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## Histiocytic disorders

**Kenneth L. McClain<sup>1,†</sup>, Camille Bigenwald<sup>2</sup>, Matthew Collin<sup>3</sup>, Julien Haroche<sup>4</sup>, Rebecca A. Marsh<sup>5</sup>, Miriam Merad<sup>2</sup>, Jennifer Picarsic<sup>6</sup>, Karina B. Ribeiro<sup>7</sup>, Carl E. Allen<sup>1</sup>**

<sup>1</sup>Texas Children's Cancer Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

<sup>2</sup>Department of Oncological Sciences and Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

<sup>3</sup>Human Dendritic Cell Lab, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, Great Britain

<sup>4</sup>Department of Internal Medicine, Institut E3M French Reference Centre for Histiocytosis, Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, Sorbonne Université, Paris, France

<sup>5</sup>Division of Bone Marrow Transplantation and Immune Deficiency Cincinnati Children's Hospital Medical Center, and University of Cincinnati, Cincinnati, OH, USA

<sup>6</sup>Division of Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

<sup>7</sup>Faculdade de Ciências Médicas da Santa Casa de São Paulo, Department of Collective Health, São Paulo, Brazil.

### Abstract

The historic term 'histiocytosis' meaning 'tissue cell' is used as a unifying concept for diseases characterized by pathogenetic myeloid cells that share histologic features with macrophages or dendritic cells. These cells may arise from the embryonic yolk sac, fetal liver, or post-natal bone marrow. Prior classification schemes align disease designation with terminal phenotype: for example, Langerhans cell histiocytosis (LCH) shares CD207<sup>+</sup> antigen with physiologic epidermal Langerhans cells. LCH, Erdheim-Chester disease (ECD), juvenile xanthogranuloma (JXG), and Rosai-Dorfman disease (RDD) are all characterized by pathologic ERK activation driven by activating somatic mutations in MAPK pathway genes. The title of this Primer ('Histiocytic

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<sup>†</sup>Corresponding Author: Kenneth McClain MD, PhD: klmclai@txch.org.

#### Author contributions

All authors reviewed the review concept and finalized the manuscript. Specific areas of focus: Introduction (C.E.A. and K.L.M.); Epidemiology (K.B.R. and K.L.M.); Mechanisms/pathophysiology (C.B., J.H., R.A.M., M.M., J.L.P., C.E.A. and K.L.M.); Diagnosis, screening and prevention (J.H., R.A.M., J.L.P., C.E.A. and K.L.M.); Management (J.H., R.A.M., C.E.A. and K.L.M.); Quality of life (J.H., R.M., C.E.A. and K.L.M.); Outlook (M.C. and C.E.A.); Overview of primer (K.L.M.).

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Disorders’) was chosen to differentiate the above diseases from Langerhans cell sarcoma and malignant histiocytosis, which are hyper-proliferative lesions typical of cancer. By comparison LCH, ECD, RDD, and JXG share some features of malignant cells including activating MAPK pathway mutations, but are not hyper-proliferative. ‘Inflammatory myelo-proliferative neoplasm’ may be a more precise nomenclature. By contrast, hemohagocytic lymphohistiocytosis (HLH) is associated macrophage activation and extreme inflammation, representing a syndrome if immune dysregulation. These diseases affect children and adults in varying proportions depending on which of the entities is involved.

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## Introduction

Histiocytic disorders were originally classified by histological features of pathological cells compared with presumed physiological counterparts.<sup>1, 2</sup> The term ‘histiocyte’ (Greek for ‘tissue cell’) was applied by Aschoff and Kiyono in 1913<sup>3</sup> to designate mononuclear phagocytes, cells of myeloid origin that now include blood and tissue dendritic cells (DC) and macrophages.<sup>1, 4, 5</sup> An updated contemporary classification proposes to classify histiocytoses not only by terminal phenotype, but also taking into account molecular lesions and clinical features.<sup>2</sup> (Box 1)

This Primer discusses histiocytic diseases including Langerhans cell histiocytosis (LCH), Erdheim Chester Disease (ECD), juvenile xanthogranuloma (JXG), Rosai-Dorfman disease (RDD, sometimes known as Rosai–Dorfman–Destombes disease), and hemophagocytic lymphohistiocytosis (HLH). The first four diseases have features of transformed cells, though implications of oncogenic drivers on classification as ‘cancer’ remains an area of debate. ‘Hallmark’ cancer features include oncogenic driver mutations, apoptosis resistance, self-sufficiency in growth signals and tissue invasion and metastasis.<sup>6</sup> However, these histiocytic cells have very low proliferative index and LCH has recently been characterize with senescence-associated secretory phenotype.<sup>7</sup> The histiocytosis discussed here rarely transform to lymphoma-like malignant diseases such as histiocytic sarcoma or malignant histiocytosis. LCH, ECD, JXG and RDD could reasonably be described as inflammatory myeloid neoplastic disorders.<sup>8, 9</sup> By contrast, HLH is clearly a syndrome of immune dysregulation.<sup>10</sup>

LCH primarily affects children of <10 years of age, with potential to develop lesions in virtually any organ system, most frequently bone and skin. Adults are also affected, typically in the third to fifth decades of life. Clinical presentations in children and adults are highly variable, with symptoms based on sites of disease. Lesions in spleen, liver and bone marrow are considered ‘high risk’ due to the association of increased potential for death<sup>11</sup> compared with lesions in other ‘low risk’ sites. Extent of disease is typically established by the number of lesions and organ systems involved (for example, single lesion, single system; multifocal lesions, single system; or multifocal lesions, multisystem). Patients with pituitary lesions may present with diabetes insipidus and other endocrinopathies. LCH-associated neurodegeneration (LCH-ND) is a severe complication that can arise acutely with systemic symptoms or years after the initial LCH diagnosis, with clinical signs of cerebellar dysfunction and associated abnormal MRI findings in the in cerebellum, pons

and brainstem.<sup>12</sup> Clinical responses in LCH-ND have been reported with LCH-directed chemotherapy as well as MAPK inhibitors.<sup>9, 13</sup>

ECD affects primarily adults with clinical signs and symptoms similar to LCH. Xanthelasma (yellowish deposit of cholesterol under the skin) below the eyes is a unique clinical finding. Initial evaluations and staging are similar to LCH, with attention paid to characteristic potential distribution of lesions including heart, major blood vessels, kidneys and peritoneum. ECD had a more dire prognosis than LCH before the use of IFN $\alpha$  and, more recently, MAPK pathway inhibitors.<sup>14, 15</sup> Despite substantial advances in life expectancy among patients with ECD, few are cured with current therapies.

JXG primarily occurs in children of <4 years of age, with single or multiple orange-red papules on the skin (xanthogranulomas, characterized by the presence of foamy macrophages (xanthoma cells)) that usually resolve spontaneously. Systemic disease can arise in the liver, spleen, kidney, brain, bone or other sites, is typically progressive without therapy, and is potentially fatal.

The classic presentation of RDD is in children of <10 years of age, with painless, massive enlargement of cervical lymph nodes that may spontaneously resolve. Rarely, RDD may cause bone lesions, subcutaneous nodules, proptosis (protruding eyeballs), or intra-cerebral masses. RDD probably represents a variety of conditions that share a common histological phenotype, accounting for greater variability in presentation and outcomes than other histiocytoses. Patients with progressive disease or with lesions in critical locations may require systemic chemotherapy.

HLH is a syndrome of extreme immune activation. Historically, HLH has been divided into primary (or genetic or familial) and secondary forms. Primary HLH pertains to patients with predisposing inherited defects caused by pathogenetic variants in genes that compromise cellular cytotoxicity, inflammasome function, or other cellular functions. 'Secondary' HLH refers to patients who develop a syndrome of HLH without an underlying defect in cytotoxicity, and often occurs in association with a strong immunologic stimulus such as infection, or malignancy or autoimmune/autoinflammatory condition. Patients, typically young children but occasionally adults, present with clinical and laboratory findings reflecting pathological inflammation and are at very high risk of death without prompt initiation of immune suppression. For patients with fixed immune defects associated with HLH, allogeneic hematopoietic stem cell transplant is required for long-term survival.

This Primer discusses current practice and evolving understanding of the epidemiology, mechanisms/pathophysiology, diagnosis, screening, prevention, management, quality of life and future outlook for patients with histiocytic disorders.

## Epidemiology

The Histiocytic disorders discussed in this section are rare and inconsistently documented in disease registries. High-risk LCH was tracked in the Surveillance, Epidemiology, and End Results (SEER) database, but reporting was probably incomplete. Additional resources include the International Rare Histiocytic Disorders Registry (IRHDR) that opened in 2014,

the Belgian Langerhans Cell Histiocytosis Registry, and the UK Histiocytosis Registry. These registries are important efforts to capture disease experience but have variable participation, and no data are yet publicly available.

**Langerhans cell histiocytosis (LCH)**—The current version of the International Classification of Diseases for Oncology (ICD-O-3) only includes high-risk LCH. Low-risk LCH (which includes LCH not otherwise specified (NOS), LCH unifocal and LCH multifocal) is not included.<sup>16</sup> In the USA and Canada, cancer registries collect information about all forms of LCH. Most LCH cases occur during childhood, with age-adjusted incidence rates (in children of 0–14 years of age) varying between 2.6 and 8.9 new cases per million individuals per year, globally.<sup>17–19</sup> The French National Registry of Childhood Hematopoietic Malignancies reported a standardized incidence rate (in children of 0–14 years of age) of 5 cases per million individuals between 2000–2004, with a slight predominance among male children.<sup>17</sup> In Sweden, a population-based study described an annual incidence rate of 8.9 per million children of 0–14 years of age.<sup>19</sup> Review of SEER programme databases in the USA found that African-American children had a lower relative risk compared to risk of white children developing LCH (0.41), whereas Hispanic children had a 1.63-fold higher risk than non-Hispanic children.<sup>16</sup> A WHO publication on histiocytosis stated the incidence may be increased in northern European regions compared with Asian and African regions.<sup>20</sup> Household crowding and reduced educational level (according to area-based measures) were also associated with increased risk of LCH among children and adolescents in the USA.<sup>16</sup> This finding may be linked to an increased risk of infections that can trigger immune dysregulation as a theoretical, but unproven, cause of LCH. A large case-control study conducted in the USA and Canada identified that neonatal infections were associated with an increased risk (OR=3.8) of multisystem LCH (but no specific pathogen was identified), whereas childhood vaccinations were associated with decreased risk of localized and systemic LCH. (OR=0.4).<sup>21</sup> Another case-control study of children living in Los Angeles, USA area reported an association with infections in the first year of life (OR=2.8); additionally, LCH was associated with a family history of cancer (OR=2.5) and parental exposures to heavy metals granites or wood dust in the work place (OR=2.5).<sup>22</sup> A study from the Texas Cancer Registry matched individuals in the registry with healthy individuals born on the same year and found that children born of Hispanic mothers were 51% more likely to develop LCH than children with white non-Hispanic mothers; the risk was even more pronounced when both parents were Hispanic (adjusted OR=1.80).<sup>23</sup> A GWAS study of patients with LCH and their parents found that a variant of *SMAD6* that is present with increased frequency in Hispanic populations was associated with increased risk of LCH.<sup>24</sup> A functional link between *SMAD6* and LCH is unclear, although it is intriguing that the gene is in very close proximity to *MAP2K1* (encoding ERK activator kinase 1 (MEK1)), a central component of the MAPK signaling pathway. Smoking is clearly linked to pulmonary LCH in adults, but no other co-morbidities have been identified.<sup>25</sup> Interrogation of the SEER database from 2000–2009 identified 59 adult patients with disseminated LCH, of which 51 were white, 3 were black and 4 were of other ethnicities.<sup>26</sup> It is likely that since SEER data were limited to adult disseminated LCH, the overall incidence for adult LCH is higher than the 0.07 per million per year estimated

from this study. It seems probable that genetic and/or ethnic background has a role in susceptibility to LCH through mechanisms that are currently unknown.

**Erdheim Chester disease (ECD)**—The global incidence or prevalence of ECD is not known; however, the number of ECD cases world-wide since 1930 is estimated to be ~1,500, by taking into account the published case reports and series, the various cohorts of patients followed both in Europe and the USA, and the numbers from the ECD Global Alliance patient’s association (assuming unique cases among registries). ECD primarily affects adults (mean age at diagnosis of 55 years), with a male preponderance (3:1) Childhood ECD is rare (<20 reported cases world-wide)<sup>27</sup>. Pediatric ECD could be considered disseminated JXG in most cases.<sup>28</sup> ECD, like JXG, RDD and HLH, is not reportable by cancer registries. The Histiocyte Society Rare Disease Registry is attempting to characterize submitted cases.

**Juvenile Xanthogranuloma (JXG)**—Data from the Kiel Tumor Registry suggest that JXG occurs at a frequency of ~1 per million children.<sup>29</sup> The male:female ratio is 1.4:1, with 35% of patients having lesions at birth and 71% developing lesions within the first year of life<sup>29</sup>. There is a 3:1 predominance of females among patients with JXG with disseminated lesions.<sup>30</sup> There are several case reports of children with neurofibromatosis type 1 and JXG, consistent with the known role of MAPK pathway activation in pathogenesis of both diseases.<sup>31</sup>

**Rosai–Dorfman Disease (RDD)**—Rosai–Dorfman Destombes Disease (RDD) was first described in 1965 by Destombes,<sup>32</sup> who reported four cases of children or young adults with massive lymphadenopathy. Four years later, Rosai and Dorfman analyzed 34 cases of the same entity, which they named ‘sinus histiocytosis with massive lymphadenopathy’.<sup>33</sup> These patients had massive cervical lymphadenopathy, with a benign, but prolonged clinical course.

