ALK+ histiocytosis: a new clinicopathologic spectrum highlighting neurologic involvement and responses to ALK inhibition

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Abstract:
ALK-related histiocytosis (formerly ALK-positive histiocytosis) is a rare subtype of histiocytic neoplasm first described in 2008 in three infants with multisystemic disease involving the liver and hematopoietic system. This entity has subsequently been documented in case reports and series to occupy a wider clinicopathologic spectrum with recurrent KIF5B-ALK fusions. The full clinicopathologic and molecular spectra of ALK-related histiocytosis remain, however, poorly characterized. Here, we describe the largest study of ALK-related histiocytosis to date, with detailed clinicopathologic data of 39 cases, including 37 cases with confirmed ALK rearrangements. The clinical spectrum comprised distinct clinical phenotypic groups: infants with multisystemic disease with liver and hematopoietic involvement, as originally described (Group 1A: 6/39), other patients with multisystemic disease (Group 1B: 10/39), and patients with single-system disease (Group 2: 23/39). Nineteen patients of the entire cohort (49%) had neurologic involvement (seven and twelve from Groups 1B and 2, respectively). Histology included classic xanthogranuloma features in almost one third of cases, whereas the majority displayed a more densely cellular, monomorphic appearance without lipidized histiocytes but sometimes more spindled or epithelioid morphology. Neoplastic histiocytes were positive for macrophage markers and ALK, and often conferred strong expression of phosphorylated-ERK, confirming MAPK pathway activation. KIF5B-ALK fusions were detected in 27 patients, while CLTC-ALK, TPM3-ALK, TFG-ALK, EML4-ALK and DCTN1-ALK fusions were identified in single cases. Robust and durable responses were observed in 11/11 patients treated with ALK inhibition, ten with neurologic involvement. This study presents the existing clinicopathologic and molecular landscape of ALK-related histiocytosis, and provides guidance for the clinical management of this emerging histiocytic entity.

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ALK-related histiocytosis: a new clinicopathologic spectrum highlighting neurologic involvement and responses to ALK inhibition

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KEY POINTS

- ALK-related histiocytosis is a distinct entity associated with KIF5B-ALK fusions and characterized by frequent neurologic involvement
- ALK inhibition induces robust and durable responses in patients with ALK-related histiocytosis
ABSTRACT

ALK-related histiocytosis (formerly ALK-positive histiocytosis) is a rare subtype of histiocytic neoplasm first described in 2008 in three infants with multisystemic disease involving the liver and hematopoietic system. This entity has subsequently been documented in case reports and series to occupy a wider clinicopathologic spectrum with recurrent KIF5B-ALK fusions. The full clinicopathologic and molecular spectra of ALK-related histiocytosis remain, however, poorly characterized. Here, we describe the largest study of ALK-related histiocytosis to date, with detailed clinicopathologic data of 39 cases, including 37 cases with confirmed ALK rearrangements. The clinical spectrum comprised distinct clinical phenotypic groups: infants with multisystemic disease with liver and hematopoietic involvement, as originally described (Group 1A: 6/39), other patients with multisystemic disease (Group 1B: 10/39), and patients with single-system disease (Group 2: 23/39).

Nineteen patients of the entire cohort (49%) had neurologic involvement (seven and twelve from Groups 1B and 2, respectively). Histology included classic xanthogranuloma features in almost one third of cases, whereas the majority displayed a more densely cellular, monomorphic appearance without lipidized histiocytes but sometimes more spindled or epithelioid morphology. Neoplastic histiocytes were positive for macrophage markers and ALK, and often conferred strong expression of phosphorylated-ERK, confirming MAPK pathway activation. KIF5B-ALK fusions were detected in 27 patients, while CLTC-ALK, TPM3-ALK, TFG-ALK, EML4-ALK and DCTN1-ALK fusions were identified in single cases. Robust and durable responses were observed in 11/11 patients treated with ALK inhibition, ten with neurologic involvement. This study presents the existing clinicopathologic and molecular landscape of ALK-related histiocytosis, and provides guidance for the clinical management of this emerging histiocytic entity.
INTRODUCTION

Histiocytic disorders are a group of rare diseases characterized by the accumulation of macrophage-, dendritic cell-, or monocyte-differentiated cells in various tissues and organs.\(^1,2\) With the advent of molecular technologies, recurrent genetic alterations have been identified in several histiocytoses,\(^3-17\) reframing the conceptualization of these disorders from one of primary inflammatory conditions to that of clonal neoplastic diseases.\(^18,19\) These alterations primarily affect genes encoding protein kinases of the intracellular mitogen-activated protein kinase (MAPK) signalling pathway, leading to constitutive activation of this pathway (Figure 1).\(^11\) However, downstream ERK activation has also been observed in histiocytoses without detected MAPK pathway mutations,\(^3,11,13\) leading to the notion that histiocytic neoplasms are uniformly characterized by dependence on MAPK signalling.\(^20\) This has led to the clinical implementation of pharmacological inhibitors of the MAPK signalling pathway (BRAF and/or MEK inhibitors) for the treatment of patients with severe and/or relapsed/refractory histiocytic disease.\(^20-27\) In addition to MAPK pathway mutations, recurrent activating genetic alterations in genes encoding protein kinases of the PI3K/AKT/mTOR signalling pathway (i.e. PIK3CA) or receptor tyrosine kinases (i.e. CSF1R, NTRK1, RET or ALK) have been identified in a subset of histiocytic neoplasms.\(^6,13\) Furthermore, significant associations between specific kinase alterations and clinicopathologic phenotypes have recently been observed.\(^6\) These findings pave the way for molecular (sub)classification of histiocytic neoplasms,\(^1,6,8,28\) and highlight the potential for targeted therapy beyond BRAF or MEK inhibition in histiocytosis patients.

In 2008, Chan et al. first described three infants with a novel type of systemic histiocytosis characterized by ALK immunoreactivity in large CD163\(^+\) histiocytes with variable expression of other histocyte/dendritic cell markers (S100, Fascin, Factor XIIIa).\(^20\) Clinically, the
disease was characterized by liver and hematopoietic involvement and could be confused with a storage disorder or leukemia. The disease tended to resolve slowly, sometimes even without any specific treatment but only supportive care. One case demonstrated a \textit{TPM3-ALK} translocation. Over the following years, several individual case reports and small case series about ALK-positive histiocytosis have been published\textsuperscript{30–49}, confirming the rare but indefinite occurrence of this emerging histiocytic entity, hereinafter referred to as ALK-related histiocytosis. Most notably, Chang \textit{et al.} described a series of ten cases\textsuperscript{43}, including the original three cases reported by Chan \textit{et al.}, with eight having molecular confirmation of ALK rearrangements. Together, these reports have documented ALK-related histiocytosis in both older children and adults with single- or multisystemic disease, expanding beyond the initial observation of the disease as a systemic disorder of infancy. Moreover, the frequent presence of \textit{KIF5B-ALK} fusions has been reported\textsuperscript{43}, and successful treatment of some patients with ALK inhibition has been described\textsuperscript{6,27,33,34,39,40,43,50,51}. Due to the modest number of reported patients, and particularly few cases with clinical information and/or successful molecular analysis, the full clinicopathologic and molecular spectra of ALK-related histiocytosis remain, however, incompletely characterized. Accordingly, here we describe the results of the largest study of ALK-related histiocytosis to date, outlining the clinicopathologic features of 39 patients, including 37 cases with proven \textit{ALK} rearrangements.
MATERIALS AND METHODS

