molecular analysis of (specific) histiocytic disorders has only rather recently found its way into clinical practice, the three patients described in this study may comprise only the tip of the iceberg of all histiocytosis patients in our PALGA cohort (1971–2019) with an additional haematological malignancy bearing the same genetic alteration(s). Accordingly, all three patients were diagnosed with their first presenting disorder from 2017 onwards. In addition, this study is limited by the fact that PALGA diagnostic terms do not exist for all histiocytic disorders, such as ICH, and that pathologists may sometimes assign incorrect or non-specific coding terms to relevant pathology reports. As a consequence, this study may not include all patients with a histologically confirmed histiocytic disorder. Finally, haematological malignancies are not always histologically confirmed with a trephine biopsy, but may also be diagnosed based on laboratory tests, cytological evaluation and/or flow cytometric analysis alone. Thus, not all haematological malignancies of the patients from our study may have been registered by PALGA. Despite these limitations, we could still identify three patients with different histiocytic neoplasms associated with diverse haematological malignancies bearing identical oncogenic mutations, underscoring the rare but indefinite occurrence of this phenomenon across the spectrum of histiocytic neoplasms.

Importantly, the detected variant allele frequencies point out that the identified *RAS* mutations were present in both the specified histiocytic neoplasms and myeloid leukaemias, suggesting a common clonal origin. Nevertheless, we acknowledge the lack of flow sorted cells to unequivocally demonstrate the phenotype of the mutated cells [31]. Notably, we could exclude the possibility of a germline *RAS* variant in Cases 2 and 3. For Case 1, the probability of a germline variant is very low, considering the VAF of 30–42% of the *KRAS* mutation that was detected in four separate NGS analyses (Table 2). Unfortunately, we could not establish whether the MM of Case 3 harboured the same *NRAS* mutation as the ECD and AML of this patient due to the absence of cryopreserved cells. Yet, other studies have already demonstrated shared genetic alterations in histiocytic neoplasms associated with various lymphoid neoplasms (Table 3), including MM (see supplementary material, Table S3) [51]. Putative mechanisms for this phenomenon include the existence of a common HSPC, and dedifferentiation or transdifferentation of lineage-committed haematopoietic cells [69]. In accordance with a previous observation in LCH [19], lymphoid neoplasms often preceded the histiocytic neoplasm in the reported cases (see supplementary material, Tables S2 and S3; $n = 45/57$), whereas myeloid neoplasms were more frequently diagnosed concurrently ($n = 7/34$) or after the diagnosis of the histiocytic neoplasm ($n = 14/34$). In addition, interesting differences in association patterns between the different histiocytic neoplasms were recognisable in our comprehensive overview of published cases (Table 3; see supplementary material, Tables S2 and S3), with ECD only being associated with myeloid leukaemia ($n = 10/10$) and malignant histiocytoses often associated with lymphoid neoplasms, and particularly FL ($n = 20/52$).

In addition to the development of clonally related histiocytic neoplasms and lymphoid or myeloid malignancies through trans-/dedifferentiation or from a common haematopoietic precursor cell or clone, alternative mechanisms may explain the high prevalence of haematological malignancies in (especially adult) histiocytosis patients. A multi-institutional cohort study of adult patients with ECD or mixed histiocytosis by Papo *et al* demonstrated that, in 10/12 patients with an additional myeloid malignancy that were molecularly characterised using NGS, the histiocytic neoplasm and myeloid malignancy harboured distinct kinase mutations [23]. These findings suggest that the two neoplasms were not clonally related and arose at different stages of haematopoietic development in these patients. Interestingly, the histiocytosis patients with an additional myeloid neoplasm were significantly older at ECD diagnosis than the histiocytosis patients without an additional myeloid neoplasm. Therefore, the authors hypothesised that the high prevalence of (seemingly unrelated) myeloid malignancies in (particularly older) adult histiocytosis patients could be due to the well-described association between aging and clonal hematopoiesis (CH) [70], given that CH is known to be associated with increased frequency of myeloid neoplasms because of acquisition of somatic mutations in genes commonly mutated in myeloid neoplasms. Accordingly, prospective analysis of bone marrow samples by NGS revealed that almost half of adult ECD patients harbour mutations in myeloid cancer- and CH-associated genes [71,72], such as *TET2*. These mutations may represent early events in the development of ECD and of additional myeloid malignancies in ECD patients. In this hypothetical model, the histiocytic neoplasm and additional myeloid malignancy may independently derive from a different CH subclone, although development from a shared (e.g. *TET2* mutated [15,72]) CH (sub)clone is also possible.