Since the original publication of the RDD registry,<sup>34</sup> which included 423 cases in 1990, there have been >1,200 additional cases reported.<sup>34</sup> Although RDD is most frequent in children and young adults, it can occur at any age (mean age at diagnosis 20.6 years). There are no studies to define incidence of RDD in children compared with adults.<sup>33</sup> RDD is more common in boys, and the classic presentation of chronic benign cervical lymphadenopathy was initially described in sub-Saharan African boys and may be more common in African-Americans than in other ethnic groups in the USA.<sup>35</sup> However, the cutaneous form is more frequent in females.<sup>36</sup>

**Hemophagocytic Lymphohistiocytosis (HLH)**—Data on the epidemiology of HLH is scarce, which makes it difficult to summarize differences in the incidence or prevalence in different ethnic groups. The first report from Sweden estimated the primary form of HLH to occur in 1 in 50,000 live born children<sup>37</sup> and a more recent Swedish study reported an annual incidence of 0.12 per 100,000 children <15 years of age.<sup>38</sup> A single center study in the USA estimated a frequency of 1 case of HLH for every 3,000 general hospital admissions to a large pediatric referral hospital and a cross-sectional prevalence of 1.07 cases per 100,000 persons less than 18 years old across Texas.<sup>39, 40</sup> A nationwide survey in Japan found an annual incidence of 1 per 800,000 individuals of all ages, with a male/

female ratio of 0.94 and <5% being familial cases.<sup>41</sup> More than 56% of the patients were under 15 years of age, and a majority of all patients had Epstein–Barr virus (EBV) or other infections. For unknown reasons, EBV-associated HLH is more common in Asia than in US and Europe.<sup>42, 43</sup> Additionally, EBV-associated HLH in people of Asian descent and from Central or South America frequently includes atypical T cell infection that is unusual in other populations.<sup>44</sup> Inherited defects in genes regulating effector immune function are more common in infants than older children.<sup>45</sup> The incidence of HLH in adults is not known, but is increasingly recognized over the past decade, with cancer-associated HLH being more common in adults than children, most frequently lymphomas or hematologic malignancies.<sup>46,47</sup>

### Mechanisms/pathophysiology

LCH, ECD, JXG, RDD and HLH are characterized by the accumulation of cells with macrophage and/or dendritic cell (DC) phenotype in various tissues. All of these histiocytic disorders also present with an intense inflammatory infiltrate that has a key role in generating the organ-specific lesions and clinical symptoms. However, the pathological cell may have a different origin and/or phenotype in each condition. Whereas LCH, ECD, JXG and RDD have overlapping pathophysiology with accumulation of clonal mononuclear phagocytes, the origin of HLH disease is very different, as it primarily involves activated CD8<sup>+</sup> T cells that in turn drive macrophage activation and extreme systemic inflammation.

**Langerhans cell histiocytosis**—LCH lesions contain varying proportions of the clonal pathological CD1a<sup>+</sup>, CD207<sup>+</sup> dendritic cells (Langerhans cells) within an intense inflammatory infiltrate composed of macrophages, lymphocytes, eosinophils, multinucleated giant cells and, less commonly, neutrophils and plasma cells. Given the intense inflammatory infiltrate, the nature of LCH as a reactive inflammatory disorder versus neoplastic disorder was debated.<sup>48</sup>

In 1994, two different groups identified clonality of LCH cells using X-linked polymorphic DNA probes.<sup>49, 50</sup> However, for many years, a pathogenetic genetic lesion remained elusive.<sup>51</sup> In 2010, a recurrent V600E mutation in the serine/threonine-protein kinase B-raf (encoded by *BRAF*; noted throughout this Primer as *BRAFV600E*) was identified in 57% of LCH lesions.<sup>52</sup> *BRAF* is a central kinase of the RAS-RAF-MEK-ERK mitogen-activated protein kinase (MAPK) signal-transduction pathway involved in numerous cell functions. The *BRAFV600E* mutation renders the MAPK pathway constitutively active. This pathway is an evolutionarily conserved signaling cascade that transmits signals from cell surface receptors that normally execute programs related to cell cycle progression, differentiation, protein translation and resistance to cell death.<sup>53</sup> Additional, mutually exclusive, clonal somatic activating mutations in genes of the MAPK pathway, including *MAP2K1*, *ARAF*, *NRAS* and *KRAS*, as well as alternative *BRAF* mutations have been subsequently described in LCH lesions.<sup>54–57</sup> (Figure 1A) In patients with multiple lesions, the same somatic mutations are identified in every lesion, and in patients who relapse, the original mutation is consistently re-identified, supporting clonal nature of LCH. MAPK pathway activation is described as a universal feature of LCH, as confirmed by consistent ERK phosphorylation, even in cases without identified MAPK activating mutations.<sup>52, 58</sup>

Earlier models considered LCH cells to originate from cutaneous Langerhans cells (LCs), mostly owing to phenotypic similarities and the fact that the intracytoplasmic Birbeck granules (which can be found in LCH cells) were originally thought to be exclusive to epidermal LC.<sup>59</sup> Ultrastructurally, the Birbeck granule is an intra-cytoplasmic rod-shaped structure of variable length with a central, periodically striated lamella.<sup>60</sup> In the 2000s, the Birbeck granules were associated with expression of *CD207*, which encodes C-type lectin domain family 4 member K, an endocytic receptor (also known as CD207 antigen or Langerin) and was also expressed in both LC and LCH cells. Birbeck granules are now thought to be subdomains of the endosomal recycling compartment that forms where Langerin accumulates, and thus provides access to the non-classical antigen-processing pathway. Where physiological Langerin expression was initially thought to be restricted to epidermal LC, subsequent studies identified multiple lineages with potential to express Langerin, including dermal dendritic cells.<sup>61, 62</sup> (Figure 2A) Additionally, transcriptional profiling of CD207<sup>+</sup> cells from LCH lesions identified a signature more consistent with immature myeloid precursors than with differentiated epidermal LCs.<sup>8</sup> Together, multiple origins of CD207<sup>+</sup> cells and the fact that the gene expression signature of LCH cells is similar to that of relatively immature myeloid cells suggested that LCH may have potential to arise from multiple hematopoietic precursors other than differentiated epidermal LC.

**Role of the cell of origin and mutations.:** The discovery of the *BRAFV600E* mutation provided the opportunity to track LCH cells in the hematopoietic system. *BRAFV600E* mutation was identified not only at the tissue site but also localized to CD34<sup>+</sup> hematopoietic progenitor cells and myeloid precursors in some patients with LCH. Ability to detect *BRAFV600E* in bone marrow and peripheral blood (in patients with *BRAFV600E*<sup>+</sup> lesions) was associated with disseminated high-risk LCH.<sup>63, 64</sup> The percentage of haematopoietic progenitor cells or peripheral blood mononuclear cells harboring the mutation was extremely low, <0.1% in most cases.<sup>63, 65, 66</sup>

Based on these observations, we proposed a hypothesis that clinical presentation of LCH is defined at least in part by the cell of origin and specific mutations.<sup>9</sup> Hence, if the *BRAFV600E* mutation affects a very immature hematopoietic progenitor, the disease will be disseminated, whereas if the *BRAFV600E* mutations occurs in a mature myeloid cell, the disease may have more restricted tissue distribution. This hypothesis was validated by generating a mouse model in which the *BRAFV600E* mutation was constitutively expressed in CD11c<sup>+</sup> cells (CD11c is a marker of myeloid DC progenitors and monocyte). The mice developed an aggressive LCH-like phenotype.<sup>63</sup> Strikingly, the phenotype of the disease was less severe when the *BRAFV600E* mutation was expressed in CD207<sup>+</sup> cells (CD207 is expressed on mature epidermal Langerhans cells and some dermal DC). Notably, single cell study of LCH lesions identified transcription patterns consistent with heterogeneous differentiation within LCH lesions, likely reflecting impact of local factors on pathogenic cells.<sup>67</sup>

Further, the specific mutation probably has a role in clinical presentation and outcomes. For example, two retrospective series noted increased risk of treatment failure or relapse in patients with *BRAF V600E*.<sup>68, 69</sup> Similarly, *BRAF V600E* was highly associated with potential to develop LCH-ND.<sup>12, 64</sup> The overall mutation burden in LCH is quite low

(median of 1 mutation in an exon in a single gene per patient).<sup>58</sup> However, adults tend to have more somatic mutations in additional genes compared to children, and in some cases LCH can arise as part of myelodysplasia resulting from clonal hematopoiesis.<sup>70</sup> Rarely, LCH can also arise as a clonal phenotype along with other hematopoietic malignancies.<sup>71, 72</sup>

**Altered signaling.:** Some mechanisms downstream of MAPK activation that drive LCH pathogenesis have been described. Interestingly, *BRAFV600E* is not associated with increased proliferation; rather, pathogenetic DCs accumulate and persist within LCH lesions.<sup>73</sup> *BRAFV600E* expression in myeloid DCs inhibits expression of C-C chemokine receptor type 7 (CCR7), which is required for antigen-presenting cells to migrate to draining lymph nodes. Further, DC in LCH lesions upregulated the expression of Bcl-2-like protein 1 (also known as BCL-xL), conferring resistance to cell death.<sup>7, 73</sup> (Figure 2B) Additionally, by mechanisms that have not yet been defined, DCs in LCH lesions recruit activated T cells that become exhausted, contributing to pathological local and systemic inflammation.<sup>74, 75</sup>

*BRAFV600E* oncogene-induced senescence has been proposed as key mechanism for LCH pathophysiology.<sup>7</sup> Indeed, in a mouse model in which the *BRAFV600E* mutation is expressed under the *Scf* promoter (*Scf* is expressed on short-term and long-term hematopoietic stem cells), the mutated hematopoietic stem cells have a proliferative disadvantage compared to non-mutated hematopoietic stem cells, explaining the low fraction of mutant allele observed in patients with LCH. Moreover, the mutated hematopoietic stem cells predominately differentiate toward the mononuclear phagocyte compartment in a cell intrinsic and extrinsic manner. These mutated hematopoietic progenitors present all the canonical features of senescence (low proliferative capacity, high Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) expression, high activity of senescence-associated  $\beta$ -galactosidase, and secretion of multiples pro-inflammatory cytokines and matrix metallo-proteinases). This senescence program is conserved in tissues and may be responsible for key features of LCH, such as the accumulation of poorly proliferative mononuclear phagocytes in tissues and the large infiltration of immune cells and subsequent fibrotic injuries.

**LCH-associated neurodegenerative disease.:** A progressive LCH-associated neurodegenerative disease (LCH-ND) arises in up to 10% of patients, sometimes years after initial disease is presumed to be cured.<sup>76</sup> Aetiology of this phenomenon has not been well understood. Earlier studies described brain biopsies with enriched CD8<sup>+</sup> T cells at sites of neurodegeneration, leading to speculation that LCH-ND could be an autoimmune or paraneoplastic phenomenon.<sup>77</sup> More recently, *BRAFV600E*<sup>+</sup> peripheral blood mononuclear cells have been identified in patients with LCH-ND without other systemic lesions.<sup>12</sup>

Further, *BRAFV600E*<sup>+</sup> microglia-like cells are identified near blood vessels in areas of neurodegeneration (in patients with history of *BRAF-V600E*<sup>+</sup> lesions).<sup>12</sup> Moreover, these cells express markers of senescence.<sup>7</sup> Notably, two studies found *BRAFV600E* mutation to be highly correlated with risk of LCH-ND<sup>12, 69</sup>. Further, in patients without active systemic LCH, persistent *BRAFV600E*<sup>+</sup> peripheral blood mononuclear cells were highly specific for patients with LCH-ND.<sup>12, 7</sup> A mouse model in which *BRAF-V600E* is expressed on yolk-sac cells that seed embryonic brain with microglia resulted in progressive neurodegeneration.<sup>78</sup> However, LCH-ND cells arise only after (or concurrent with) systemic lesions, and



the microglia-like cells do not express some features of tissue-resident microglia (for example, P2Y purinoceptor 12 (P2RY12)). Thus, we hypothesize that LCH-ND arises from hematopoietic cells, clonal to systemic LCH lesions that have potential to migrate to the brain.