Patient selection and confirmation of diagnosis

Fifty-two cases from different hospitals throughout North America, Europe, and Australia were compiled for this international retrospective study. Six cases were previously published\(^6,30,31,39,44,47\), and are reported with updated follow-up (when available). All cases underwent central review of pathology slides (J.P. & J-F.E.) for confirmation of diagnosis. Additional immunohistochemical and/or molecular analyses were performed when deemed appropriate. Criteria for a diagnosis of ALK-related histiocytosis and inclusion in the study were the following: (i) histologic confirmation of a histiocytosis\(^1\), with expression of at least two macrophage/histiocyte markers (including CD163, CD68, CD14 and/or CD4) by the lesional cells, and (ii) molecular confirmation of ALK rearrangement and/or (iib) classic infantile systemic disease with diffuse strong ALK immunoreactivity, as previously described\(^29\). Cases with ALK rearrangements in which immunohistochemistry revealed histiocytes to be disparate from lesional ALK\(^+\) cells without immunoreactivity for macrophage/histiocyte markers were termed “atypical ALK-rearranged histiocyte-rich tumors” and not included in the primary study cohort. Also, histiocytosis cases with ALK immunoreactivity but without ALK rearrangements by comprehensive molecular analysis (i.e. RNA sequencing) were not included in the primary study cohort. Cases excluded for these two reasons are characterized separately. Written informed consent for this study was obtained from the patients and/or their legal representative when required, or a waiver of consent was obtained from the relevant Institutional Review Board for retrospective research.

Clinical and radiologic assessment and data collection

Clinical information was extracted from the medical records by the treating physicians and provided in a pseudonymized fashion according to a standardized de-identified case report.
form. Retrieved data included demographic characteristics, presenting symptoms, sites of
disease involvement, treatments, and treatment outcomes. First- and second-or-further line
treatments were categorized as observation with supportive care, surgical resection,
radiotherapy, conventional systemic therapy (i.e. cytotoxic chemotherapy, corticosteroids,
immunosuppression and/or interferon-alpha), ALK inhibition, or a combination of these. As
previously published for Erdheim-Chester disease (ECD) and Langerhans cell histiocytosis
(LCH)\textsuperscript{27,52,53}, responses to treatment as documented in the medical record were classified as
complete response (complete resolution of disease), partial response (partial resolution of
disease), stable disease (no significant change in lesions), or progressive disease (growth of
known lesions or appearance of new lesions). Responses were extracted from the clinical
medical records and based on formal clinical interpretations of ultrasound, computed
tomography (CT), magnetic resonance imaging (MRI) and/or positron emission tomography
(PET)-CT when available. The less favorable radiologic response was prioritized if multiple
imaging methods were used that showed discrepant responses. Objective response was
defined as partial or complete response. Whether or not progression or relapse of disease was
subsequently observed was dichotomously captured. Progression was defined as progressive
disease or death from any cause.

**Histopathologic analysis**

Histopathologic information was collected in a standardized de-identified case report form.
Morphology and immunophenotype were reviewed for all cases (J.P. & J-F.E.).
Immunohistochemistry (IHC) was performed as part of initial diagnosis at participating
institutions, and consequently was executed using varying staining techniques and
monoclonal antibodies. If ALK, CD163, CD68, S100 and/or CD1a IHC was not originally
performed, these stains were done in the process of central review when sufficient tumor
tissue was available. The ALK clones that were used for each case are shown in Supplementary Table 1. The phosphorylated-ERK (p-ERK) immunostains were all performed using clone D13.14.4E (Cell Signaling, The Netherlands).

**Molecular pathologic analysis**

Molecular pathologic information was collected in a standardized de-identified case report form. Molecular analysis was performed as part of initial diagnosis at referring institutions or done in the process of central review, when sufficient tumor tissue was available. For analyses performed during central review, fluorescence in situ hybridization (FISH) analysis was performed using CytoCell® dual color ALK break-apart probes (Oxford Gene Technology, UK), as previously described. Targeted RNA sequencing was performed using customized Archer® FusionPlex® next-generation sequencing (NGS) panels, which allow the detection of both fusions with known or unknown fusion partners. For NGS analysis at the European referral center Ambroise Paré Hospital (Paris, France), RNA was isolated using the Maxwell RSC RNA FFPE kit (Promega, USA), and RNA concentrations were measured using the Qubit RNA HS Assay kit (Thermo Fisher Scientific, USA). Successful NGS required 20-250 ng RNA per reaction. Generated libraries were sequenced using a MiSeq system (Illumina, USA), and produced reads were analyzed with Archer Analysis software. Archer® RNA NGS at the North American referral center Memorial Sloan Kettering Cancer Center (New York, USA) was performed as previously described. Clonality assessment of T-cell receptor (TCR) or immunoglobulin (Ig) gene rearrangements was performed using standardized multiplex PCR assays, as previously described.

**Statistical analysis**
Data were analyzed using descriptive statistics. Continuous variables were summarized with medians and ranges, and categorical variables were summarized with frequencies and proportions. Frequency of objective responses were summarized for treatment modality by patient group and for the entire cohort, and the frequency of disease progression or relapse following therapy was summarized.
RESULTS

Thirty-nine patients meeting criteria for ALK-related histiocytosis were identified and included in the study (Tables 1-3; Supplementary Tables 1-3). In addition, three patients with atypical ALK-rearranged histiocyte-rich tumors were recognized (Table 3), and ten patients were identified with histiocytoses demonstrating ALK immunoreactivity but no ALK rearrangement by RNA sequencing (Supplementary Table 4). These thirteen patients without complete criteria for ALK-related histiocytosis diagnosis were excluded from the primary study cohort and are described separately.

Clinical features

The 39 patients with ALK-related histiocytosis comprised 24 females and fifteen males and included 31 children and eight adults (Table 1). Their age at presentation ranged from zero days to 41 years – one infant had hepatosplenomegaly, anemia and thrombocytopenia only seven hours after birth. Moreover, the 31 pediatric cases consisted of 21 children of three years or younger, including thirteen infants (age < 1 year). The 39 patients had diverse organ system manifestations (Figures 2-6), and were classified into two distinct clinical phenotypic groups: patients with multisystem disease (Group 1, N = 16) and patients with single-system disease (Group 2, N = 23). Within Group 1 were infants with systemic disease with liver and hematopoietic involvement, as originally described (Group 1A, N = 6), and other patients with multisystemic disease, not fitting the original infantile disease presentation (Group 1B, N = 10).

Infants with systemic disease with liver and hematopoietic involvement (Group 1A)

Patients in Group 1A ranged from zero days to five months of age, and presented with hepatomegaly, anemia and thrombocytopenia. In addition, 5/6 had splenomegaly, 5/6
displayed leukocytosis and some had measurable liver dysfunction, such as elevated liver enzymes, high bilirubin and/or decreased serum protein and albumin levels. In three cases, focal lesions were seen in the liver (Figure 2). Coagulation profiles were available for 4/6 patients (Supplementary Table 2), revealing prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT) and decreased serum fibrinogen in Case 3. Small numbers of ALK-positive histiocytes were observed in the bone marrow of all five patients that had a bone marrow biopsy performed. Regarding additional sites of disease, 2/6 had renal involvement, 1/6 had interstitial lung involvement requiring prolonged oxygen supplementation, and 1/6 had skin involvement (Table 3).