Unfortunately, all three patients described in this manuscript died with active (histiocytic) disease, despite treatment in tertiary referral hospitals according to current standard-of-care protocols. Similarly, many of the previously reported patients with a histiocytic neoplasm and additional haematological cancer bearing the same genetic alteration(s) died within several months or a few years after diagnosis of the last presenting disorder (see supplementary material, Tables S2 and S3). Collectively, these observations underscore the poor prognosis for such patients. Novel therapeutic options are thus urgently needed. Recent studies have demonstrated safety and remarkable efficacy of BRAF [73–78] and MEK [78,79] inhibition in patients with *BRAF*-mutated or *BRAF* wildtype histiocytic neoplasms, respectively. Moreover, dramatic responses to selective inhibition of RET (Selpercatinib) or ALK (Crizotinib) have been observed in two patients with histiocytic neoplasms driven by rare rearrangements involving one of these genes [10], and high response rates have been described in ECD patients treated with the mTOR inhibitor Sirolimus [80]. Although there is a well-described risk of paradoxical activation of ERK signalling in (pre)malignant cells bearing MAPK pathway mutations other than $BRAF^{V600E}$ upon BRAF inhibition [81,82], the use of targeted therapeutics in patients with histiocytoses and additional haematological malignancies bearing the same kinase alterations may result in a beneficial response across both conditions [23]. Accordingly, one patient with

Table 3. Overview of reported patients with histiocytic neoplasms and additional haematological malignancies bearing the same genetic alteration(s) as evidenced by DNA sequencing and/or DNA methylation profiling.