**Erdheim Chester Disease**—ECD is characterized by macrophage-like CD68<sup>+</sup>, CD14<sup>+</sup>, CD163<sup>+</sup>, and CD1a<sup>-</sup>, CD207<sup>-</sup> cells. This immunohistochemical pattern in combination with specific clinical findings (for example, “hairy” kidney, sheath around the aorta, and tibial hyperintensity on FDG-PET, see Diagnosis, screening and prevention, below) supports the diagnosis of ECD.<sup>79</sup> Like LCH, clonality has also been confirmed in ECD. Some patients have mixed clinical and histological features of LCH and ECD, supporting a potential shared cell of origin.<sup>79</sup> (Figure 2A) ECD histiocytes harbor *BRAFV600E* or alternative mutations activating the MAPK pathway.<sup>80</sup> Moreover, *BRAFV600E* mutation has also been identified in CD34<sup>+</sup> hematopoietic progenitors from patients with ECD<sup>65, 66</sup>. (Figure 1A) Hence, LCH and ECD share many pathological features, such as infiltration of myeloid cells that carry a mutation in genes involved in the MAPK pathway, and presence of the same mutation at the hematopoietic progenitor level, but are very distinct epidemiologically and clinically. In addition to *BRAFV600E* in approximately 50% of ECD, recurrent MAPK gene mutations have been reported in *MAP2K1*, *ARAF*, *NRAS*, and *KRAS*, and translocations of *BRAF*, *ALK*, and *NTRK1*.<sup>81</sup> (Figure 1) Interestingly, PI3K-AKT-activating mutations in *PI3KCA* (not mutually exclusive with MAPK pathway mutations) have also been reported in ECD.<sup>82</sup> Expression of the *BRAFV600E* mutation in mouse hematopoietic stem cells and human umbilical cord stem cells transplanted into immune deficient mice gives rise to a LCH-like phenotype but not an ECD-phenotype.<sup>7, 83</sup> Either the cell of origin of ECD is different from that of LCH or there is an additional event required to cause LCH rather than ECD. Mechanisms underlying varying histological manifestations and clinical presentations of LCH and ECD for individual patients are still not known. The CCR7 down-regulation found in dendritic cells from LCH lesions has not been studied in ECD, as CCR7 is not a key chemokine for macrophages.<sup>73</sup> The overexpression of anti-apoptotic proteins observed in LCH as part of the senescence programme, has not been studied in ECD lesions.<sup>7, 73</sup> The number of ECD cells within histological lesions is classically limited, but in some cases the lesions are more densely infiltrated with pathologic histiocytes. As opposed to the pediatric population with LCH (in which individuals with *BRAFV600E* mutation have a worse outcome than individuals with a different mutation) the prognosis of patients with ECD carrying a *BRAF* mutation, to date, is the same as for patients with alternative mutations.<sup>64, 84</sup> However, *BRAF-V600E* is associated with increased risk of cardiovascular involvement, including aortic, cardiac and pericardial infiltration in ECD.<sup>28, 84</sup>

**Juvenile Xanthogranuloma**—Very little is known regarding pathogenetic mechanisms of JXG. The origin of JXG cells is unknown, but JXG cells share some common features with dermal macrophages including expression of fascin and coagulation factor XIII. There are cases of mixed LCH and JXG phenotype, supporting potential common cell of origin. Whole exome or targeted DNA and/or RNA sequencing of pediatric patients with JXG found mutations activating MAPK pathway, including recurrent *BRAFV600E*, *NTRK1* fusions, and mutations in *MAP2K1* and *CSF1R*.<sup>58, 81, 85</sup> (Figure 1A). Additionally, JXG

can arise in the setting of neurofibromatosis with germline *NF1* mutations,<sup>31</sup> with some case reports of JXG and juvenile myelomonocytic leukemia (JMML) in patients with *NF1*.<sup>86, 87</sup> A unique group of infants with an aggressive form of JXG with spleen, liver, and bone marrow showed infiltration with histiocytes with activating *ALK* fusions.<sup>88</sup>

**Rosai Dorfman Disease**—The causes of RDD are not known. Among the histiocytoses, RDD has relatively indistinct diagnostic criteria, based on absence of *ECD*, *JXG* or *LCH*-defining cell markers and presence of emperipolesis, a relatively non-specific phenomenon of trafficking by viable lymphocytes through histiocytes, a characteristic but non-specific finding. RDD probably represents heterogeneous conditions with shared terminal phenotype. However, at least some cases are associated with *MAPK* pathway activating mutations, including in *ARAF*, *NRAS*, *KRAS*, *MAP2K1*, and *CSF1R*, though overall mutation frequency is much lower than in *LCH*, *ECD* and *JXG*.<sup>80, 89, 90 81</sup> (Figure 1A) Hereditary syndromes such as H-syndrome and Faisalabad histiocytosis have been reported to exhibit pathological findings that are compatible with RDD and are associated with germline mutations in *SLC29A3*.<sup>91–93</sup> In addition to characteristic histiocytes, there is an abundance of plasma cells in RDD lesions, and RDD can arise along with enriched *IgG4+* cells in some cases.<sup>94</sup> It is not known if *IgG4+* RDD represents a distinct sub-class of RDD or if the presence of *IgG4+* cells reflects non-specific immune activation.

**Hemophagocytic Lymphohistiocytosis**—HLH differs substantially from the rest of the histiocytoses reviewed in this Primer as a syndrome of immune dysregulation that is classically driven by pathologic activation of cytotoxic T cells that in turn drives extreme systemic immune activation. ‘Hemophagocytic’ refers to the characteristic histologic feature of bone marrow and tissue macrophages that engulf red blood cells. HLH manifests with fevers, cytopenias and splenomegaly, along elevations of characteristic inflammatory biomarkers such as ferritin and soluble *IL-2* receptor alpha (*sIL-2R $\alpha$* ). HLH can occur for a variety of reasons. ‘Primary’ HLH refers to patients with predisposing inherited inborn errors of immunity that intrinsically cause a predisposition to the development of HLH. Some patients will develop severe HLH at a very young age, such as infants with pathogenetic mutations in *PRF1* or *UNC13D* that lead to complete absence of perforin or *Munc13–4* function, whereas other patients may develop milder HLH or develop HLH at older ages, such as patients with pathogenetic mutations in *STX11* or *XIAP*. (Figure 1 B) Age of presentation and clinical severity of HLH has been associated with functional severity of cytotoxic gene defects.<sup>95, 96</sup> However, there is tremendous variability in the phenotype associated with each genetic disorder, even within families with the same specific genetic variants. ‘Secondary’ HLH is driven by persistent immune activation, such as with malignancy, infection, or autoimmune disease, rather than by an intrinsic defect in immune function.

Several inherited inborn errors of immunity associated with HLH are characterized by defective T cell and NK cell granule-mediated cytotoxicity and are often classified as Familial HLH disorders. (Box 2). Autosomal recessive mutations in *PRF1* result in defective perforin, a protein carried by cytotoxic granules of NK cells and T cells that is required for forming pores in the membrane of target cells.<sup>97</sup> Autosomal recessive mutations

in *UNC13D* (encoding Protein unc-13 homolog D, also known as Munc13–4), *STX11* (encoding Syntaxin 11), and *STXBP2* (encoding Syntaxin-binding protein 2), impair the complex process whereby cytotoxic granules traffic to and fuse with the plasma membrane and extrude their contents into the extracellular space at the immunological synapse.<sup>98–100</sup> Some conditions associated with lysosomal trafficking defects and hypopigmentation (for example, Griscelli Syndrome and Chediak-Higashi Syndrome) also affect cytotoxic lymphocyte degranulation and, patients may be at risk for developing HLH (Box 2).<sup>101, 102</sup> An infection or other immunological trigger may initiate the syndrome of HLH in these disorders by the abnormal persistence of antigen presenting cells and expansion of hyperactive polyclonal CD8<sup>+</sup> T cells that secrete IFN- $\gamma$ , TNF $\alpha$ , and other cytokines, with resultant activation of macrophages leading to a vicious cycle of lymphohistiocytic proliferation and hypercytokinemia.<sup>103–105</sup> The magnitude of pathological inflammation distinguishes HLH from other hyperinflammatory conditions.<sup>106</sup>

Cytotoxic function-deficient mice with knockout mutations in genes that are that are required for cytotoxic lymphocyte function (e.g. *Prf1*) develop an HLH-like disease when infected with lymphocytic choriomeningitis virus.<sup>99, 107–109</sup> In these HLH mouse models, if CD8<sup>+</sup> T cells were depleted or when IFN- $\gamma$  was neutralized, the HLH phenotype was abrogated, whereas phenotype was not rescued by depletion of NK cells were depleted or other pro-inflammatory cytokines were neutralized, suggesting that CD8<sup>+</sup> T cells and IFN- $\gamma$  are essential in HLH pathophysiology. The HLH-like phenotype is thought to result from the failure of the cytotoxic T cells to eliminate activated antigen presenting cells, leading to continued stimulation of the cytotoxic T lymphocytes. Although defects of NK cell cytotoxic activity alone are not sufficient to induce HLH in mouse models<sup>99</sup>, NK cells probably have a role in HLH pathophysiology. When NK cell cytotoxicity is conserved, the HLH phenotype is less severe, through reduction of the cytotoxic CD8<sup>+</sup> T proliferation.<sup>99, 110</sup> The importance of IFN- $\gamma$  as master-regulator of inflammation is further supported by clinical responses to antibody therapy directed against IFN- $\gamma$  in patients with HLH.<sup>111</sup>

There are additional inherited disorders that cause HLH by more complex or alternative mechanisms that can also be considered primary HLH disorders (Box 2). These disorders include X-linked lymphoproliferative disease type 1 (due to mutations in *SH2D1A*),<sup>112–114</sup> X-linked lymphoproliferative disease type 2 (due to mutations in *XIAP* (*also known as BIRC4*)),<sup>115</sup> other diseases that are often classified as EBV-susceptibility diseases that reflect T cell dysfunction, including ITK116 CD27,<sup>117</sup> and MAGT1118), and diseases caused by mutations in *CDC42*<sup>119</sup> or *NLRC4*<sup>120, 121</sup>. Persistent and/or extreme EBV viremia in turn can induce HLH. The full discussion of the pathophysiology of these diseases is outside the scope of this Primer, but general mechanisms are listed in Box 2. Notably, patients with *XIAP* deficiency and activating variants in *NLRC4* have dysregulated inflammasome function, implying over-exuberant inflammasome-derived cytokine production can also drive HLH. The spectrum of genetic disorders associated with HLH is expanding, with increased recognition that a wide spectrum of inborn errors of immunity can be associated with HLH, usually in the setting of infectious immune challenges.<sup>45, 122 123</sup> Inborn errors of metabolism can also occasionally be complicated by HLH.<sup>124</sup> This occurrence may be due to abnormal macrophage activation due to the accumulation of non-degraded substrates. Rheumatological disorders (autoimmune and/or

auto-inflammatory) with exuberant hyper-cytokinemias can also be complicated by HLH, which may be called macrophage activation syndrome (MAS) in this setting.<sup>125</sup>

## Diagnosis, screening and prevention

### Clinical Presentations

**LCH.:** Clinical presentations for LCH are widely variable depending on sites of disease and the effect on specific organ systems.<sup>9, 10, 126–128</sup> (Figure 3 , Supplementary Figure 1) LCH most frequently manifests in the skin, where lesions may present as pruritic or ulcerative skin rashes that are easily mistaken for more common rashes, especially in infants (Figure 4A), in whom LCH can arise as skin-limited disease, or the skin can be one of many organs involved in more severe multisystem disease.<sup>129</sup> Chronic inner ear and/or mastoid lesions may present with chronic drainage that could be confused with otitis externa. In women, LCH of the vulva can mimic other genitourinary conditions. LCH can also involve the gastrointestinal track anywhere from mouth to anus, with weight loss and chronic diarrhoea as possible signs of intestinal involvement. In the mouth, LCH can involve the gingiva, causing early eruption of deciduous teeth or loss of mature teeth. LCH frequently arises in bone, where it can cause local pain and/or mechanical damage. (Figure 4B) Back pain with vertebra plana (complete collapse of a vertebra) is a classic presentation. Adults with smoking history may present with acute pneumothorax or may develop symptoms of obstructive pulmonary disease if lung LCH and associated parenchymal damage progresses.<sup>25</sup> (Figure 4D) Disseminated LCH can involve liver, spleen and/or bone marrow. (Figure 4 C) Initially liver disease may present with hepatomegaly and signs of acute hepatitis. Long-standing disease can progress to sclerosing cholangitis (diffuse inflammation of the bile ducts) with jaundice and other signs of liver failure. (The spleen can be diffusely infiltrated or have focal lesions, clinically manifesting as splenomegaly. Patients with bone marrow infiltration may present with clinical signs of cytopenias (bruising, anemia, and infections) and in some cases may develop hyperinflammatory symptoms of secondary HLH.<sup>130</sup> Sudden onset of diabetes insipidus is a classic presentation of LCH involving pituitary gland. Mass central nervous system (CNS) lesions can cause focal neurological deficits and/or headache, whereas LCH-ND typically presents with signs of cerebellar dysfunction.<sup>76, 131</sup> (Figure 4E)

**Erdheim Chester Disease.:** ECD can affect nearly every organ system and is frequently a multi-system disease, leading to a vast array of symptoms and signs in adults. (Figure 3) The natural history of ECD is difficult to evaluate, owing to the wide spectrum of presentations and treatments. Untreated ECD can be severe and fatal, particularly in those with multi-system disease. The rare occurrences of spontaneous regression and self-healing that can occur in LCH are not observed in patients with ECD. The mean time from symptom onset to diagnosis in a recent series of 261 patients was 2.7 years.<sup>28</sup> ECD may be heralded decades before the diagnosis by diabetes insipidus supposedly of unknown origin.<sup>84</sup> Iconic radiographical and clinical signs of ECD include the ‘hairy kidney’, sheath of fibrotic tissue with histiocytes around the aorta, sclerosis of long bones, right atrial pseudotumors, and xanthelesma on the eyelids (Figure 5). Like LCH, neurological involvement in ECD may have a tumoural or neurodegenerative presentation. A retrospective analysis of all CNS manifestations (both clinical and only detected by imaging) in 253 patients with ECD

showed CNS involvement in 97 individuals (38%).<sup>132</sup> A review of 74 brain MRIs identified three non-exclusive patterns: tumoural (66%), degenerative (50%) and vascular (18%).<sup>132</sup>

**JXG.:** JXG most often presents in children with cutaneous flesh colored, yellow-orange, brown or purple macules or papules, which may occur anywhere on the body.<sup>133</sup> (Figure 6) Some patients have isolated or very few cutaneous lesions, whereas others have hundreds, with potential to become quite large in some cases. JXG is present in one or two sites in 75% of patients.<sup>133</sup> Extra-cutaneous sites include the liver (22%), lung (16%), soft tissue (16%), spleen (11%), eye (9%), oral cavity (7%), lymph nodes (7%), brain (7%), adrenal glands (7%), gastrointestinal tract (7%), bone marrow (7%) and heart (4%).<sup>30, 134, 135</sup> Disseminated JXG can be aggressive and potentially fatal.