Other patients with multisystemic disease (Group 1B)

Group 1B contained five children and five adults who presented with disseminated disease involving various organs (Table 1), including three infants without hematopoietic involvement. The most commonly involved organs were the nervous system (7/10), bone (7/10), lungs (7/10), liver (5/10), skin (4/10), and lymph nodes (4/10). All three pediatric cases with neurologic involvement had multiple masses in the brain (Figure 4A-E); adult cases had lesions of cranial and/or spinal nerves (3/4; Figure 4G-I), intramedullary spinal cord and/or leptomeningeal enhancement (2/4; Figure 4F), and/or parenchymal brain masses (1/4). Reminiscent of the localization of bone lesions in ECD, bone involvement included bilateral lesions in femora and/or tibiae in 7/7 patients (Figure 3A-D). Lung involvement was uniformly characterized by pulmonary nodules, ranging in size from micronodules to significant tumors (Figure 3H-K). All five patients with liver involvement had focal lesions on imaging.

Patients with single-system disease (Group 2)
Patients in Group 2 were 20 children and three adults, including twelve cases with neurologic involvement (52%). Patients with neurologic involvement presented with diverse neurological symptoms, including seizures (3/12), ataxia (3/12), headaches (3/12), vomiting (2/12), hypotonicity (2/12), unclear paroxysmal neurologic symptoms (“spells”) (1/12), torticollis (1/12), trigeminal neuralgia (1/12), paresis (1/12) and diplopia (1/12). Tumoral lesions were widely distributed in the central and peripheral nervous system (Figure 4J-S; Table 3). In addition, two patients had cerebrospinal fluid (CSF) monocytosis, including one case with a very low level of ALK rearrangements in the CSF by FISH analysis. The eleven patients with non-neurologic disease comprised one case with a single lung tumor, two cases with solitary bone lesions, and eight children with localized skin lesions or soft tissue tumors in varying locations (Figure 5; Table 3).

Treatments

Group 1A

Two of six patients did not receive histiocytosis-directed therapy but were managed with supportive care including transfusions (Table 2). In both cases, spontaneous resolution of disease was observed. Three other patients were treated with chemotherapy. Two experienced progressive disease, including one patient that had worsening of ascites and died from coagulopathy and sepsis. The other received second-line systemic therapy with dexamethasone, anakinra and azathioprine and obtained a complete response. The third patient achieved a partial response to first-line chemotherapy, relapsed with biopsy-proven renal involvement at five months after completion of treatment, and subsequently received a second round of chemotherapy. The remaining patient was lost to follow-up.

Group 1B
First-line therapy consisted of conventional systemic therapy alone in 6/10, and ALK inhibition with or without conventional therapy in 4/10 patients (Table 2). Conventional systemic therapy led to an objective response in 3/6 patients, two of whom had sustained complete responses to chemotherapy and one of whom died from (likely immunosuppression-related) sepsis following a partial response to high-dose corticosteroids (Table 3). The 3/6 patients with stable or progressive disease following conventional systemic therapy were treated with ALK inhibition as second-line therapy, with objective responses in all three. ALK inhibition, with or without chemotherapy, led to an objective response in 4/4 patients in the first-line setting. Responses to ALK inhibition were durable in 7/7 patients, with no events of progression or relapse on treatment (Figure 7).

Group 2
First-line treatment consisted of surgical resection in 13/23, radiation in 1/23, conventional systemic therapy with or without local therapy in 4/23, and ALK inhibition in 1/23 patients (Table 2). Furthermore, 1/23 patients was observed without treatment, 1/23 was not treated yet, and 2/23 were lost to follow-up. Of those with local therapy, 2/14 had progressive disease. Of patients treated with conventional systemic therapy, 3/4 had an objective response, and 1/4 had progressive disease. The patient treated with ALK inhibition had an objective response. Second-line treatment was given to four patients. ALK inhibition was administered in 2/4 patients with objective response, while the two others received chemotherapy with progressive disease. Third-line treatment with ALK inhibition was initiated in one of these patients with objective response (Figure 5A-D). Responses to ALK inhibition were durable in 4/4 patients (Figure 7).

Histopathologic features
The morphology of ALK-related histiocytoses varied and included classic xanthogranuloma features with plump foamy histiocytes and variable Touton giant cells in 12/39 (31%) patients (Figure 8A-B) to a more densely cellular, monomorphic appearance without lipidized histiocytes in the majority of cases (Figure 8C and 8E), some with more spindled/streaming or epithelioid histocyte morphology (Supplementary Table 1). Nuclear features included ovoid nuclei that frequently displayed either a slight fold or indentation (i.e. “cup shape”) of its nuclear contour. Mild atypia was limited to 6/39 cases, often with an epithelioid phenotype, which can be confused with malignant histiocytosis; however, mitotic counts and Ki-67 proliferation indexes were low to moderate. Bone marrow involvement was typically a small amount and not a diffuse infiltrative process as in leukemia. The morphology was that of plump histiocytes with indented nuclei and a JXG-like phenotype in normo- to hypercellular bone marrow with relatively preserved hematopoiesis and occasional eosinophilia, mild myelofibrosis, and/or megakaryocytic hyperplasia.

The ALK staining pattern did not appear to correlate with molecular alteration, as the frequent KIF5B-ALK fusion was noted with all stain patterns; however, no (convincing) nuclear ALK staining was observed. Four cases displayed focal weak ALK staining and 3/39 had exclusive cytoplasmic Golgi dot-like staining in the lesional histiocytes (Figure 8I; Table 4), despite confirmed ALK fusions. Almost half of cases had variable Rosai-Dorfman disease (RDD)-like histologic features including S100 expression (18/39) and emperipolesis (13/39). When tested for OCT-2 expression, 14/23 (61%) were positive. When tested for p-ERK (18/39) and Cyclin D1 (19/39) expression (Supplementary Table 1), the lesional histiocytic cells often showed corresponding, strong expression (Figure 8K-L).

Molecular pathologic features
ALK rearrangements were confirmed in 37/39 patients (Table 1). In two infants from Group 1A with classic systemic disease, similar to the cases described by Chan et al.\textsuperscript{29}, no ALK rearrangements were detected by FISH; however, the material was insufficient for comprehensive analysis by RNA sequencing. \textit{KIF5B} was the most common ALK fusion partner and identified in 27 cases, with exon 24 of \textit{KIF5B} fused to exon 20 of \textit{ALK} in 25/27 cases (Supplementary Table 3). In addition, \textit{CLTC-ALK}, \textit{TPM3-ALK}, \textit{TFG-ALK}, \textit{EML4-ALK} and \textit{DCTN1-ALK} fusions were detected in single cases (Supplementary Figure 1).

### Atypical ALK-rearranged histioyte-rich tumors: a potential diagnostic pitfall

Three cases were identified without macrophage/histiocyte marker expression by the \textit{ALK\textsuperscript{+}} tumor cells, but with prominent intermixed CD163 expressing (reactive) macrophages (Supplementary Figure 2), which can be mistaken for ALK-related histiocytosis. Moreover, by hematoxylin and eosin stain, the tumor cells often had similar morphology as those seen in ALK-related histiocytosis, consisting of plump mononucleated cells with eosinophilic cytoplasm. In addition, the lesional cells demonstrated p-ERK and/or Cyclin D1 expression (Supplementary Figure 2). Despite extensive immunohistochemical and molecular pathologic analysis, no definitive diagnosis could be made for the three cases. The \textit{ALK\textsuperscript{+}} cells lacked immunoreactivity for many lineage markers, including CD30 and smooth muscle actin (Supplementary Table 1). Moreover, clonality assessment of TCR and Ig gene rearrangements did not reveal high monoclonal peaks (Supplementary Methods), arguing against a type of lymphoma. CD34 immunostains did reveal a remarkable nested, endocrinoid to hemangiopericytoma-like pattern with abundant blood capillaries in all three cases (Supplementary Figure 2). None had the \textit{KIF5B-ALK} fusion, but rather harbored \textit{ALK} fusions involving \textit{EML4} or \textit{SQSTM1}.
Clinically, one of the three patients was an adult with Group 1B-like multisystemic disease involving the CNS, left lung and bone (Case A1; Supplementary Figure 3A-D). This patient initially had an objective response to ALK inhibition, but then demonstrated disease progression and switched to chemotherapy (Figure 7; Table 3). The remaining two cases had Group 2-like single-system disease with localized soft tissue tumors. Both were treated with surgery; however, one relapsed with subcutaneous and liver metastases (Case A2; Supplementary Figure 3E-F) and received systemic treatment with ALK inhibition with stable disease after three months of Crizotinib.