Nr	P/A	Histiocytic neoplasm	Associated haematological malignancy	Shared genetic alteration(s)[1]	Reference
1	P	LCH	T-ALL	• *KRAS* p.G12V, *BRAF* p.G464V, *NOTCH1* p.Q2501X, *CREBBP* p.R2066H and 14 VUS; • Homozygous CN loss at 9p21.3, including *CDKN2A/2B*	[27]
2	P	LCH	T-ALL	*NOTCH1* p.Q2405X[2]	[41]
3	A	LCH	HCL	*BRAF* p.V600E	[28]
4	A	LCH	HL	*BRAF* p.V600E	[42]
5	A	LCH	CMML	*BRAF* p.V600E	[83]
6	A	LCH	AML NOS	*ASXL1*, *IDH2* and *STAG2* mutations	[44]
7	A	LCH	AML NOS	• Trisomy 8; • *KRAS* p.A146T	[45]
8	A	LCH	PMF	*JAK2* p.V617F	[43]
9	A	Mixed LCH/ECD	ET	*JAK2* p.V617F	[23]
10	A	Mixed LCH/ECD	AML-M4	*TET2* p.L1819X and *SRSF2* p.L95P	[15]
11	A	ECD	AML-M5[3]	*NRAS* p.Q61R	This study
12	A	ECD	AML-M5	*BRAF* p.V600E, *SI* p.C635, *DNAH6* e61-23, *NDUFB4* NULL and *ENSG00000252849* NULL	[29]
13	A	ECD	AML NOS[4]	*BRAF* p.V600E	[46]
14	A	ECD	CMML	*BRAF* p.V600E, *TET2* and *SRSF2* mutations	[46]
15	A	ECD	CMML	*KRAS* p.G12D and *ASXL1* p.G642fs	[30]
16	A	ECD	CMML	*KRAS* p.G12D and *DNMT3A* p.Y623fs	[30]
17	A	ECD	CMML	*KRAS* p.G12D, *ASXL1* p.Y591X, *ROBO2* p.T982M, *CLDN1* p.A124T, *THBS4* p.R591W and *SYNC* p.E56K	[31]
18	A	ECD	CMML	*NRAS* p.G13D	[30]
19	A	ECD	CMML	*NRAS* p.Q61R	[23]
20	A	ECD	CMML	*NRAS* p.Q61R	[46]
21	A	ICH	CMML	*NRAS* p.G12V	This study
22	A	ICH	CMML	*KRAS* p.G12R	[47]
23	A	ICH	CMML	*TET2* p.Q1466X and p.Q1523X, *ASXL1* p.K618X and *ZRS2* p.Q100X	[48]
24	P	JXG	JMML	*PTPN11* p.E76K	[49]
25	P	Mixed LCH/LCS	T-ALL	*NOTCH1* p.C1693R and p.Q2441X	[50]
26	P	Mixed non-LCH/HS	T-ALL	• *ACACB* p.A507T, *ARHGEF11* UTR_C>T, *IGFN1* p.G12V, *MEIS1* p.G64G, *ODAM* p.R22H and *PPP5C* p.D453N; • CN loss at 9p21.3, including *CDKN2A/B*	[26]
27	A	HS	CMML	*KRAS* p.A59E	This study
28	N/A	HS	CMML[5]	*TP53* mutation	[51]
29	A	HS	MDS[6]	*TP53* and *BCOR* mutations	[52]
30	P	HS	B/myeloid MPAL	• Mutations in *ADCY1*, *C9orf9* and *IGHV1-69*; • CN loss at 14q, 12p and 9p, including homozygous deletion of *CDKN2A*, and gain of chromosome X	[53]
31	P	HS	T-ALL	CN loss at 14q and 9p, including *CDKN2A*	[53]
32	P	HS	T-ALL	CN loss at 9p, including *CDKN2A*	[53]
33	P	HS	T-ALL	*MYC* rearrangement	[54]
34	A	HS	T-ALL	• *MYC* rearrangement; • *NRAS* p.G12D, *PTEN* p.R234fs, *PHF6* p.R116X, *NOTCH1* p.R592H, *ROS1* p.R2072Q, *B2M* p.H71R and *LRP1B* p.L2794I	[32]
35	A	HS	HCL	• *BRAF* p.V600E; • CN loss at 7q, 10q, 11q, 17p (including *TP53*) and 17q	[25]
36	A	HS	CLL	Mutations in *BRAF* and *ATM*, 12 VUS and del(11q)	[55]
37	A	HS	FL	• *BCL2* rearrangement, and gain of 9q and 19q; • *CREBBP* p.I1471T and p.F1484V, *KMT2D* p.D3061fs and p.L5318fs, *TNFRSF14* p.W12X and *IGLL5* p.S58G and p.S61C	[39]
38	A	HS	FL	*BCL2* rearrangement	[17]
39	A	HS	FL	*BCL2* rearrangement	[17]
40	A	HS	FL	*BCL2* rearrangement	[17]
41	A	HS	FL	*BCL2* rearrangement	[17]
42	A	HS	FL	*BCL2* rearrangement	[17]
43	A	HS	FL	*BCL2* rearrangement	[17]
44	A	HS	FL	*BCL2* rearrangement	[18]
45	A	HS	FL	*BCL2* rearrangement	[38]
46	N/A	LCS	FL[7]	• *BCL2* rearrangement and *MYC* extra copies; • *CREBBP* p.1640_1641del[8]; • CN gain at 22q11.23	[56]
47	A	LCS	MZL	Trisomy 12 and del(11q14.1q24.1)	[57]
48	A	LCS	CLL	• *NRAS* p.Q61K, *NOTCH1* p.Q2403X, *KMT2D* p.E2989X and *MAP2K1* p.C121S; • CN loss at 9p21, including *CDKN2A/B*	[51,58]
49	A	IDCS	CLL/SLL	Trisomy 12	[59]
50	A	IDCS	DLBCL	• *MYC* rearrangement; • 29 mutations in exon 2 of *MYC*, *TP53* p.S94X, *PTEN* splicing mutation, *BAIAP3* p.D780Y, *PRPH2* p.N199S, *PCDHB1* p.S243L; CN gain at 6p, 10p and 16p, CN loss at chromosome 18, and uniparental disomy of 10q (including *PTEN*)	[60]
51	A	Atypical non-LCH	PTCL NOS	*NRAS* p.Q61K	[61]
52	A	Atypical non-LCH	AML-M0	*RUNX1* p.R166X and p.P425L	[62]
53	A	MPDCN	MDS-MLD	*PTPN11* p.R501K	[63]
54	A	BPDCN	AML NOS	*TET2* p.C1642fs and p.A1810fs and *SRSF2* p.P95H	[64]
55	A	BPDCN	CMML	*TET2* p.G523fs, *SRSF2* p.P95L, *PHF6* p.Q251H, *PLCXD3* p.T282M, *TRMT61B* p.Y219C[9], *STK3* p.R178fs[9], *SLC25A10* p.R263C, *DIP2A* p.R309W[9], *SARDH* p.G641E and *PLP1* p.T116M	[65]
56	A	BPDCN	CMML	*TET2* p.Y1244fs and p.Q810X and *SRSF2* p.P95H	[66]
57	N/A	BPDCN	CMML	*TET2* mutation	[67]
58	A	BPDCN	MDS-RARS	*TET2* mutation	[68]