**RDD.:** RDD may occur as an isolated disorder or in association with autoimmune, hereditary, and malignant diseases.<sup>136</sup> A review of 47 patients with RDD found that most had chronic disease activity.<sup>137</sup> Extranodal RDD has been documented in 43% of cases, with the most frequent sites being skin, soft tissue, the upper respiratory tract, multifocal bone, kidney, eye and retro-orbital tissue usually with lymphadenopathy.<sup>34</sup> Head and neck involvement has been reported in 22% of patients, most commonly affecting the orbit, nasal cavity or the parotid gland.<sup>138</sup> Unlike neurological LCH and ECD, neurodegenerative findings have not been reported in patients with RDD. CNS RDD are mass lesions that can be asymptomatic and, therefore, discovered incidentally, or they may arise with clinical symptoms of central endocrinopathies, seizures, or headaches.<sup>139 137</sup>

**HLH.:** It is important to distinguish between the syndrome of HLH and an underlying genetic disease which predisposes to HLH.<sup>140</sup> The syndrome of HLH refers to a constellation of clinical and laboratory manifestations. Diagnostic criteria for the syndrome of HLH were established by the Histiocyte Society and include fever, splenomegaly, cytopenias, hypertriglyceridemia and/or hypofibrinogenemia, observation of hemophagocytosis, decreased NK-cell function, elevated ferritin, and elevated sIL-2R $\alpha$  levels (Box 3).<sup>141</sup> There are several genetic inborn errors of immunity which predispose patients to develop the syndrome of HLH. Such diseases can be referred to as primary or genetic HLH disorders. Other common manifestations include CNS involvement in 30–73% of patients<sup>142</sup> (sometimes as an isolated manifestation<sup>143</sup>), and hepatitis or acute liver failure, which occurs in 90% of patients<sup>144</sup>. Patients who develop HLH in the absence of a primary/genetic HLH disorder are often referred to as having secondary HLH, though distinctions between presumed ‘primary’ and ‘secondary’ can be blurred with infectious triggers in many ‘primary’ cases, and complex or undiscovered genetic predisposition in presumed ‘secondary’.<sup>45</sup>

### Diagnostic Tests and Imaging

**LCH.:** Initial evaluations typically include studies to identify potential organ damage and systemic inflammation including complete blood count, liver panel, complete metabolic panel, ferritin, and prothrombin time (PT) or partial thromboplastin time (PTT).<sup>10</sup> For screening, skeletal x-ray of the whole body with 4 views of skull, chest x-ray, and/or abdominal ultrasonography may identify lesions. We favor <sup>18</sup>F-fluorodeoxyglucose (FDG)-

PET–CT scans to define the extent of disease because of sensitivity relative to other imaging modalities and ability to define metabolic responses to therapy.<sup>145, 146</sup> Chest CT is appropriate for pulmonary lesions, owing to need for high resolution imaging. MRI is preferred for spine and brain imaging, though maxillo-facial bone sites are best imaged by CT.<sup>131</sup> Bone marrow evaluations are appropriate for young children or for patients presenting with cytopenias. For diagnostic procedures, excisional biopsy is preferred to fine needle aspiration or core biopsies, owing to complex architecture of lesions. More limited biopsies may give an inaccurate representation of the histology, as pathological cells may not be evenly distributed in the sample. While excisional biopsies are appropriate, aggressive excisions including wide margins are contraindicated for LCH, as these can impair tissue remodeling. Unlike other malignancies or neoplastic disorders, bone has potential to repair with successful LCH therapy.<sup>127</sup> Targeted sequencing of MAPK pathway genes may identify pathogenetic mutations that support LCH diagnosis and inform disease risk and potential therapeutic options. It is possible to evaluate for BRAFV600E+ peripheral blood mononuclear cells or bone marrow cells with high sensitivity techniques.<sup>147</sup> Next generation sequencing approaches should take into account potential for low variant allele frequency (median 8% pathogenic cells in LCH lesions).<sup>63</sup>

There are several differential diagnoses for the LCH presentations that may require tissue biopsy to differentiate. LCH skin rashes mimic a wide variety of more common cutaneous diseases such as psoriasis, seborrhea (excessive discharge from the sebaceous glands), viral exanthema (widespread rash), and the ‘blueberry muffin’ presentation of neuroblastoma and myeloid leukemias. Orbital bone involvement causing proptosis (extreme bulging out of the eyes), or other osteolytic bone lesions may mimic sarcomas, neuroblastoma, and leukemias. Benign lesions similar to LCH include disorders of bone and connective tissue including dermal bone cysts, myofibromatosis<sup>148</sup>, fibrous dysplasia<sup>149</sup>, and cranial fasciitis.<sup>150</sup> Hepatomegaly and elevated liver enzymes could be caused by a vast number of primary hepatic diseases. Diabetes insipidus can be idiopathic or also associated with germ cell tumors, sarcoidosis, lymphocytic hypophysitis (infiltration of the pituitary with lymphocytes), or tuberculosis. The MRI images of the LCH neurodegenerative syndrome are similar to those of patients with encephalitis, sequelae of acute disseminated encephalomyelitis (ADEM), and drug overdoses. Pneumothorax caused by a number of primary pulmonary conditions with cysts and interstitial lung abnormalities can be confused with LCH and *vice versa*. LCH rarely presents along with other hematologic malignancies (leukemia or lymphoma), frequently sharing pathogenic somatic mutations beyond the typical MAPK repertoire. Biologically, LCH in context of bi-phenotypic leukemia or lymphoma is likely distinct from more typical presentations.<sup>72</sup>

**ECD.:** Evaluations for cytopenias, renal and hepatic insufficiency, endocrinopathies, immunological assessment (T cell and B cell subsets and immunoglobulins), and inflammatory markers (sedimentation rate and C-reactive protein) should be performed.<sup>28, 151</sup> The most frequent radiographical sign of ECD is long-bone osteosclerosis (abnormal bone density), seen in 80–95% of patients but symptomatic in only 38%.<sup>28</sup> It was historically evaluated by bone scan, but PET–CT may be more effective and also allow extra-osseous evaluations.<sup>152, 153</sup> Abdominal CT scans reveal the iconic fibrotic infiltration

around the kidneys ('hairy kidneys') in 63% of patients.<sup>28</sup> (Figure 5A) CT scans may also reveal sheathing of the aorta and pericardial disease sometimes leading to tamponade. (Figure 5B) Right atrium pseudotumor, another classical feature of ECD, can be observed by cardiac MRI in 36% of patients.<sup>154</sup> Biopsies should be evaluated for mutations of genes in the MAPK and PI3K-AKT pathways (Figure 1).

Several diseases have features similar to ECD. Takayasu arteritis has some features comparable to aortic abnormalities in patients with ECD. However, radiological findings differ between Takayasu arteritis and ECD: the entire wall is affected in Takayasu arteritis, whereas the adventitial and periadventitial periaortic spaces, but not the wall itself, are affected in patients with ECD.<sup>155</sup> Radiological characteristics can also help distinguishing between ECD and mediastinal and retroperitoneal fibrosis. Idiopathic retroperitoneal fibrosis is classically not circumferential, infiltrating the anterior and lateral sides of the aorta, but sparing the posterior space. IgG4-related disease, a multi-system condition that may be confused with ECD, is characterized histologically by dense lymphoplasmacytic infiltrates rich in IgG4<sup>+</sup> plasma cells, abundant fibrosis with a storiform (cartwheel) pattern, lymphoid follicles, tissue eosinophilia and obliterative phlebitis (vascular inflammation resulting in obstruction). Serum IgG4 levels may be elevated as they are in approximately half the patients with IgG4-related disease.<sup>156</sup> Other histiocytoses (such as RDD) should also be considered in the differential diagnosis.

**JXG.:** For infants with only a few cutaneous lesions (Figure 6) and no systemic symptoms or signs, evaluations should include a complete blood count, liver enzyme panel, basic metabolic panel, and ophthalmological examination. Patients with a large number of lesions and/or systemic symptoms or abnormalities of the aforementioned laboratory tests should have MRIs of the brain and abdomen to evaluate for possible systemic lesions. When cytopenias are present, bone marrow aspirates and biopsies are indicated. Targeted or whole exome sequencing may be informative. (Figure 1)

JXG lesions may be mistaken for atheromas or lipomas, or LCH. Urticaria pigmentosa, neurofibromatosis type I (NF1), and myelogenous leukemia have also been associated with JXG and can all be differentiated by biopsies.<sup>157</sup> Patients with NF1 and JXG may have highly increased risk of developing juvenile myelomonocytic leukemia.<sup>87</sup>

**RDD.:** Laboratory analyses should include a complete blood count, liver function tests, basic metabolic panel, serum immunoglobulins and erythrocyte sedimentation rate.<sup>136</sup> Staging evaluation includes a skeletal survey for osteolytic lesions and a PET scan to locate affected lymph nodes, periorbital or sinus masses, pulmonary, spleen, liver, and bone lesions. (Figure 7) Biopsy is required for diagnosis, and excisional biopsy is preferred owing to complex and heterogeneous histology. Targeted sequencing or whole exome sequencing may be informative in some cases. (Figure 1)

Several differential diagnoses for RDD exist. Sinus involvement or proptosis from retro-orbital RDD presents much like neuroblastoma, sarcomas, or LCH. The lytic osseous lesions of RDD are often not as symmetrical as those observed in LCH and can occur in any bone. CNS disease can mimic a meningioma. Histopathological characteristics resembling RDD

have been described in 41% of patients with autoimmune lymphoproliferative syndrome type Ia, with *TNFRSF6* heterozygous germline mutations affecting the *FAS* gene, and in patients with autoimmune hemolytic anemia, systemic lupus erythematosus, and juvenile idiopathic arthritis.<sup>158, 159</sup> RDD may arise in patients with H-syndrome and Faisalabad histiocytosis, associated with germline mutations in *SLC29A3*.<sup>91, 92</sup> Some patients with the IgG4 related disease have features of RDD.<sup>160</sup> RDD probably represents a common histological endpoint from many conditions.

**HLH:** A sample diagnostic algorithm is given in Figure 8. Hemophagocytosis alone has limited diagnostic sensitivity and specificity, as it can be observed in patients without HLH and can be absent in patients with HLH.<sup>161–163</sup> Ferritin and sIL-2R $\alpha$  may be elevated in many settings, but highly elevated inflammatory markers should prompt more in depth evaluations for HLH.<sup>39, 164, 165</sup> Inherited cytotoxic defects are associated with decreased NK cell function; however, these findings can be non-specific in very young children or temporarily depressed in acutely ill patients. Tests that evaluate perforin expression or lymphocyte CD107 (also known as Lysosome-associated membrane glycoprotein 1 upregulation) can be used to more reliably screen patients for perforin deficiency or pathogenetic variants in the genes that are crucial for degranulation.<sup>166, 167</sup> Newer measurements, such as C-X-C motif chemokine 9 (CXCL9), a marker of IFN $\gamma$  activity, and IL-18, which reflects inflammasome activation, may become helpful in identifying patients with pathological inflammation that can be used to reflect magnitude of pathological activation, which may distinguish HLH from other conditions.<sup>168, 169</sup>

All patients with HLH should be evaluated for malignancy and infection, which are especially common in adults.<sup>170, 171</sup> HLH can arise either at diagnosis or during periods of immune dysregulation resulting from chemotherapy or HCT.<sup>172</sup> Leukemias and lymphomas are predominantly associated with HLH, though solid tumors have been reported.<sup>170, 173</sup> HLH may occur in up to 1% of hematological malignancies.<sup>172</sup> PET scan or CT–MRIs of the entire body can be helpful to rule out associated malignancies. MRI of brain and (where possible) cerebrospinal fluid evaluations can support identification of CNS pathology in patients with HLH. (Figure 9) HLH can be associated with infections varying from common triggering viruses such as EBV to virtually any viral, bacterial, fungal, mycobacterial, or parasitic infection. Rheumatological or metabolic disorders should be considered.<sup>124, 125</sup> Patients should be evaluated for underlying genetic HLH disorders and inborn errors of immunity.<sup>45</sup> Rapid screening tests can help define some primary HLH disorders. Flow cytometric assays are available to screen for perforin, SAP (signaling lymphocytic activation molecule (SLAM)-associated protein, also known as SH2 domain-containing protein 1A), and XIAP (X-linked inhibitor of apoptosis) deficiencies.<sup>167, 174–176</sup> Degranulation (CD107a) assays screen for genetic diseases that compromise granule-mediated cytotoxicity.<sup>177</sup> IL-18 levels can be useful when considering XIAP deficiency and pathological variants in *NLRCA*, or MAS in the setting of rheumatological disorders.<sup>168, 178, 179</sup> Genetic HLH panels are frequently used, but broad primary immune deficiency gene panels or whole exome sequencing offer the benefit of capturing additional inborn errors of immunity or metabolic diseases.<sup>45, 180</sup>



A diagnosis of the syndrome of HLH does not exclude important underlying disorders or mimicking disorders. For HLH, differential diagnoses may also include concurrent diagnoses. Patients with hyperinflammatory conditions such as those with sepsis, the systemic inflammatory response syndrome (SIRS), multi-organ failure from many causes, metabolic storage diseases, malignancies, and immune deficiencies may present with many of the signs and symptoms of HLH. The term ‘macrophage activation syndrome’ (MAS) is the subject of ongoing nomenclature debate, but typically describes pathological inflammation in the setting of autoimmune or autoinflammatory disease or cancer. MAS is diagnosed using criteria established by a collaborative effort that examined discriminating features for MAS in patients with systemic juvenile idiopathic arthritis<sup>181</sup>.