**ALK-immunoreactive histiocytoses without ALK rearrangements**

Ten patients were identified with histiocytoses demonstrating variable ALK immunoreactivity (Supplementary Figure 4) but no ALK rearrangement by RNA sequencing. Both clinically and histologically, these cases represented the full spectrum of histiocytic neoplasms, from L-, C-, R- to M-group (Supplementary Table 4). When compared to ALK-related histiocytoses, they more often showed nuclear ALK staining. Other MAPK activating somatic mutations were identified in 5/10 patients.
DISCUSSION

We present here a collaborative international study of ALK-related histiocytosis with detailed clinicopathologic data of 39 cases, including 37 cases with confirmed ALK rearrangements. This study defines a new clinicopathologic spectrum, and highlights frequent neurologic involvement. Histology included classic xanthogranuloma features in almost one third of patients, whereas the majority displayed a more densely cellular, monomorphic appearance without lipidized histiocytes. We affirm the frequent occurrence of KIF5B-ALK fusions and expand the molecular spectrum by describing single cases with CLTC-ALK, TPM3-ALK, TFG-ALK, EML4-ALK or DCTN1-ALK fusions. Finally, we show dramatic and durable responses in 11/11 patients treated with ALK inhibition (Figure 7), ten with neurologic involvement.

We noted an apparent predilection for females in our cohort of ALK-related histiocytosis cases, consistent with our overview of the existing literature (Supplementary Table 5) and the female predilection observed in ALK-rearranged tumors other than non-small cell lung cancer, such as ALK-rearranged inflammatory myofibroblastic tumors. This female predilection is in clear contrast to the male predominance in LCH, ECD, and JXG; particularly in BRAF p.V600E associated CNS lesions with JXG histology.

Our Group 1A patients were similar to the cases originally described by Chan et al. In addition to liver, spleen and hematopoietic involvement, our patients had lesions in the lungs, kidneys and/or skin. Unlike in LCH, in which liver, spleen and/or hematopoietic involvement constitutes “risk organ” involvement associated with increased mortality, this clinical phenotype in ALK-related histiocytosis does not necessarily comprise high-risk disease as two of six patients from Group 1A had spontaneous regression of disease with only...
supportive care. However, of the three other patients with clinical follow-up, two required second-line systemic therapy and one died, underscoring the life-threatening condition that this disease can still represent29.

Other patients with multisystemic ALK-related histiocytosis (Group 1B) have been variably reported previously (Supplementary Table 5), sometimes as ECD or systemic JXG. The nervous system was the most frequently involved organ in our patients from this group, followed by the lungs, bone, liver, skin, and lymph nodes. Although there are similarities between patients from this group and patients with ECD, particularly bilateral bone lesions in the legs, many other stigmata of ECD were not observed. For example, perinephric soft tissue thickening (“hairy kidneys”), periaortic encasement, diabetes insipidus and right atrial infiltration are frequent in ECD62,67, but were absent in our cohort. Moreover, ECD patients are generally older, with a median age of 55 years2,63, as opposed to a median age of 14.5 years of our Group 1B patients. Apart from the specific phenotypic entity that is ECD, there exists the constellation of “extracutaneous/systemic JXG”68,69, referring to the rare disease in childhood with JXG histology involving extracutaneous tissues. In a recent series of “systemic JXG”, associations were observed between specific molecular alterations and clinical phenotypes, with ALK rearrangements overrepresented in female cases and cases with lung involvement68. These findings are compatible with ours, raising the question whether ALK-related histiocytosis is distinct from “systemic JXG” without ALK rearrangements.

Altogether, while there is overlap in histomorphology and immunophenotype, we believe that sufficient evidence is available for the designation of ALK-related histiocytosis as a separate histiocytic entity from both ECD and “extracutaneous/systemic JXG”, as previously proposed by others45.
The findings in our patients with single-system disease (Group 2) were similar to that which has been observed previously (Supplementary Table 5), with the nervous system and skin/soft tissue as common sites of disease and surgical resection often being definitive. We expand the clinical spectrum by describing patients with localized bone involvement. When compared to previously reported cases with single-system disease, our cohort includes more cases with isolated neurologic involvement (52% vs. 19%), and more children (87% vs. 38%). Given the retrospective nature of our study, we cannot rule out some selection bias influencing the clinical spectrum of the disease in our cohort. For example, our cohort did not include adult female patients with isolated breast masses, as recently described by Kashima et al. and Osako et al. Yet, our study did include two patients with multisystemic disease including breast masses (Table 3; Figure 3R), supporting the recurrent involvement of this anatomic site.

Eleven patients in our cohort were treated with diverse ALK inhibitors, either as first- or second-line therapy, and sustained objective responses were observed in 11/11 patients (Figure 7). These data corroborate the favorable outcomes of ALK inhibition observed in seven ALK-related histiocytosis patients outside of our cohort33,34,40,43,50,51. Our patients were treated with various ALK inhibitors at variable doses; however, the impression of their efficacy is unequivocal. The responses to ALK inhibition stand in contrast to outcomes with conventional systemic therapy, which conferred an objective response in only 7/13 (54%) patients as first-line therapy and 2/5 (40%) patients as second-line therapy (Table 2). However, given that this is a retrospective study, it remains undefined whether ALK inhibition should be implemented as first-line treatment or when disease is refractory to conventional therapies. Also, our study does not address the optimal treatment regimen or duration for ALK inhibition, which is an enduring question for targeted therapy of
histiocytosis more broadly, as cessation of BRAF inhibition in histiocytosis patients has been shown to lead to early relapses in most cases\textsuperscript{22,26,70}.

As illustrated by the three “atypical ALK-rearranged histiocyte-rich tumors”, stringent histopathologic assessment is essential before making a diagnosis of ALK-related histiocytosis. Diagnosis requires diffuse expression of macrophage/histiocyte markers, preferably CD163, by the lesional ALK\textsuperscript{+} tumor cells. ALK-rearranged tumors with prominent intermixed histiocytic infiltrates disparate from ALK\textsuperscript{+} cells without immunoreactivity for macrophage/histiocyte markers are a potential diagnostic pitfall, and should be assessed for the possibility of representing another ALK-rearranged entity, such as inflammatory myofibroblastic tumors, which typically do not harbor $KIF5B$-$ALK$ fusions\textsuperscript{71,72}. Future studies may elucidate whether “atypical ALK-rearranged histiocyte-rich tumors” without characteristics of established (ALK-rearranged) entities comprise a new entity. In all three of our cases, the CD34 immunostain revealed a remarkable nested pattern with abundant blood capillaries (Supplementary Figure 2), which was also observed in a case previously reported as ALK-positive histiocytosis with unusual morphology and a $TRIM33$-$ALK$ fusion\textsuperscript{32}. Clinically, this patient had a large mesenteric tumor, highly similar to Case A2 from our study (Supplementary Figure 3E-F). Finally, the monomorphic dense infiltration of histiocytes seen in the majority of our ALK-related histiocytosis cases should be distinguished from epithelioid fibrous histiocytoma\textsuperscript{73}.