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multi-organ LCH and CMML-1 harbouring the same *BRAF* p.V600E mutation was successfully treated with the BRAF inhibitor Vemurafenib [83]. Furthermore, one patient with *NRAS* p.Q61R mutated ECD and CMML-1 who was treated with Trametinib – a MEK inhibitor – has been reported by Papo *et al* [23]; they showed a complete metabolic response of the ECD by PET CT after 2 months, in addition to improved monocyte and platelet counts.

Shared *RAS* mutations were found in some other previously reported cases with a histiocytic neoplasm and haematological malignancy bearing the same genetic alteration(s) as well (Table 3), and were primarily detected in histiocytic neoplasms associated with myeloid leukaemia (n = 11/15). In contrast, shared *NOTCH1* mutations or shared *CDKN2A* deletions were almost exclusively present in patients with histiocytic neoplasms associated with T-cell acute lymphoblastic leukaemia (n = 4/5 and n = 5/8, respectively; see supplementary material, Tables S2 and S3). As expected, shared *BCL2* rearrangements were frequently detected in patients with malignant histiocytoses associated with FL (n = 19/20; see supplementary material, Tables S2 and S3), and shared *TET2* mutations were abundant in BPDCN associated with additional myeloid malignancies (n = 5/5), as these are the most prevalent genetic alterations in FL [84] and BPDCN [85], respectively. Although limited by their descriptive nature, these distinct distribution patterns suggest that mutations in particular genes may specifically predispose to the development of an additional clonally related haematological malignancy or secondary histiocytic neoplasm.

In addition to the shared genetic alteration(s), another interesting finding is the additional somatic mutations detected only in the secondary neoplasm. These mutations may have contributed to the divergent lineage differentiation of the common HSPC, or the dedifferentiation or lineage switch of the primary lymphoid [17,69] or histiocytic [58,61,86] neoplasm. For example, in Case 1, the *MAP2K1* and *RAF1* mutations were (presumably) only present in the HS and not in the CMML, suggesting that these mutations may have contributed to the development of the HS from a common *KRAS* mutated HSPC or primary CMML clone. Similar situations of one or more shared genetic alterations and additional unique mutations in the histiocytic neoplasm and/or associated haematological malignancy have been reported in other cases [15,18,23,25–27,29,31,32,39,41,44,45,47,52–57,59,60,63–67,87–95]. The histiocytic neoplasms often harboured unique mutations in genes encoding proteins of the MAPK signalling pathway, including *NRAS* [52,55], *KRAS* [39,53,56], *BRAF* [15,23,44,45,54,92,94] and *RAF1* [32], again demonstrating the importance of constitutive MAPK pathway activation in the pathogenesis of the histiocytic neoplasms [3,96].