### Pathology

**LCH.:** The diagnosis of LCH is relatively straightforward, with biopsy demonstrating pathological round CD1a<sup>+</sup>–CD207<sup>+</sup> histiocytes having reniform nuclei with infiltrating immune cells, predominantly T cells. (Figure 10).<sup>62, 182</sup> CD207<sup>+</sup> cells may be abundant or scarce (median of 8% lesional cells).<sup>63</sup> The cells should be distinguished from the physiological normal inflammatory CD207<sup>+</sup> dendritic cells, which have a branching morphology owing to their antigen-presenting role. Relative expression of CD1a and CD207 can vary; notably, it can be dim or absent in brain, liver and bone marrow lesions.<sup>183</sup> The presence of a macrophage phenotype with an MAPK activating mutation can be consistent with LCH despite lack of classic antigens. In one study, *BRAFV600E*<sup>+</sup> cells were identified in > 50% of bone marrow aspirates clinically reported as normal.<sup>63</sup>

**ECD.:** ECD lesions show infiltration of foamy histiocytes surrounded by fibrosis or xanthogranulomatosis. (Figure 10) Touton giant cells are frequently, but not always, observed.<sup>151</sup> Microscopically, ECD is indistinguishable from the fibrohistiocytic JXG subtype<sup>184, 185</sup> and depends on distinct clinical-radiographical features for an integrated diagnosis. Immunophenotype of ECD and JXG includes expression of CD163, CD68, CD14, Factor XIIIa (less consistently in xanthomatous cells) and often fascin.<sup>85</sup>

**JXG.:** The classic JXG cells are large with a vacuolated cytoplasm and small round or indented nuclei. (Figure 10) Like ECD cells, these cells stain with CD163, CD14, CD68, fascin, Factor XIIIa, CD4, but not CD1a.<sup>184</sup> A subset of JXG-like lesions may harbor *ALK* gene rearrangements most commonly *KIF5B-ALK* with *TPM3-ALK* also described.<sup>88</sup>

**RDD.:** The diagnostic feature of RDD is a large histiocytic cell with ample pale foamy cytoplasm, often described as ‘watery-clear’, and a large hypochromatic nucleus with prominent nucleolus. (Figure 10) Histologically RDD is characterized by large histiocytes with ample pale cytoplasm, often with emperipolesis (that is, viable lymphocytes and plasma cells trafficking through cytoplasm of RDD histiocytes). The biological or clinical significance of emperipolesis is not well understood but may be a mechanism of immune evasion. The engulfed cells are found in a membrane-bound vesicle in the macrophage cytoplasm and are not phagocytosed. Emperipolesis in characteristic cells distinguishes RDD from other causes of massive lymphadenopathy. The RDD cells do not present any cytologic atypia, have very few mitoses figures, selectively stain for S100, fascin and

macrophage markers (CD14, CD69, or CD163) and lack DC markers (CD1a and CD207)<sup>186</sup> In affected lymph nodes, histiocytes are mainly located within the sinuses.<sup>184</sup> Lesions can contain numerous plasma cells, and systematic evaluation for associated IgG4-related disease is recommended if there is an elevated plasma IgG/IgG4 levels ratio is >40%.<sup>156</sup> The cytomorphology and immunophenotype should be considered together with the pattern of tissue involvement, as small focal areas of RDD-like cells do not equate to disease.

**HLH:** Bone marrow studies are important not only to evaluate for hemophagocytosis (Figure 11) but also to characterize possible bone marrow failure, malignancy, metabolic storage disease and/or infection. Hemophagocytosis is a diagnostic criterion, but has limited sensitivity and specificity. Activated macrophages, some with hemophagocytosis, may also occur in a number of diseases affecting lymph nodes, spleen, CNS, liver, bone marrow, and thymus, together with increased CD8<sup>+</sup> T-cells.<sup>187</sup> Staining with anti-CD68 PGM-1 clone or anti-CD163 antibodies can highlight activated macrophages in bone marrow or other tissues.

## Management

Treatment goals and strategies for histiocytic disorders vary depending on the disease, and optimal therapeutic strategies remain uncertain for most patients with histiocytic disorders. Cytotoxic therapies may be used in attempts to cure patients with systemic LCH, ECD, JXG and RDD with therapeutic goal to clear the pathogenic clone. MAPK pathway inhibitors are being evaluated for MAPK-driven histiocytoses. Early trials with LCH and ECD demonstrate very high response rates, but disease may recur with cessation of MAPK inhibition.<sup>68, 69, 188, 189</sup> Immune modulation may be used to control pathological inflammation and symptom management. Prompt initiation of immune suppression is central to treatment of HLH, and HCT is required to cure patients with intractable immune dysregulation.

**LCH**—Patients with isolated bone lesions or limited skin rash may be treated with local approaches, whereas patients with multifocal and/or CNS disease typically require systemic therapy.

**Single bone lesions.:** Patients with isolated bone lesions may be treated with curettage (operative removal of diseased tissue) alone, and/or with steroid injection.<sup>190</sup> Lesions in the facial bones or skull base are considered ‘CNS-Risk’, owing to association with developing pituitary gland disease and LCH-ND, and are, therefore, treated with systemic chemotherapy with same strategies as multifocal disease.<sup>131</sup> For bone lesions, wide excision with ‘clean margins’ may impair bone remodeling and is not indicated. Radiation therapy is rarely used for bone lesions in children due to potential impact on growth and efficacy of alternatives, but may be applied more broadly for adults with single bone lesions that are inaccessible to surgery or interventional radiology.

**Lung.:** Smoking cessation, without pharmacological therapy, may be effective for some adult patients with isolated lung disease.<sup>191</sup> However, others with extensive cysts and nodules are preferably treated with systemic chemotherapy. Additionally, such patients

should be evaluated thoroughly for extra-pulmonary lesions, and patients with multi-system disease typically require chemotherapy.<sup>192</sup>

**Skin.:** Isolated cutaneous LCH may resolve with no treatment in some infants.<sup>129</sup> Progressive disease may respond to oral hydroxyurea, methotrexate with or without 6-mercaptopurine (6-MP), or thalidomide.<sup>193–195</sup> Radiation therapy and surgical excision are rarely used owing to potential toxicity and limited utility for wide-spread disease.

**LCH of pituitary, parenchymal brain lesions and LCH-ND.:** In patients who present with isolated diabetes insipidus, polydipsia (excessive thirst) and polyuria (excessive urination) without other signs of LCH, it may be challenging to differentiate LCH of the pituitary gland or brain from germinoma or pituitary hypophysitis (inflammation of the pituitary gland). Suspected or proven LCH lesions that progress on MRI may be treated with systemic chemotherapy.<sup>196–198</sup> Parenchymal or neurodegenerative LCH lesions may respond to chemotherapy or MAPK inhibitors.<sup>12, 68, 199</sup>

**Multifocal disease.:** Treatment regimens evolved from studies by the German, Austrian, and Dutch Pediatric Leukemia Study Group and the Histiocyte Society over the past four decades. The current standard of care for multifocal site, low risk or high risk LCH is 12 months of vinblastine and prednisone, with 6-mercaptopurine (6-MP) added for high-risk patients.<sup>11</sup> While overall survival has greatly improved over the past decades, one year progression-free survival for low-risk patients is 50% and for high-risk patients 33%. The Japanese LCH Study Group have used a multi-drug protocol including vincristine, cytarabine, prednisolone, methotrexate, and 6MP.<sup>200</sup> The one year progression free-survival for this study was 76% for high-risk patients and 94% for low-risk patients. By 5 years the progression-free survival was 46% for high-risk and 70% for low risk. Other drugs used to treat LCH, including cytarabine, clofarabine, and cladribine, have reported efficacy for multi-site disease in case series and single-arm trials.<sup>197, 198, 201</sup> On the basis of the current understanding of LCH deriving from a myeloid precursor, therapies including nucleoside analogs such as cytarabine, cladribine, and clofarabine, may be effective, owing to the sensitivity of myeloid malignancies to these drugs. For a similar reason, oral hydroxyurea, historically effective in myeloid neoplastic disorders, was evaluated and found to elicit responses in some patients with skin and bone LCH.<sup>193</sup> The Histiocyte Society LCH-IV Trial is currently testing the effect of 24 versus 12 months of therapy for both multi-focal low risk and high risk LCH ([NCT02205762](#)). A multi-institutional randomized phase 3 trial is testing frontline cytarabine versus standard vinblastine–prednisone for pediatric LCH ([NCT02670707](#)).

Adult patients have been treated with similar regimens. Cladribine was one of the first reported regimens and has gained popularity in patients with pulmonary LCH.<sup>202, 203</sup> Some groups have found vinblastine/prednisone is safe and effective in adults, though neuropathy and steroid toxicity seem more significant in adults than children.<sup>204, 205</sup> Cytarabine monotherapy has been reported as well-tolerated and effective in case series of adults with bone disease.<sup>204</sup> Other published approaches include MACOP-B chemotherapy and Zoledronic acid.<sup>206, 207</sup>

**Recurrent or progressive LCH:** Data to guide salvage therapy strategies for LCH are primarily limited to case series and early phase trials. (Table 1) Nucleoside inhibitors alone or in combination have efficacy and toxicity correlated to dose.<sup>197, 198, 201, 208, 209</sup> As discussed above, mutually exclusive activating MAPK pathway mutations have been identified in almost all patients with LCH. Case series and early phase trials have reported near universal responses to MAPK pathway inhibition.<sup>9, 14, 68, 69, 80, 188, 189, 210</sup> Many patients, even those with high risk site involvement, achieve a complete metabolic response by PET scan. Patients with the early onset of neurodegenerative changes on MRI and clinical deficits may have marked radiological improvement and variable degrees of clinical improvement; by contrast, patients with long-standing neuropathology may have limited potential for clinical improvement. Patients frequently fail to achieve complete responses with MAPK inhibition, and early reports suggest that those who do achieve clinical complete responses have a high risk of relapse with cessation of therapy.<sup>68, 69</sup>

The BRAF-V600E and MEK inhibitors are generally tolerated but can cause serious adverse reactions to the skin, tendons, joints, eyes, and heart, which require dose reduction or change in therapy. Second malignancies have been described in some adult patients, but so far none in children.<sup>211</sup> The effect of long-term MAPK inhibition in children is not known. Optimal timing and duration of MAPK inhibition versus chemotherapy is also uncertain and merits further evaluation with prospective studies.

**Erdheim Chester Disease**—ECD was historically considered a fatal diagnosis, with nearly 60% of patients dying within 3 years of diagnosis.<sup>154</sup> Interferon  $\alpha$  (IFN $\alpha$ )-based regimens have improved survival,<sup>212</sup> and BRAF and MEK inhibitors have further improved outcomes.<sup>188, 213</sup> A review of the Pitié-Salpêtrière hospital cohort reported a 5-year survival rate of 79%.<sup>84</sup> PEGylated IFN- $\alpha$  is better tolerated than the non-PEGylated form. The therapeutic response of IFN based regimens (measured by PET-CT and conventional imaging) is usually much slower and less complete than that observed with targeted therapies.<sup>28, 84</sup> Assessments should be made every 6 months. IFN- $\alpha$  treatment is associated with numerous adverse effects, such as fatigue and depression, affecting half of the patients.<sup>15</sup> In cases of mild or non-severe ECD (for example, without heart and/or CNS involvement) and contraindications to or adverse effects to IFN- $\alpha$ , the alternative regimens include the IL-1 receptor antagonist anakinra,<sup>214</sup> the anti-TNF- $\alpha$  monoclonal chimeric antibody infliximab,<sup>215</sup> and the mammalian target of rapamycin (mTOR) inhibitor sirolimus with steroids.<sup>216</sup>

In 2012, the first patients with multi-system refractory ECD or combined ECD-LCH with the *BRAF*V600E mutation were successfully treated with the BRAF-V600E inhibitor vemurafenib.<sup>213</sup> Subsequently, over 80 such patients were treated with BRAF-V600E or MEK inhibitors with excellent outcomes, as measured with the PET Response Criteria in Solid Tumors (PERCIST).<sup>14, 189, 210</sup> These results led to FDA approval of vemurafenib for *BRAF*-mutated ECD. When therapy was stopped, relapses occurred after a median of six months in 75% of the patients, though re-treatment with the same MAPK inhibitors following relapse was generally effective.<sup>189</sup> The most frequent side effects in ECD case series are similar to those noted above for LCH, including photosensitivity and keratosis pilaris, spinocellular carcinoma, melanoma, sarcoidosis-like disease, drug reaction with

eosinophilia and systemic symptoms (DRESS), arthralgias, pancreatitis, and QT-segment prolongation. Myelodysplastic syndrome and pancreatic adenocarcinoma have also been reported.<sup>70, 217</sup> Vemurafenib toxicity is usually dose limited and is the main reason for using lower doses in ECD patients than in the melanoma trials. Dabrafenib, another BRAF-V600E inhibitor, has also been reported to be effective with similar range of toxicities.<sup>218</sup>