Supported by our ten excluded histiocytosis cases demonstrating variable ALK immunoreactivity but no ALK rearrangement by RNA sequencing, molecular confirmation of ALK rearrangement should be performed whenever possible to confirm the diagnosis of ALK-related histiocytosis. The ten excluded cases also provide rationale for changing the
name of this entity from “ALK-positive” to “ALK-related” histiocytosis; this distinction is important because cases with ALK staining but without ALK rearrangements may not benefit from ALK inhibition\textsuperscript{74,75}. ALK IHC should particularly be applied in the diagnostic workup of infants with classic multisystemic disease and patients with tumorous lesions in the nervous system, lungs, bone and/or soft tissue with non-LCH histology. Yet, pathologists should be aware of the variable staining intensity and pattern, with variations further compounded by the ALK clone and protocol used. Comprehensive molecular analysis (i.e. RNA sequencing) should be performed in ALK IHC negative cases when the detection of an ALK rearrangement could have treatment consequences, as we noted in seven confirmed ALK-rearranged cases weak or exclusive Golgi dot-like cytoplasmic ALK staining that could be misinterpreted as negative.

Overall, this study defines a new clinicopathologic spectrum of ALK-related histiocytosis with frequent neurologic involvement and durable responses to ALK inhibition. The findings support the recognition of ALK-related histiocytosis as a distinct entity from both ECD and (extracutaneous/systemic) JXG. Comprehensive genomic analysis of patients with histiocytic neoplasms is now a necessary adjunct to detailed morphologic and immunophenotypic assessment, as this may directly affect disease classification and therapeutic decision making.

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AUTHOR CONTRIBUTIONS

P.G.K., J.P., E.L.D. and J-F.E. conceived the study and drafted the manuscript. All other authors contributed cases and helped revise the manuscript, meeting ICMJE criteria for authorship. J.P. and J-F.E. performed the central pathology review and provided histologic images. P.G.K., J.P., B.H.D., Z.H-R., L.H-J. and J-F.E. performed additional immunohistochemical and/or molecular analyses. P.G.K. made the figures. A.G.S.H. participated in data discussions. A.G.S.H. and O.A. provided supervision. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

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### TABLE 1. Clinical and molecular characteristics of the distinct clinical phenotypic groups of ALK-related histiocytosis and the overall cohort.

|                        | Group 1A (N = 6) | Group 1B (N = 10) | Group 2 (N = 23) | Overall (N = 39) |
|------------------------|------------------|------------------|------------------|------------------|
| **Age at presentation**|                  |                  |                  |                  |
| Median age (range)     | 1.5 months (0-5 months) | 14.5 years (0-41 years) | 7 years (0-41 years) | 3 years (0-41 years) |
| Child                  | 6 (100%)         | 5 (50%)          | 20 (87%)         | 31 (79%)         |
| Adult                  | 5 (50%)          | 3 (13%)          | 8 (21%)          |                  |
| **Gender**             |                  |                  |                  |                  |
| Female                 | 4 (67%)          | 7 (70%)          | 13 (57%)         | 24 (62%)         |
| Male                   | 2 (33%)          | 3 (30%)          | 10 (43%)         | 15 (38%)         |
| **Organ involvement**  |                  |                  |                  |                  |
| Nervous system         |                  |                  |                  |                  |
| Liver                  | 6 (100%)         | 5 (50%)          | 11 (28%)         |                  |
| Lung                   | 1 (17%)          | 7 (70%)          | 9 (23%)          |                  |
| Bone                   | 7 (70%)          | 2 (9%)           | 9 (23%)          |                  |
| Skin                   | 1 (17%)          | 4 (40%)          | 4 (17%)          |                  |
| Soft tissue            |                  | 3 (30%)          | 4 (17%)          | 7 (18%)          |
| Hematopoietic system   | 6 (100%)         | 5 (50%)          | 11 (28%)         | 6 (15%)          |
| Spleen                 | 2 (33%)          | 2 (20%)          | 11 (28%)         |                  |
| Kidney                 |                  | 2 (20%)          | 4 (10%)          | 5 (13%)          |
| Lymph node             | 4 (40%)          | 4 (40%)          | 4 (10%)          |                  |
| Breast                 | 2 (20%)          | 2 (20%)          | 2 (5%)           |                  |
| Pancreas               | 2 (20%)          | 2 (20%)          | 2 (5%)           |                  |
| Other*                 | 2 (20%)          | 2 (20%)          | 2 (5%)           |                  |
| **ALK rearrangement**  |                  |                  |                  |                  |
| KIF5B-ALK              | 1 (17%)          | 7 (70%)          | 19 (52%)         | 27 (69%)         |
| CLTC-ALK               | 1 (17%)          | 1 (10%)          | 1 (3%)           |                  |
| TPM3-ALK               | 1 (10%)          | 1 (10%)          | 1 (3%)           |                  |
| TFG-ALK                |                  | 1 (10%)          | 1 (3%)           |                  |
| EML4-ALK               |                  | 1 (4%)           | 1 (3%)           |                  |
| DCTN1-ALK              |                  | 1 (4%)           | 1 (3%)           |                  |
| ALK-FISH+              | 2 (33%)          | 1 (10%)          | 2 (9%)           | 5 (13%)          |
| Not confirmed          | 2 (33%)          |                  |                  | 2 (5%)           |
| **Median follow-up (range)** | 3.75 years (0-7 years) | 2 years (0-6 years) | 14 months (0-18 years) | 21 months (0-18 years) |
| **Status at last follow-up** |                     |                  |                  |                  |
| Alive with             |                  |                  |                  |                  |
| no evidence of disease | 4 (67%)          | 5 (50%)          | 14 (61%)         | 23 (59%)         |
| regressive disease     | 4 (40%)          | 5 (22%)          | 9 (23%)          |                  |
| stable disease         |                  | 1 (4%)           | 1 (3%)           |                  |
| recent diagnosis       |                  | 1 (4%)           | 1 (3%)           |                  |
| Dead                   | 1 (17%)          | 1 (10%)          |                  | 2 (5%)           |
| Lost to follow-up      | 1 (17%)          |                  | 2 (9%)           | 3 (8%)           |

Abbreviations: FISH, fluorescence in situ hybridization. *, cervix, thyroid, salivary glands and colorectum.
TABLE 2. Treatments and outcomes of the different clinical groups of ALK-related histiocytosis and the overall cohort.

|                      | Group 1A (N = 6) | Group 1B (N = 10) | Group 2 (N = 23) | Overall (N = 39) |
|----------------------|------------------|------------------|------------------|-----------------|
|                      | Number of cases  | Objective response | Progression or relapse | Number of cases  | Objective response | Progression or relapse | Number of cases  | Objective response | Progression or relapse |
| First-line           |                  |                  |                  |                  |                  |                  |                  |                  |
| Conventional systemic therapy | 3 (50%) | 1/3 (33%) | 3/3 (100%) | 6 (60%) | 3/6 (50%) | 2/6 (33%) | 13 (33%) | 7/13 (54%) | 6/13 (46%) |
| Surgical resection   |                  |                  |                  |                  |                  |                  |                  |                  |
| ALK inhibition        |                  |                  |                  |                  |                  |                  |                  |                  |
| Observation & supportive care | 2 (33%) | 2/2 (100%) | 0/2 (0%) | 4 (40%) | 4/4 (100%) | 0/4 (0%) | 5 (13%) | 5/5 (100%) | 0/5 (0%) |
| Radiotherapy          |                  |                  |                  |                  |                  |                  |                  |                  |
| Unknown or not treated yet | 1 (17%) |                  |                  | 3 (13%) |                  |                  | 4 (10%) |                  |                  |
| Second- and further-line |            |                  |                  |                  |                  |                  |                  |                  |
| ALK inhibition        |                  |                  |                  |                  |                  |                  |                  |                  |
| Conventional systemic therapy | 3 (50%) | 2/3 (67%) | 1/3 (33%) | 3 (30%) | 3/3 (100%) | 0/3 (0%) | 6 (15%) | 6/6 (100%) | 0/6 (0%) |