In conclusion, our data further support the existence of a common haematopoietic cell-of-origin in at least a proportion of patients with a histiocytic neoplasm and additional haematological malignancy, and emphasise the importance of adequate (bone marrow) staging, prospective molecular analysis and long-term follow-up of each histiocytosis patient. Future studies should investigate whether the shared genetic alterations can be traced in HSPCs, more downstream committed precursor cells and/or mature blood cells (e.g. classical monocytes of CMML). In addition, the temporal effect of particular driver mutations on the differentiation of such haematopoietic cells needs to be explored to further unravel the mechanisms underlying the co-occurrence of histiocytic neoplasms and additional haematological malignancies.

Table 3. Abbreviations: P, paediatric; A, adult; N/A, not available; LCS, Langerhans cell sarcoma; non-LCH, non-Langerhans cell histiocytosis; NOS, not otherwise specified; MPDCN, mature plasmacytoid dendritic cell neoplasm; T-ALL, T-cell acute lymphoblastic leukaemia; HCL, hairy cell leukaemia; PMF, primary myelofibrosis; ET, essential thrombocytosis; JMML, juvenile myelomonocytic leukaemia; MPAL, mixed phenotype acute leukaemia; MZL, marginal zone lymphoma; DLBCL, diffuse large B-cell lymphoma; PTCL, peripheral T-cell lymphoma; MLD, multilineage dysplasia; RARS, refractory anaemia with ring sideroblasts.

[1]Genes mutated, deleted and/or translocated in two or more patients are depicted in bold.

[2]A *NRAS* p.G12S mutation and homozygous deletion at 9p21, including *CDKN2A*, was also detected in the T-ALL sample using WES and SNP array analysis (Kato M, *et al. Br J Haematol* 2016). LCH specimens were not available for these analyses.

[3]The patient was initially diagnosed with concurrent ECD and multiple myeloma. Molecular analysis of the multiple myeloma was precluded by the absence of cryopreserved material.

[4]AML with at least phenotypic monocytic differentiation.

[5]The patient also had a mediastinal germ cell tumour (MGCT). The existence of a common precursor was suggested by the demonstration of the same *TP53* mutation in all three neoplasms and identical chromosomal aberrations in the HS and the MGCT.

[6]The patient had a history of a metastatic non-seminomatous germ cell tumour with yolk sac component (NSGCT) with identical mutations in *TP53* and *BCOR*, along with isochromosome 12p (shared with the MDS) and a unique mutation in *RRAS2*.

[7]The patient also developed DLBCL seven years before and several months after LCS diagnosis. Molecular analysis of DLBCL specimens was precluded by the absence of material.

[8]In addition, 11 variants that are presumably germline polymorphisms were detected.

[9]These mutations are depicted as such in Figure 2A of the manuscript (Patnaik MM, *et al. Blood Cancer J* 2018), but other mutations in *TRMT61B* (p.T219C), *STK3* (p.N207fs) and *DIP2A* (p.R373W) are described in the main text of the article.

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Author contributions statement

HSL, BR, KH, JAML, PM and MDL cared for the patients and provided clinical data. KMH, STP, RMV and KHL performed histopathological analyses and molecular testing of FFPE tissue specimens. PJMV performed molecular testing of peripheral blood, bone marrow and saliva samples. AHB helped in selecting the PALGA search strategy and assisted in obtaining the pathology specimens for histopathological review and additional analyses. PGK, PCWH and AGSH wrote the PALGA study protocol. PGK analysed the PALGA dataset. PCWH performed the central pathology review. AGSH supervised the study. PGK, PCWH and AGSH drafted the manuscript. PGK made the figures and tables. All authors contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL ONLINE

Figure S1. Coverage of various genes by the different NGS panels used to analyse the affected tissue specimens of the three cases presented in this study

Table S1. Available PALGA diagnostic terms for the various histiocytic disorders

Table S2. Reported patients with histiocytic neoplasms associated with additional haematological malignancies bearing the same genetic alteration(s) as demonstrated by DNA sequencing and/or DNA methylation profiling

Table S3. Reported patients with histiocytic neoplasms associated with additional haematological malignancies bearing the same genetic alteration(s) as demonstrated by techniques other than DNA sequencing and/or DNA methylation profiling

Case 1. Detailed sequencing data

Case 2. Detailed sequencing data

Case 3. Detailed sequencing data