The robust efficacy of the MEK inhibitor cobimetinib in three patients with multi-system and refractory ECD was reported in 2018.<sup>219</sup> In 2019, cobimetinib was shown to be effective in 12 patients with ECD (overall response rate 89%).<sup>188</sup> Treatment efficacy was not affected by genotype, and responses were observed in patients with a range of activating MAPK pathway gene mutations (*ARAF*, *BRAF*, *KRAS*, *RAF1*, *MAP2K1* and *MAP2K2*). The most frequent adverse effects of cobimetinib are nausea, acneiform rash, and rhabdomyolysis.<sup>189</sup>

**Juvenile xanthogranuloma**—Most skin-limited JXG lesions involute spontaneously, but excision of a single lesion or a limited number of lesions may be indicated in some cases, if the lesion is cosmetically unacceptable or perhaps endangers vision or impairs swallowing. Our preference for treatment of patients with systemic JXG is clofarabine. We reported three such patients were successfully treated with moderate doses of clofarabine that can typically be administered in outpatient settings.<sup>201</sup> A range of therapies including cladribine and combined cytarabine with cladribine have also been reported with responses.<sup>220, 221</sup> Patients with *ALK* mutations can be cured by treatment with crizotinib, an inhibitor of the *ALK* tyrosine kinase receptor, although one patient had a spontaneous recovery.<sup>88</sup> The many therapies given to patients with JXG reflect the current lack of data to guide therapeutic strategies. Patients with JXG are eligible to participate in Cobimetinib trials discussed previously.

**Rosai-Dorfman disease**—Patients with the classic manifestations of RDD present with limited lymphadenopathy or subcutaneous nodules with slow progression or remissions and relapses over many years, but they typically do not require treatment. Such limited involvement is rarely life-threatening, but some patients have a fatal outcome from involvement of the brain, liver, kidney, or spleen or massive lymphadenopathy causing respiratory or vascular compromise.<sup>136, 222</sup> Individuals with autoimmune diseases such as systemic lupus erythematosus, autoimmune hemolytic anemia, or autoimmune hepatitis are more likely to have extensive disease and a higher fatality rate than patients with classic RDD. Patients with extra-nodal disease have a protracted clinical course and require systemic chemotherapy.<sup>136, 137</sup> No standard of care has been established, and the therapeutic approach is usually determined on a case-by-case basis.

Surgery is generally limited to biopsy, whereas debulking might be required in patients with upper airway obstruction or intracranial lesions.<sup>136</sup> Radiotherapy has little efficacy in most cases, although it can be beneficial in refractory orbital RDD with visual disturbances.<sup>136</sup> Systemic corticosteroids are usually helpful in decreasing nodal size and improving symptoms, but they are immunosuppressive, and most patients relapse after cessation of therapy.<sup>136</sup>

A review of 40 RDD cases from the 1970's until the mid-1990's found variable response with a variety of chemotherapeutic agents.<sup>222</sup> In one report, only two of ten patients given chemotherapy reached complete remission, both with a combination of low-dose methotrexate and 6-mercaptopurine.<sup>223</sup> There are case reports of successful treatment with a combination of prednisone, oral methotrexate, thioguanine, and leucovorin<sup>224</sup>, IFN $\alpha$ ,<sup>225</sup> acyclovir,<sup>226</sup> thalidomide<sup>227</sup>, and the tyrosine kinase inhibitor imatinib.<sup>228</sup> Cladribine or clofarabine have been found to be effective in some cases of recurrent, refractory, or severe RDD.<sup>201, 229</sup> The efficacy of the anti-CD20- specific monoclonal antibody rituximab has been described and seems particularly interesting in cases of associated autoimmune hemolytic anemia or systemic lupus erythematosus.<sup>230</sup> Radiotherapy and temozolomide have been used in neurological RDD.<sup>139</sup> A recent case series reports that MEK inhibitor cobimetinib was effective in two patients;<sup>231</sup> MEK inhibitor could be considered for severe, refractory RDD and/or RDD with severe life threatening presentation (such as CNS RDD).

**Hemophagocytic lymphohistiocytosis**—The initial management of HLH hinges on suppressing the hyperinflammatory process and treating the underlying trigger. Subsequently, prompt preparation for allogeneic hematopoietic stem cell transplantation is important for patients with many of the genetic HLH disorders, or those who have refractory or relapsing disease that suspected to be a genetic disorder. HLH is typically fatal without intervention. Treatment should begin quickly, with the exception that corticosteroids or etoposide should be withheld until evaluations for malignancy are completed (if feasible), as they could interfere with the ability to diagnose hematological malignancies. Exceptions to the requirement for HLH-specific treatment include patients who have secondary HLH that is expected to improve with treatment of the underlying trigger. For instance, individuals with visceral leishmaniasis may respond to antimicrobial therapy alone, and HLH that develops with malignancy may improve with treatment of the malignancy. In some cases, these interventions will result in improvement of the secondary hyperinflammatory manifestations.

The most commonly used HLH treatments are dexamethasone and etoposide according to the HLH-94 protocol, which was studied for the treatment of HLH by the Histiocyte Society in a multi-center trial.<sup>232</sup> Etoposide has specific activity against activated T cells, which may account for evolution of this treatment approach.<sup>233</sup> Cyclosporine is not recommended for early treatment of HLH owing to the risk of toxicity and the observation that early initiation of cyclosporine did not improve outcomes in the HLH-2004.<sup>141</sup> Cyclosporine may be useful to prevent disease reactivation in patients who have achieved remission.<sup>232</sup> Intrathecal treatment is recommended for central nervous system HLH that does not completely respond to systemic therapy. Not all patients with HLH require etoposide; corticosteroids alone may suffice in patients with mild disease. An alternative approach includes corticosteroids and anti-thymocyte globulin (ATG).<sup>234</sup> ATG is a polyclonal antibody preparation made by immunizing horses or rabbits with human thymocytes. When administered to patients, ATG depletes human T cells. Treatment with etoposide and ATG resulted in complete responses in ~50–75% of patients.<sup>141, 234, 235</sup> Rituximab or other B-cell depleting therapy should be considered for EBV-associated HLH.<sup>236</sup> In contrast to patients with HLH, patients with MAS associated with autoimmune or auto-inflammatory disease are typically treated with

IL-1 inhibitors, corticosteroids, and cyclosporine and are not treated with etoposide unless inflammation control is insufficient with conventional therapies.<sup>237</sup>

Newer treatments for HLH include biologic agents. Emapalumab is a monoclonal antibody directed against interferon gamma that was approved by the Food and Drug Administration for refractory, recurrent, or progressive HLH based on a clinical trial that demonstrated responses in 63% of previously treated patients.<sup>111, 238</sup> Alternative approaches to immune suppression targeting specific immune cells, immune activation pathways and/or cytokines are also under investigation.<sup>132, 238</sup> Alemtuzumab, a monoclonal antibody against CD52, has been used as salvage therapy.<sup>239, 240</sup> Ruxolitinib, a Janus kinase (JAK) inhibitor that blocks multiple cytokine signaling pathways, was effective in a number of patients with HLH and murine studies.<sup>241–245</sup> Anakinra (an IL-1R antagonist) was effective in secondary HLH.<sup>246</sup> Tadekinig alfa is a recombinant human IL-18 binding protein<sup>247</sup> that may be effective for some forms of MAS.

Allogeneic haematopoietic stem cell transplant is generally reserved for patients with severe genetic HLH disorders, such as those caused by mutations in *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *RAB27A*, *LYST*, and *SH2D1A*, and can also be considered for patients with refractory or relapsing HLH that is thought to be a genetic disorder of the immune system. Reduced intensity conditioning regimens were often used in the past decade owing to the high mortality associated with fully myeloablative regimens in HLH. However, reduced intensity conditioning can frequently be associated with mixed chimerism and graft failure. A national trial with reduced intensity condition resulted in relatively high overall survival, but also high rates of secondary graft failure.<sup>248</sup> Patients are at risk of HLH recurrence if donor chimerism declines to <5–30% of whole blood or T cell populations.<sup>248, 249</sup> New developments in reduced toxicity myeloablative approaches may improve sustained donor chimerism while still minimizing toxicity.<sup>250–252</sup> Propective studies are required to optimize survival, engraftment, and morbidity in HCT in this high risk population.<sup>252</sup>

**Global variations**—Diagnosing histiocytosis in developing settings may be extremely difficult because of the limited numbers of physicians who are aware of the diagnoses and the scarce resources for radiographical and other diagnostic and molecular tests, especially regarding histochemical stains to differentiate these diseases from more common benign and malignant entities. Available treatments may be extremely limited, based on need to deliver systemic chemotherapy along with required supportive care. Oral therapy with MAPK inhibitors could be effective, but currently challenging to deliver owing to limited drug access and high cost. For HLH, the need for haematopoietic stem cell transplantation for definitive cure for many patients is also a challenge.

### Quality of life

**LCH**—Long-term quality of life studies report long-term sequelae in the majority of LCH survivors, with risks of sequelae associated with severity and duration of disease.<sup>253, 254</sup> Long-term morbidity may have been increased by historic uncertainty of LCH as an inflammatory disorder, leading to tolerance of ‘smoldering’ disease and practice of treating relapses symptomatically. Specific long-term challenges including behaviour problems and

learning in children, especially those with brain lesions and LCH-ND. Approximately 5% of all patients with LCH develop LCH-associated changes in the cerebellum, pons or basal ganglia (detectable with MRI), and some of them develop a neurodegenerative syndrome with ataxia, dysmetria (poor handwriting), dysarthria (unclear speech), and behavioural, or learning disabilities<sup>131, 255</sup> Even adults with isolated pulmonary disease have been reported to struggle with long-term anxiety.<sup>256</sup> The majority of patients with diabetes insipidus will need life-long supplemental vasopressin, and half of these individuals will also need supplemental thyroid, adrenal, growth, or gonadal hormones.<sup>196</sup> Some patients with bone lesions can lead to permanent dysfunction, especially at vertebral sites where vertebra plana can cause instability of the spine.<sup>257</sup> Pulmonary LCH can lead to destruction of sufficient pulmonary tissue that 50–90% of patients may experience dyspnea on exertion, need supplemental oxygen, and rarely lung transplant.<sup>258–260</sup> If sclerosing cholangitis occurs with fibrosis of large parts of the liver, liver transplant may be required.<sup>261</sup> A caregiver for a girl with LCH shares their story (Box 4).

**Erdheim Chester disease**—Prior to modern therapies, life expectancy for patients with ECD was >60% 3 years after diagnosis.<sup>154</sup> Outcomes have improved with IFN- $\alpha$  and MAPK inhibitor therapeutic strategies, though patients typically require ongoing therapy. Long-term morbidities arise from both chronic disease and toxicities of long-term therapy.<sup>153</sup> A patient-reported outcome tool was developed for ECD, the Erdheim-Chester Diseases Symptom Scale (ECD-SS). Sixty-two of 63 symptoms in the inventory were endorsed by at least 1 of 50 ECD participants, and neurologic or psychologic symptoms were reported by 92%. This study highlights the unmet need of management of neuro-psychiatric symptoms in this population.<sup>262</sup>

**Juvenile Xanthogranuloma**—Most patients with a few JXG lesions will have spontaneous resolution, and the disease never recurs. Others with more extensive disease can have long term problems from structural damage by brain lesions or possibly hepatic failure from large tumors<sup>263</sup>. Our experience is that long-term consequences of JXG are comparable to LCH.

**Rosai-Dorfman Destombes disease**—Most RDD patients have no long term problems from isolated adenopathy that resolves spontaneously or with modest treatment. The chronic nature of RDD can lead to discomfort or dysfunction based on sites of disease. In general, our experience is that long-term consequences of RDD are comparable to LCH. In the largest series, 17/238 (17%) patients died as the result of complications of their disease.<sup>34</sup>

**Hemophagocytic lymphohistiocytosis**—HLH can substantially affect a patient's quality of life, as it is a life-threatening condition. Treatment usually requires many weeks to months of therapy. Most patients also require treatment of an underlying trigger, and some patients develop adverse effects of HLH such as bleeding complications, organ failure, and infections, which are inadequately quantified in the literature. Most patients require hospitalization, though data regarding general length of stay is limited. Intensive care needs are common, and even with treatment, mortality remains high. Similar to other rare and serious diseases that require care at specialized centers, patients and families may have to



travel to tertiary care medical centers, a requirement that places additional financial and social stressors on all those involved. For patients with an underlying genetic HLH disorder, allogeneic HCT unfortunately leads to additional hospitalization time away from home and confers significant additional mortality risk, approximately 30%.<sup>264</sup> Late effects including organ damage, decreased motor skills, learning disabilities, and other long-term issues may be substantial. However, with successful treatment and transplantation, many patients can live normal lives.