1, including two patients treated with a combination of ALK inhibition and chemotherapy; 2, including two patients treated with chemotherapy following surgical resection; 3, including two patients who received systemic corticosteroids before surgical resection.
TABLE 3. Individual patient data of cases with ALK-related histiocytosis (*N = 39*) or atypical ALK-rearranged histocyte-rich tumors (*N = 3*).

| Nr | Gen f/variant | Sex | Age | Sites of disease | First-line treatment | Response | Progression | Second- and further-line treatment | Last response | Therapy ongoing | Outcome (follow-up#) |
|----|---------------|-----|-----|------------------|----------------------|----------|-------------|-----------------------------------|--------------|----------------|---------------------|
| GROUP 1A: INFANTS WITH MULTISYSTEMIC DISEASE WITH LIVER AND HEMATOPOIETIC INVOLVEMENT |
| 1* | N/A           | F   | 0 days | Liver, hematopoietic system, spleen, lung, possibly kidney | IVIG (2 days), corticosteroids (tapered after diagnosis) and supportive care (transfusions) | CR        | No          | -                                 | -            | No            | Alive with no disease (25 years) |
| 2* | ALK, FISH+    | F   | 27 days | Liver, hematopoietic system, spleen, kidney at relapse | Chemotherapy (VBL/PRED, 6 months) | PR        | Yes         | Chemotherapy                    | CR           | No            | Alive with no disease (7 years) |
| 3  | KIF5B, ALK    | M   | 1 month | Liver, hematopoietic system, spleen, kidney, skin | Chemotherapy (VBL/DEX/MTX, 3 weeks) | PO        | Yes         | Chemotherapy (2-CA, 1 week)      | PO           | No            | Died from complications and sepsis (1 month) |
| 4  | N/A           | F   | 2 months | Liver, hematopoietic system, spleen | Supportive care (transfusions) | CR        | No          | -                                 | -            | No            | Alive with no disease (45 years) |
| 5  | ALK, FISH+    | F   | 4 months | Liver, hematopoietic system | N/A | N/A | N/A | N/A | N/A | N/A | (not to follow up) |
| 6  | QTC, ALK      | M   | 5 months | Liver, hematopoietic system, spleen | Corticosteroids (PRED 40mg/m^2, 2.5 weeks), followed by chemotherapy (VBL - tapered PRED, 2.5 weeks) | PD        | Yes         | Dexmethylasone + anakinra (1 month), followed by anakinra (3 months) + azathioprine (6 months) | CR           | No            | Alive with no disease (4 years) |
| GROUP 1B: OTHER PATIENTS WITH MULTISYSTEMIC DISEASE |
| 7  | TNN-ALK       | F   | 3 months | Bone, lung, liver | Chemotherapy (VBL/PRED/OMP, 18 months) | CR        | No          | -                                 | -            | No            | Alive with no disease (2 years) |
| 8  | ALK, FISH+    | F   | 9 months | Lung, skin, kidney | Chemotherapy (VBL/PRED, 12 months) | CR        | No          | -                                 | -            | No            | Alive with no disease (2 years) |
| 9* | KIF5B, ALK    | M   | 10 months | CNS, lung, liver, soft tissue (peritoneum) | Chemotherapy (VBL/PRED, combined with ALK inhibition (Alectinib)) | CR        | No          | -                                 | -            | Yes (VBL/PRED + Alectinib) | Alive with no disease (13 months) |
| 10 | KIF5B, ALK    | F   | 2 years | CNS, bone, lung, liver, skin, soft tissue (perineal mass), lymph node, kidney, breast, pancreas | Chemotherapy (VBL/PRED, 6 week induction) | SD        | No          | ALK inhibition (Lorlatinib)      | PR           | Yes (Lorlatinib) | Alive with refractory disease (21 months) |
| 11 | KIF5B, ALK    | F   | 10 years | CNS, bone, lung, lymph node, cervix, thyroid, submandibular salivary gland | Chemotherapy (2 CA, 3 cycles) | CR        | No          | -                                 | -            | Yes (Crizotinib) | Alive with no disease (2 years) |
| 12 | KIF5B, ALK    | F   | 19 years | CNS/PNS, bone, lung, liver, lymph node, breast, pancreas | Chemotherapy (Crizotinib) | PR        | No          | -                                 | -            | Yes (Crizotinib) | Alive with refractory disease (2 years) |
| 13 | TFG, ALK      | M   | 21 years | Liver, skin, colonrectum | Corticosteroids (high dose) | PR        | Yes***      | -                                 | -            | No            | Died from aspiration (2 months) |
| 14 | KIF5B, ALK    | F   | 28 years | CNS/PNS, bone | ALK inhibition (Alectinib) | PR        | No          | -                                 | -            | Yes (Alectinib) | Alive with refractory disease (5 months) |
| 15 | KIF5B, ALK    | M   | 29 years | CNS, bone, lymph node, kidney, skin, soft tissue (omentum/peritoneum) | Corticosteroids, followed by peglated interferon-α (with escalation to 180μg/week, 4 months) | SD        | No          | ALK inhibition (Brigatinib)      | CR           | Yes (Brigatinib) | Alive with no disease (25 years) |
| 16* | KIF5B, ALK    | F   | 41 years | CNS, bone, lung, skin, soft tissue (omentum/peritoneum), lymph node | Interferon-α (6 months) | PD        | Yes         | ALK inhibition (Cezotinib)       | PR           | Yes (Cezotinib) | Alive with refractory disease (6 years) |
| Nr | ALK rearrangement | Analysis method(s) | Classic XG-like | Emperipolesis | ALK IHC | Staining | Staining pattern |
|----|------------------|-------------------|----------------|-------------|--------|---------|-----------------|
| 1  | Not confirmed   | ALK-FISH negative; KIF5B-ALK RT-PCR negative; no RNA-seq (insufficient material) | No | Yes | Positive | Light cytoplasmic; membranous |
| 2  | ALK-FISH+       | FISH              | No | Yes abundant | Positive | Diffuse cytoplasmic; membranous |
| 3  | KIF5B-ALK       | Archer FusionPlex targeted RNA-seq | No | No | Positive | Granular cytoplasmic with focal Golgi dot-like accentuation; no membranous |
| 4  | Not confirmed   | ALK-FISH negative; no RNA-seq (insufficient material) | Yes | Yes abundant | Positive | Scant cytoplasmic; strong membranous |
| 5  | ALK-FISH+       | FISH              | Yes | Yes | Positive | Weak cytoplasmic; strong membranous |
| 6  | CLTC-ALK        | Archer FusionPlex targeted RNA-seq | No | Yes | Positive | Diffuse dark cytoplasmic (clone 5A4) to more light granular cytoplasmic (clone ALK1); membranous (both) |