## Outlook

**Inherited and acquired genetics**—In all histiocytic disorders, the future will be shaped by questions and opportunities arising from molecular genetics. The discovery of somatic *BRAFV600E* and other mutations affecting the MAPK pathway in LCH have changed our understanding of the neoplastic histiocytic disorders LCH, ECD, JXG and RDD. For LCH, there is an association of increased risk of mortality and neurological consequences for those patients with the *BRAFV600E* mutation. Although most patients with LCH, ECD, JXG and RDD have only one driver mutation, others may have up to over a dozen, and each additional mutation may have a role in pathogenesis.<sup>81</sup> In adults, these histiocytic disorders may arise as a result of the accumulation of novel mutations through clonal hematopoiesis.<sup>53</sup> However, many questions remain concerning the origin of mutations, the range of biological effects and the clinical utility of genotyping and mutation detection as biomarkers.

HLH includes a spectrum of disorders of constitutive genetic defects, in which clinical exome sequencing has already widened the number of genotypes known to be at risk, far beyond those classical defects associated with impaired cytotoxicity. This number will continue to expand through primary immunodeficiency screening programmes, together with the increasing application of sequencing to study older children and adults with late-onset or secondary HLH.

**Cell of origin question**—The ‘cell of origin’ question is familiar yet problematic to the field of oncogenesis. Although it is highly attractive to pinpoint a mutational hit to a cellular compartment, by definition, neoplastic transformation completely skews developmental architecture so that it may be difficult to map a malignant clone to any healthy counterpart. In the case of histiocytosis, somatic activation of the MAPK pathway seems to confer little proliferative advantage to stem or progenitor cells, and children with multi-system<sup>7</sup> LCH have a stable, low burden of mutated clones.<sup>58, 81</sup> This finding is in stark contrast to the progressive clonal dominance of events such as *BCR-ABL1* translocation that are ultimately leukemogenic. In the periphery, the accumulation of lesional histiocytes is clearly driven by ERK activation. Mouse models suggest that retention in the tissues and inhibition of apoptosis correlate with accumulation of histiocytes, in keeping with the typically low proliferation rate observed in clinical lesions.<sup>7, 73</sup> Multisystem LCH and ECD are associated with the presence of somatic mutation in monocytes, myeloid dendritic cells and their precursors. Systemic involvement and the propensity to recur suggest that lesional histiocytes arise from self-renewing progenitor or stem cells in the bone marrow in these patients. By contrast, the mutation is not usually detected in the peripheral blood or bone marrow of patients with single system disease or isolated lesions.<sup>63</sup> Spontaneous healing

and complete responses to minimally cytoreductive therapy in these individuals, suggest the the mutation must arise in a non-self-renewing cell, such as a committed progenitor that seeds the periphery, producing multiple lesions or a large rapidly growing solitary mass, but the disease never becomes systemic or recurring. The potential clinical utility of peripheral blood mutation detection as an indicator of disease risk has been highlighted in several retrospective surveys.<sup>81</sup> Integration with clinical risk factors in prospective studies is in progress.

**Response to inhibitors**—Much remains unknown about the consequences of ERK activation in haematopoietic progenitors. The lack of any clonal advantage is corroborated by the failure of RAF and MEK inhibitors to eradicate the neoplastic clone, despite inducing remarkable clinical improvement in almost all patients.<sup>68, 69, 189</sup> Although inhibitor therapy can be dialled down to a low level and has been life-saving for an increasing number of infants and adults with refractory disease, the rapid relapse that occurs with treatment cessation is currently a major clinical issue. It is uncertain how long children or younger adults will tolerate inhibitor therapy, and strategies to eliminate the ‘histiocytic stem cell’ are urgently needed. Suggestions range from full intensity allograft to maintenance style cytotoxic therapy with methotrexate and 6-mercaptopurine or hydroxyurea.

**The argument for a pre-natal origin**—During normal development, histiocytes arise early in pre-natal life, and many cell populations, notably microglia of the brain, are capable of self-renewal and do not require continual replenishment by hematopoiesis. It is conceivable that somatic MAPK activation could arise in a mosaic fashion during development, leading to variable clinical syndromes, depending on the site and degree of mosaicism. This mechanism was proposed for self-healing congenital histiocytosis involving neonatal Langerhans cells (Hashimoto-Pritzker disease ).<sup>9, 129</sup> A more sinister connection with neurodegenerative disease has recently been suggested by mouse models showing that microglia harbouring activating BRAF mutation cause profound neurological deficits.<sup>78</sup> The hypothesis of congenital mosaicism has a number of attractive features, such as the ability to explain localised cephalic disease that crosses tissue boundaries (for example, the skin–bone–pituitary gland lesions characteristic of the LCH variant Hand-Schuller-Christian disease) and the well-documented association between cranio-facial lesions, posterior pituitary gland failure and neurodegeneration. However, such a mechanism does not easily account for the typically late presentation of LCH-ND, which is associated with circulating BRAF-V600E<sup>+</sup> monocytes and a perivascular distribution of similar cells in areas of early, active injury of the brain, with scant evidence of microglial activation.<sup>12</sup> Although a second trigger could be invoked to explain the protracted kinetics of LCH-ND following systemic symptoms, a post-mortem study argued that persistence of mutant cells in the peripheral blood combined with pathological evidence of an infiltrative process strongly favors a hematogenous mechanism of neuro-degenerative LCH.<sup>12</sup> It should be noted that neither involvement of the haematopoietic stem cell per se nor clinical responses of neurodegeneration to inhibitor therapy exclude the possibility that congenital mosaicism is involved in pathogenesis in some way. The concurrence of BRAF mutation in histiocytosis and a second malignancy may provide a potential test case if both lineages can be traced to a common ancestral fetal cell.

**Germline genetics and second hits**—In both neoplastic histiocytoses and HLH, there is a complex interplay between constitutional germline genetics, somatic mutations and environmental second hits that will be fertile ground for future exploration and clinical translation. LCH and possibly ECD have ethnic proclivities that may be linked to loci that modify myeloid cell development or the inflammatory response. The discovery of somatic mutations in these disorders does not exclude a role for environmental triggers such as trauma, infection and other inflammatory insults. Smoking and alpha-1 anti-trypsin deficiency both predispose lungs to develop LCH that appears genetically indistinct from other types of LCH. Cutaneous LCH also frequently involves areas subject to trauma such as skin folds. As an example hypothesis, modulation of the threshold of TLR signalling by ERK activation may be a key mechanism linking sensing of the environment to pathogenesis,<sup>265</sup> but the molecular details of this interaction and potential to intervene remain untested.

In HLH, the meaning of primary and secondary are being eroded and replaced by the concept that germline predisposition, immunosuppression, inflammatory triggers and somatic mutations may all lower the threshold of hyperinflammation to a critical level. The feed-forward signals that drive this process are increasingly well understood and are likely to be targetable. A humanised antibody against the archetypal macrophage activator INF $\gamma$  recently became the first licensed drug for this indication. Anti-IL-1, anti-IL-6 and anti-IL-18 strategies including small molecule JAK inhibitors have also been empirically tested with the promise of widening the range of exit strategies for refractory disease, beyond the traditional route of allogeneic haematopoietic stem cell transplantation.

**Classification**—The histiocytoses reviewed here represent a collection of disorders that share a terminal phenotype with features shared with cells of the mononuclear phagocyte system. Advances in genomics and ontogeny over the past decade frame a model of aberrant differentiation of myeloid precursors for LCH, ECD, JXG and some cases of RDD. Debate continues if these disorders are cancer. Clearly they represent clonal disorders associated with oncogene drivers, but designation may depend on one's definition of cancer. We believe *inflammatory myeloid neoplastic disorder* appropriately characterizes this group. HLH itself represents a spectrum of disorders that share common endpoint of pathological inflammation that results from some combination of inherited disorders of immune function along with inflammatory trigger(s). The current front-line standard of care for high-risk LCH still cures <50% of patients.<sup>9</sup> Outcomes for children and adults with histiocytic disorders may be improved by treating these conditions like cancer through cooperative trials with cancer consortia networks and resources. Organized prospective trials may further inform development of precision approaches that target specific mutations and specific cells in patients with particular genetic backgrounds

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Box 1****Classification of histiocytoses****C Group**

- Cutaneous non-LCH
  - Non-XG family: includes cutaneous RDD
  - XG family: includes JXG
- Cutaneous non-LCH with major systemic component

**H Group**

- Primary HLH (monogenic inherited conditions; FIG. 1)
- Secondary HLH (non-Mendelian HLH)
- HLH of uncertain origin

**L Group**

- LCH
- ICH
- ECD
- Mixed LCH/ECD

**M Group<sup>a</sup>**

- Primary malignant histiocytoses
- Secondary malignant histiocytosis (following or in association with another haematological malignancy)

**R Group**

- Sporadic RDD
  - Classic RDD
  - Extranodal RDD
  - RDD with neoplasia or immune disease
  - Unclassified
- Familial RDD

Somatic and germline pathogenic variants associated with Histiocytic disorders are outlined in FIG. 1. ECD, Erdheim-Chester disease; HLH, haemophagocytic lymphohistiocytosis; ICH, indeterminate cell histiocytosis; JXG, juvenile xanthogranuloma; LCH, Langerhanscell histiocytosis; RDD, Rosai-Dorfman disease; XG, xanthogranuloma.

<sup>a</sup>Malignant histiocytoses are not discussed in this review, but represent hyperproliferative, dysplastic malignancies with some histological features shared with histiocytic disorders.

**Box 2 |****Inherited disorders associated with HLH<sup>268</sup>****Familial HLH**

- Mechanisms of disease: defective granule-mediated cytotoxicity
- Mutated genes
  - *PRF1* (FHL2)
  - *UNC13D* (FHL3)
  - *STX11* (FHL4)
  - *STXBP2* (FHL5)

**Pigmentary disorders associated with HLH**

- Mechanisms of disease: defective granule-mediated cytotoxicity
- Mutated genes
  - *RA827A* (Griscelli syndrome type 2)
  - *LYST* (Chediak-Higashi syndrome)
  - *AP3B1* (Hermansky-Pudlak syndrome 2)

**X-linked lymphoproliferative diseases**

- Mutated genes; mechanisms of disease
  - *SH2D1A* (XLP1); defective 2B4-mediated cytotoxicity, defective Tcell restimulation-induced cell death and absent iNKT cells
  - *XIAP* (XLP2); dysregulated NLRP3 inflammasome and increased effector cell susceptibility to cell death

**Other diseases**

- Mutated genes; mechanisms of disease
  - *NLRC4*; constitutively active NLRC4 inflammasome
  - *CDC42*; defective formation of actin-based structures; defective proliferation, migration and cytotoxicity; and increased IL-1 $\beta$  and 11–18 production

**EBV susceptibility disorders**

- Mutated genes; mechanisms of disease
  - *MAGT1*; defective Mg<sup>2+</sup> transporter, low NKG2D, and defective cytotoxicity

- *ITK*; defective tyrosine kinase function, defective cytotoxic T cell expansion and cytolytic capacity, and decreased iNKT cells
- *CD27*; *CD27* is expressed on T cells, participates in co-stimulatory signalling, interacts with *CD70* and is required for normal T cell proliferation and triggering of cytotoxicity against EBV-infected B cells; decreased iNKT cells
- *CD70*; *CD70* is expressed by EBV-infected B cells, interacts with *CD27* on T cells and is required for normal expansion and cytotoxicity of the T cells; decreased *NKG2D* and *2B4*; decreased iNKT cells
- *CTPS1*; CTP synthase 1 is involved in de novo synthesis of the CTP nucleotide (critical precursor of nucleic acid metabolism) and its deficiency leads to impaired proliferation; decreased iNKT cells
- *RASGRP1*; RASguanyl-releasing protein 1 activates RAS, which leads to MAP kinase pathway activation; defects in T cell activation, proliferation and migration; decreased cytotoxicity; decreased iNKT cells

CTP, cytidine triphosphate; EBV, Epstein-Barr virus; FHL, familial haemophagocytic lymphohistiocytosis; HLH, haemophagocytic lymphohistiocytosis; iNKT cells, invariant natural killer T cells.