**GROUP 2: NEUROLOGIC INVOLVEMENT**

| Nr | ALK rearrangement | Analysis method(s) | Classic XG-like | Emperipolesis | ALK IHC | Staining | Staining pattern |
|----|------------------|-------------------|----------------|-------------|--------|---------|-----------------|
| 17 | KIF5B-ALK        | FoundationOne Heme targeted RNA-seq | No | No | Positive | Granular cytoplasmic with Golgi dot-like accentuation in some cells; no membranous |
| 18 | ALK-FISH+        | FISH              | No | No | Positive | Diffuse cytoplasmic with Golgi dot-like accentuation; membranous |
| 19 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | No | No | Positive | Granular cytoplasmic; no membranous |
| 20 | KIF5B-ALK        | FoundationOne Heme targeted RNA-seq | No | No | Focal, weak | Focal, weak diffuse cytoplasmic with Golgi dot-like accentuation; no membranous |
| 21 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | Yes | Yes | Positive | Diffuse cytoplasmic with Golgi dot-like accentuation; no membranous |
| 22 | KIF5B-ALK        | Whole transcriptome RNA-seq | No | No | Positive | Diffuse granular cytoplasmic; no membranous |
| 23 | KIF5B-ALK        | Whole transcriptome RNA-seq | No | No | Positive | Diffuse granular cytoplasmic; no membranous |
| 24 | ALK-FISH+        | FISH              | Yes* | No | Positive | Diffuse granular cytoplasmic; no membranous |
| 25 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | Yes | Yes | Positive | Diffuse cytoplasmic with Golgi dot-like accentuation; membranous |
| 26 | KIF5B-ALK        | Ion Ampliseq RNA Fusion Lung Cancer targeted RNA-seq; whole transcriptome RNA-seq | No | Yes abundant | Positive | Diffuse cytoplasmic with Golgi dot-like accentuation; no membranous |
| 27 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | Yes | No | Positive | Diffuse granular cytoplasmic; no membranous |
| 28 | KIF5B-ALK        | FoundationOne Heme targeted RNA-seq | No | No | Focal, weak | Variable focal; weak cytoplasmic to negative; no membranous |

**GROUP 2: NON-NEUROLOGIC INVOLVEMENT**

| Nr | ALK rearrangement | Analysis method(s) | Classic XG-like | Emperipolesis | ALK IHC | Staining | Staining pattern |
|----|------------------|-------------------|----------------|-------------|--------|---------|-----------------|
| 29 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | No | No | Positive | Diffuse cytoplasmic with Golgi dot-like accentuation; no membranous |
| 30 | KIF5B-ALK        | Oncomine Comprehensive Assay Plus targeted RNA-seq | No | No | Positive | Diffuse granular cytoplasmic with Golgi dot-like accentuation; no membranous |
| 31 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | No | No | Positive | Diffuse granular cytoplasmic; no membranous |
| 32 | KIF5B-ALK        | Whole transcriptome RNA-seq | No | No | Positive | Diffuse dark cytoplasmic; subset possibly nuclear; no membranous (clone SAA, lung staining protocol); focal/Golgi dot-like cytoplasmic; no nuclear or membranous (clone SAA, lymphoma staining protocol) |
| 33 | KIF5B-ALK        | Illumina TruSight targeted RNA-seq | No | Yes | Positive | Diffuse light granular cytoplasmic with dark Golgi dot-like accentuation; no membranous |
| 34 | KIF5B-ALK        | Illumina TruSight targeted RNA-seq | No | No | Exclusive | Focal dot-like; Golgi dot-like cytoplasmic only (focal weak); no membranous |
| 35 | KIF5B-ALK        | Archer FusionPlex targeted RNA-seq | No | No | Positive | Diffuse granular cytoplasmic with Golgi dot-like accentuation; no membranous |
| 36 | KIF5B-ALK        | FoundationOne Heme targeted RNA-seq | No | No | Exclusive | Golgi dot-like cytoplasmic only (focal weak); |
| No. | Gene      | Test Method                              | Nr | XG | IHC | FISH | RNA-seq | RT-PCR | Description                                                                 |
|-----|-----------|------------------------------------------|----|----|-----|------|---------|--------|-----------------------------------------------------------------------------|
| 37  | KIF5B-ALK | Archer FusionPlex targeted RNA-seq        | Yes| Yes|    |      |         |        | Diffuse cytoplasmic with Golgi dot-like accentuation; membranous            |
| 38  | EML4-ALK  | Archer FusionPlex targeted RNA-seq        | Yes| No |    |      |         |        | Diffuse granular cytoplasmic; membranous                                    |
| 39  | DCTN1-ALK | Archer FusionPlex targeted RNA-seq        | Yes| Yes|    |      |         |        | Diffuse cytoplasmic with Golgi dot-like accentuation; membranous            |

Abbreviations: Nr, number; XG, xanthogranuloma; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; RNA-seq, RNA sequencing; RT-PCR, reverse transcription polymerase chain reaction. *, multiple areas with xanthomatous histiocytes and Touton giant cells were observed; however, a significant part consisted of an infiltration of spindly cells.
FIGURE LEGENDS

FIGURE 1. Schematic overview of downstream ALK signaling through MAPK and PI3K/AKT/mTOR signaling pathways. ALK is a classical receptor tyrosine kinase, consisting of an extracellular ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase domain. In ALK fusions such as KIF5B-ALK, the amino-terminal fusion partner is fused to the intracellular tyrosine kinase domain of ALK, leading to constitutive activation of downstream signaling, including RAS-RAF-MEK-ERK (MAPK) and PI3K/AKT/mTOR signaling pathways. MAPK pathway activation ultimately leads to phosphorylation of downstream ERK, which can enter the nucleus and increase the transcription of various effector genes, including the gene encoding for Cyclin D1 (CCND1). Translation of CCND1 messenger RNA to the Cyclin D1 protein is mTOR-dependent. Figure adapted from Emile JF, et al. Lancet 2021, with permission from the authors.

FIGURE 2. Focal liver lesions in an infant with multisystemic disease with liver and hematopoietic involvement (Group 1A). (A) Ultrasound image showing three hypoechoic lesions in liver segment three. (B-C) Coronal T2-weighted fat-suppressed MRI images showing multiple hyperintense lesions in the liver, including a large rounded lesion in liver segment three (C). (D) Coronal T2-weighted contrast-enhanced MRI image showing late contrast accumulation in the large rounded lesion in liver segment three.

FIGURE 3. Non-neurologic disease manifestations in ALK-related histiocytosis patients from Group 1B. (A-D) Fluorodeoxyglucose PET-CT images showing bilateral hypermetabolic long bone involvement, reminiscent of ECD, with objective metabolic response in Case 11 after twelve months of Crizotinib. (E) Sagittal image of the contrast-enhanced MRI scan of the spine showing multiple hyperintense lesions in the vertebral column.
bodies. **(F-G)** Axial MRI image (F) and lateral conventional radiograph (G) showing skull lesions in two children, with an appearance reminiscent of LCH. **(H-K)** Axial CT images showing nodular pulmonary involvement in three pediatric cases. **(L)** Photograph of the right axilla of an adult with a brown maculopapular exanthema that coalesces into plaques and predominates in the axillae and flanks, reminiscent of xanthoma disseminatum. **(M)** Photograph showing one of multiple scalp skin lesions in a child, which can also be observed on the MRI of the head (Figure 4A). **(N)** Ultrasound image demonstrating round, hypoechoic lesions in both lobes of the thyroid gland. **(O-P)** Axial T2-weighted (O) and diffusion-weighted (P) pelvic MRI images showing a cervical tumor with restricted diffusion in a child that presented with menorrhagia and irregular vaginal bleeding. **(Q-R)** Axial PET-CT images showing hypermetabolic focal lesions in the liver and pancreas (Q), and in the breast (R). **(S)** Coronal CT image showing a focal lesion in the left kidney.

**FIGURE 4. Neurologic involvement in ALK-related histiocytosis patients from Group 1B or 2.** **(A-E)** Axial images of the T1-weighted contrast-enhanced MRI scans of the head of two pediatric cases with multiple solid brain tumors, before and after treatment with ALK inhibition, demonstrating robust responses in both. **(F)** Sagittal image of the T1-weighted contrast-enhanced MRI scan of the spine showing leptomeningeal contrast enhancement along the descending cauda equina nerve roots. **(G-I)** Axial images of successive fluorodeoxyglucose PET-CT scans showing partial and complete response of a neuroforaminal tumor at level L5 after two cycles of cladribine (H) and subsequent treatment with Alectinib (I), respectively. Coronal images (not shown) demonstrated that the tumor followed the course of the exiting nerves, highly reminiscent of nerve sheath tumors such as neurofibromas. **(J-N)** Axial images of successive T1-weighted contrast-enhanced MRI scans of the head of a child with a left insula tumor, before and after subtotal resection and
successful treatment with Alectinib. (O-Q) Axial images of the T1-weighted contrast-enhanced MRI scans of the head of a child with a left oculomotor nerve tumor, demonstrating slight regression but continued contrast enhancement of the tumor after treatment with vinblastine/prednisone-based chemotherapy. (R) Coronal image of the T1-weighted contrast-enhanced MRI scan of the head showing a 30 x 25 x 34 mm large tumor with contrast enhancement in the prepontine cistern that followed the course of the trigeminal nerve and caused pressure on the pons. (S) Sagittal image of the T1-weighted contrast-enhanced MRI scan of the cervical spine showing a large (18 x 24 x 45 mm) intradural extramedullary tumor at level C1-C2.

**FIGURE 5. Non-neurologic disease manifestations in ALK-related histiocytosis patients with single-system disease (Group 2).** (A-D) Successive fluorodeoxyglucose PET-CT images of an adult female with a large right clavicular tumor at diagnosis (A; t = 0) and after treatment with radiotherapy (B; t = 5.5 months), 6 weeks of vinblastine/prednisone-based chemotherapy (C; t = 9.5 months), and 2 months of Alectinib (D; t = 14 months). (E-I) T1-weighted contrast-enhanced (E), T2-weighted (F), diffusion-weighted (G), apparent diffusion coefficient (H), and plain T1-weighted (I) MRI images of the left lower leg of a child at diagnosis showing a single soft tissue tumor that infiltrates the musculature and shows contrast enhancement and restricted diffusion. (J-K) Photographs of the retroauricular scalp lesion of an infant, with a clear change in clinical appearance after 3 months (K).

**FIGURE 6. Body diagram depicting recurrent anatomic sites of involvement of ALK-related histiocytosis.**
FIGURE 7. Swimmer plot of outcomes in patients with ALK-related histiocytosis (N = 11) or atypical ALK-rearranged histocyte-rich tumors (N = 2) treated with ALK inhibition. Abbreviations: VBL/PRED, vinblastine and prednisone-based chemotherapy. ALK inhibition was initiated at timepoint zero. Median time on ALK inhibition was 16 months in ALK-related histiocytosis patients (range 3-43 months). Responses were measured by CT, MRI and/or PET-CT in all patients. Dose reductions were 67% (90mg Brigatinib/day \(\rightarrow\) 30mg/day) and 50% (1200mg Alectinib/day \(\rightarrow\) 900mg/day \(\rightarrow\) 600mg/day) in Case 15 and Case 26, respectively. Case 39 developed a severe (grade 3) anaphylactic shock on the first day of Crizotinib administration, requiring the patient to be resuscitated. The patient subsequently received vinblastine/prednisone-based chemotherapy with progressive disease, and then switched to Alectinib with objective response after two months. Case A1 developed a subcutaneous gluteal metastasis during treatment with Alectinib (Supplementary Figure 3C), which was found to harbor an ALK p.I1171N mutation – a mutation known to confer secondary resistance to Alectinib\(^{78,79}\). Therefore, the patient switched to Lorlatinib, and later to Ceritinib after repeated progressive disease. Due to continuing progressive disease during treatment with Ceritinib, the patient recently stopped Ceritinib, received three weeks of bridging therapy with Lorlatinib during antalgic radiotherapy of two metastases, and subsequently started vinblastine/prednisone-based chemotherapy.

FIGURE 8. Histopathologic features of ALK-related histiocytosis. (A) Photomicrograph of the hematoxylin and eosin (HE) stained slide of a frontal bone tumor (Case 7; original magnification 200x) with classic xanthogranuloma morphology including many Touton giant cells. (B) HE image of a spinal nerve root tumor (Case 15; 200x) showing abundant lipidized (“foamy”) histiocytes. (C) HE image (Case 31; 400x) showing a more monomorphic histiocytic infiltrate in the skin dissecting through the dermal collagen bundles. (D) HE image
of a liver biopsy (Case 4; 400x) showing sinusoidal infiltration by large histiocytes (indicated by black arrows) with ALK immunoreactivity (inlet). (E) HE image of a CNS tumor (Case 18; 400x) with a monomorphic, dense infiltrate of histiocytes that demonstrate separated red and green signals on ALK break-apart FISH analysis (inlet). (F) HE image of a CNS lesion (Case 20; 100x) showing marked infiltration of the perivascular (“Virchow-Robin”) spaces by histiocytes with clear CD163 immunoreactivity (inlet). (G) CD163 immunostain of a CNS tumor (Case 18; 200x) showing diffuse strong expression by the monomorphic histiocytic infiltrate. (H) ALK immunostain (Case 7; 200x) showing strong cytoplasmic and membranous staining of lesional histiocytes and Touton giant cells. (I) ALK immunostain of a breast tumor (Case 12; 400x) showing focal, exclusive dot-like immunoreactivity that could be misinterpreted as negative. (J) S100 immunostain of a liver biopsy (Case 4; 200x) showing immunoreactivity by the large sinusoidal histiocytes. (K) P-ERK immunostain (Case 15; 400x) showing diffuse positive staining by lesional cells, as well as clear emperiploesis (intact intracytoplasmic leukocytes). (L) Cyclin D1 immunostain of an oculomotor nerve tumor (Case 21; 200x) showing cytoplasmic and strong nuclear staining in histiocytes with frequent nuclear indentations.
Figure 1

ALK inhibitors
- Crizotinib
- Alectinib
- Brigatinib
- Ceritinib
- Lorlatinib

ALK fusions
Ligand-independent constitutive activation of the intracellular ALK tyrosine kinase domain

ALK
- receptor tyrosine kinase

CBL

PTPN11

PI3K

AKT

mTOR

mTOR-dependent translation

Cyclin D1

transcription

CCND1

RAS

RAF

MEK

ERK

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Figure 4

**Group 1B**

| At diagnosis | After 3.5 months of Lorlatinib | At diagnosis | After 3 weeks of Crizotinib | After 20 months of Crizotinib |
|--------------|---------------------------------|--------------|----------------------------|-------------------------------|
| A            |                                 | B            |                            |                               |
| G            |                                 | H            |                            |                               |

**Group 2**

| At diagnosis | Post-operative | At relapse, 3 months after surgery | After 2 months of Alectinib | After 19 months of Alectinib |
|--------------|----------------|-----------------------------------|-----------------------------|------------------------------|
| J            |                | K                                 | M                           | N                            |

| At diagnosis | 2 months after surgery, before chemotherapy | 20 months after chemotherapy completion |
|--------------|---------------------------------------------|----------------------------------------|
| O            |                                             | P                                       |
| Q            |                                             | R                                       |
| S            |                                             |                                         |