**Box 3 |****Clinical criteria to diagnose HLH**

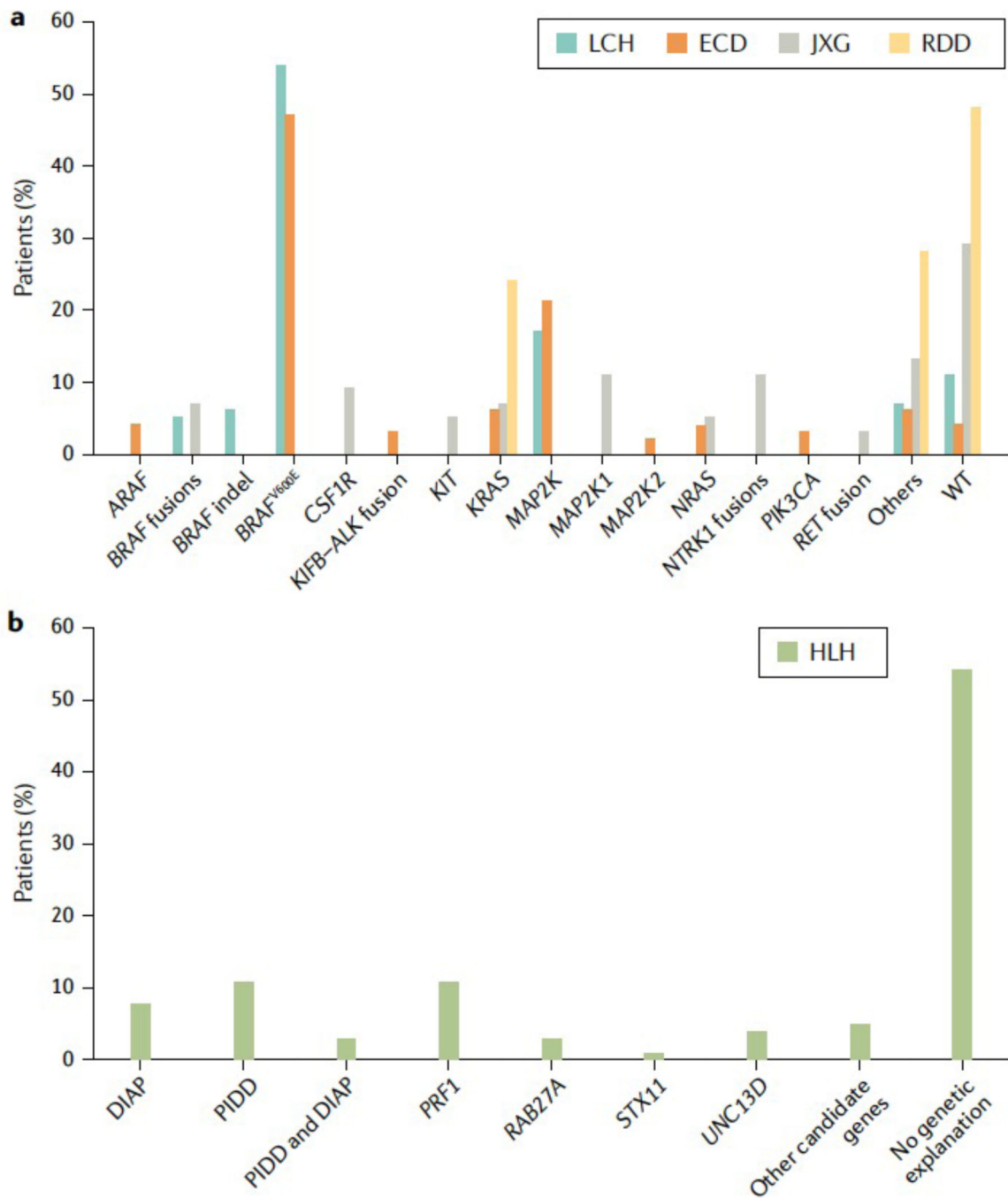
The Histiocyte Society HLH-2004 treatment trial established clinical criteria for haemophagocytic lymphohistiocytosis (HLH) for the purposes of the trial. A genetic HLH disorder can be diagnosed based on the finding of a pathogenic variant (or variants) in a known causative gene. A clinical syndrome of HLH can be diagnosed if at least five out of eight clinical and laboratory criteria are present. These criteria are as follows.

- Fever
- Splenomegaly
- Cytopenia in at least two of the following cell lineages: haemoglobin <9 g/dl (<10 g/dl in neonates), platelets (count <100 × 10<sup>9</sup>/ml) and neutrophils (count <1 × 10<sup>7</sup>/ml)
- Hypertriglyceridaemia (>265 mg/dl) or hypofibrinogenaemia (<150 mg/dl)
- Hyperferritinaemia (>500ng/ml)
- Soluble CD25 >2,400 U/ml (or elevated compared with laboratory-defined normal ranges)
- Haemophagocytosis in bone marrow, spleen, lymph nodes or liver, as observed in histological sections
- Low or absent natural killer cell cytotoxicity, as measured by a chromium release assay

Of note, a genetic defect alone does not imply that a patient has an active HLH process, only that the patient has an underlying genetic predisposition to the development of the syndrome of HLH. Diagnosis of active HLH in patients with a genetic predisposition to HLH should still be made based on the presence of signs and symptoms of hyperinflammation.

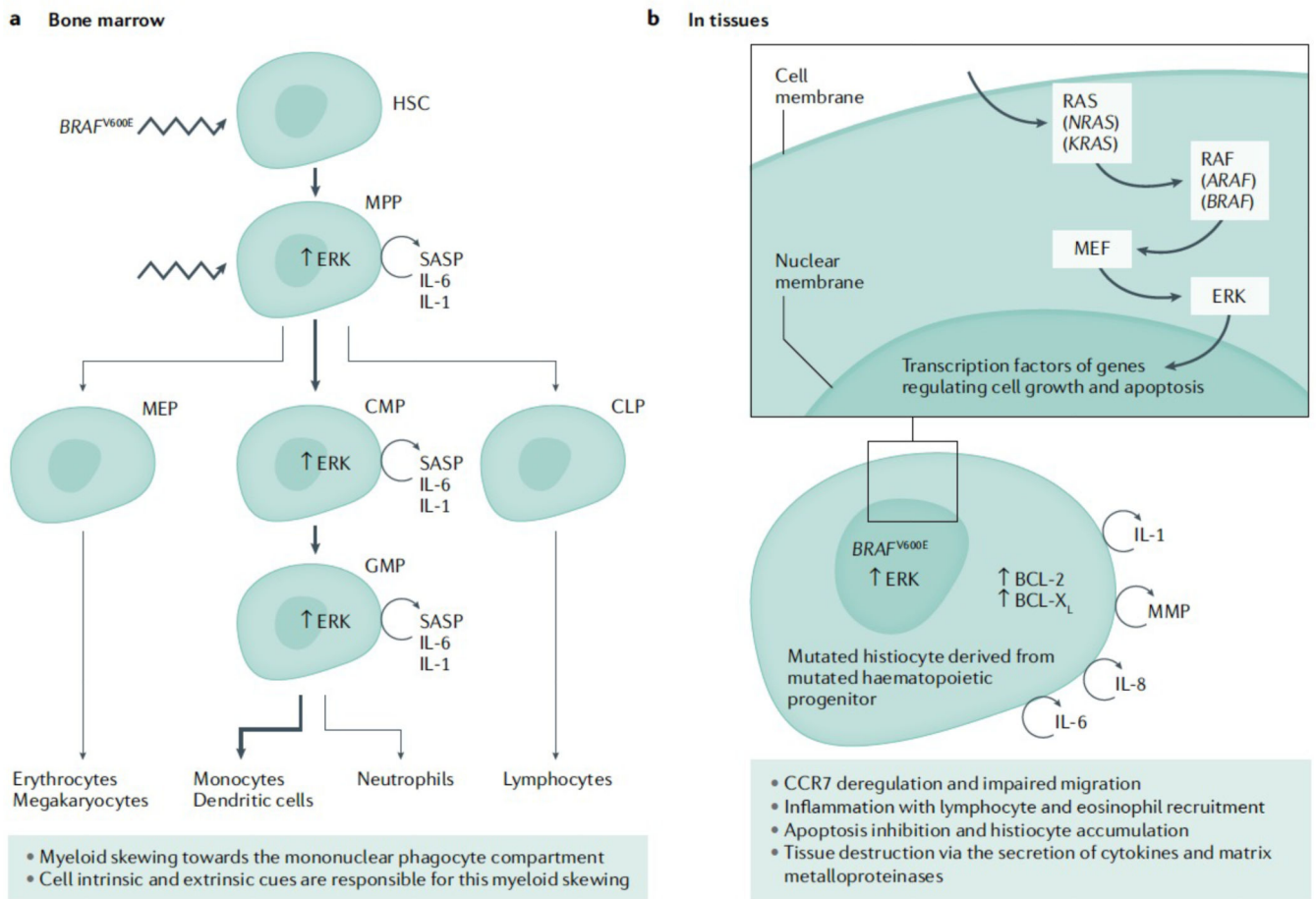
**Box 4 |****A patient's story**

Our daughter was diagnosed in October 2013. The journey for her and our family has taken many turns. Before diagnosis, we had chased Langerhans cell histiocytosis symptoms that mimic other common childhood diseases for over a year. The official diagnosis was in some way a relief and terrifying at the same time. We finally had a better understanding of how to help her get better. Unfortunately, the lack of treatment for such a long time meant that her condition had progressed to include bone lesions throughout her body, diabetes insipidus, and involvement in her spleen. We agreed to participate in a clinical trial for her treatment. The IV chemotherapy treatment and adverse effects were difficult. It was also a struggle to handle caring for a small toddler who was frequently hospitalized, another young child who was rarely permitted into the hospital, and normal work and life responsibilities. The treatment worked and her LCH was inactive until February of 2015. She was started on a stronger IV chemotherapy for 6 months. She handled this treatment better overall, and rang the 'cured' bell in September of 2015. We were overjoyed! Unfortunately, she relapsed four more times, with disease ranging from skin lesions to bone lesions, to CNS involvement. Each time we worked with her doctors to find the best treatment with the least amount of adverse effects. Her treatments have included IV chemotherapy, oral chemotherapy, BRAF inhibitors, and combinations of these different treatments. Emotionally and physically it has been draining for her and the entire family. She's now ten and has a better understanding of the implications of another relapse. She's more willing to share if she has any unexplained pain. The entire family becomes anxious before scans and follow-up appointments. But we trust her team and are excited at all the breakthroughs in the research and treatments that happened in the past eight years.



**Figure 1. Mutations found in histiocytic disorders**

Bar graphs showing the genes and mutations that have been associated with LCH, ECD, JXG, and RDD (panel A) and HLH (panel B) and the percentage of patients carrying these variants. DIAP: Dysregulated Immune Activation and Proliferation-Associated Genes, PIDD: primary immune deficiency disease; WT: wild type (no mutations found) Data from Durham<sup>81</sup> and Chinn<sup>45</sup>

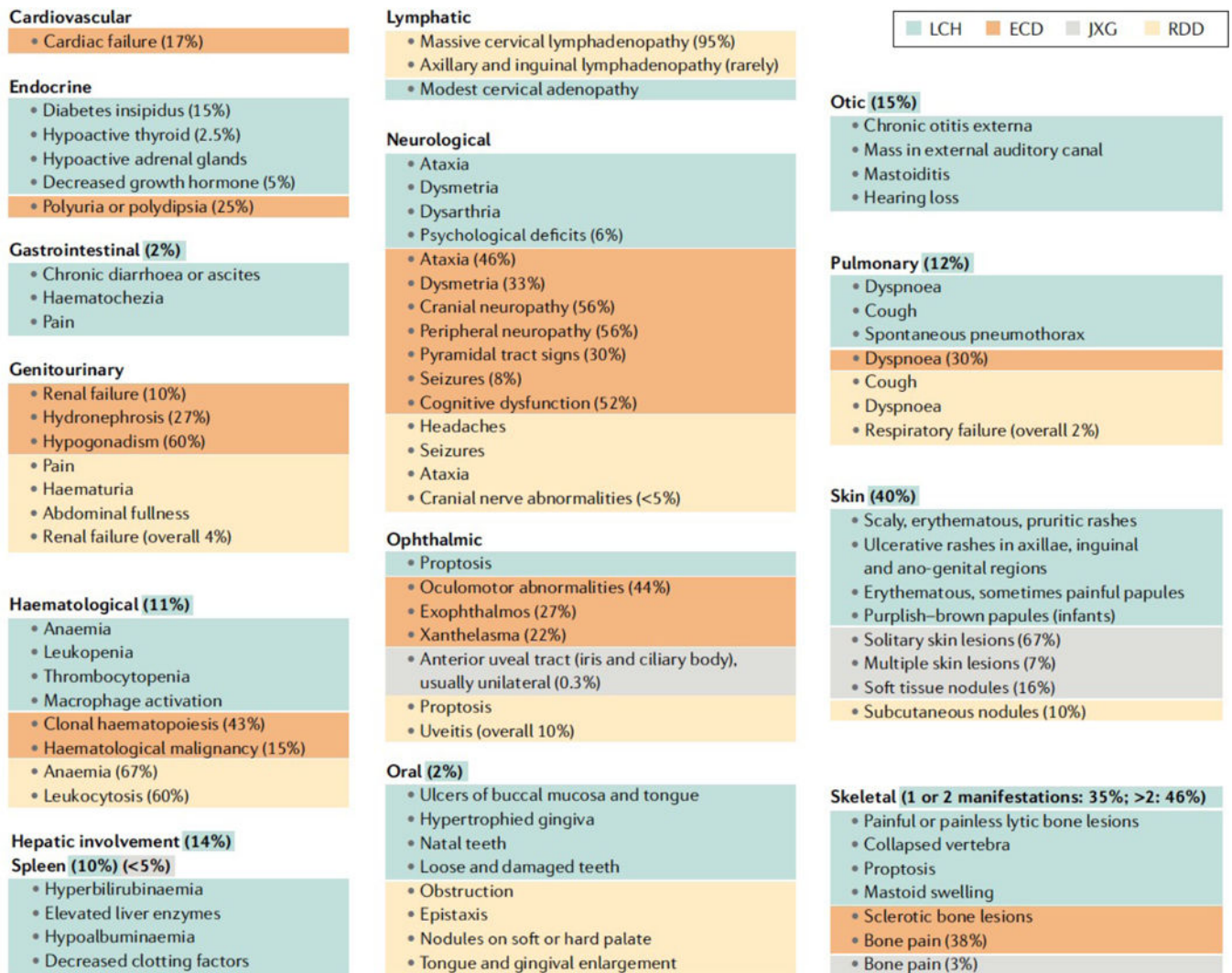


**Figure 2. Physiopathology of Langerhans cell histiocytosis**

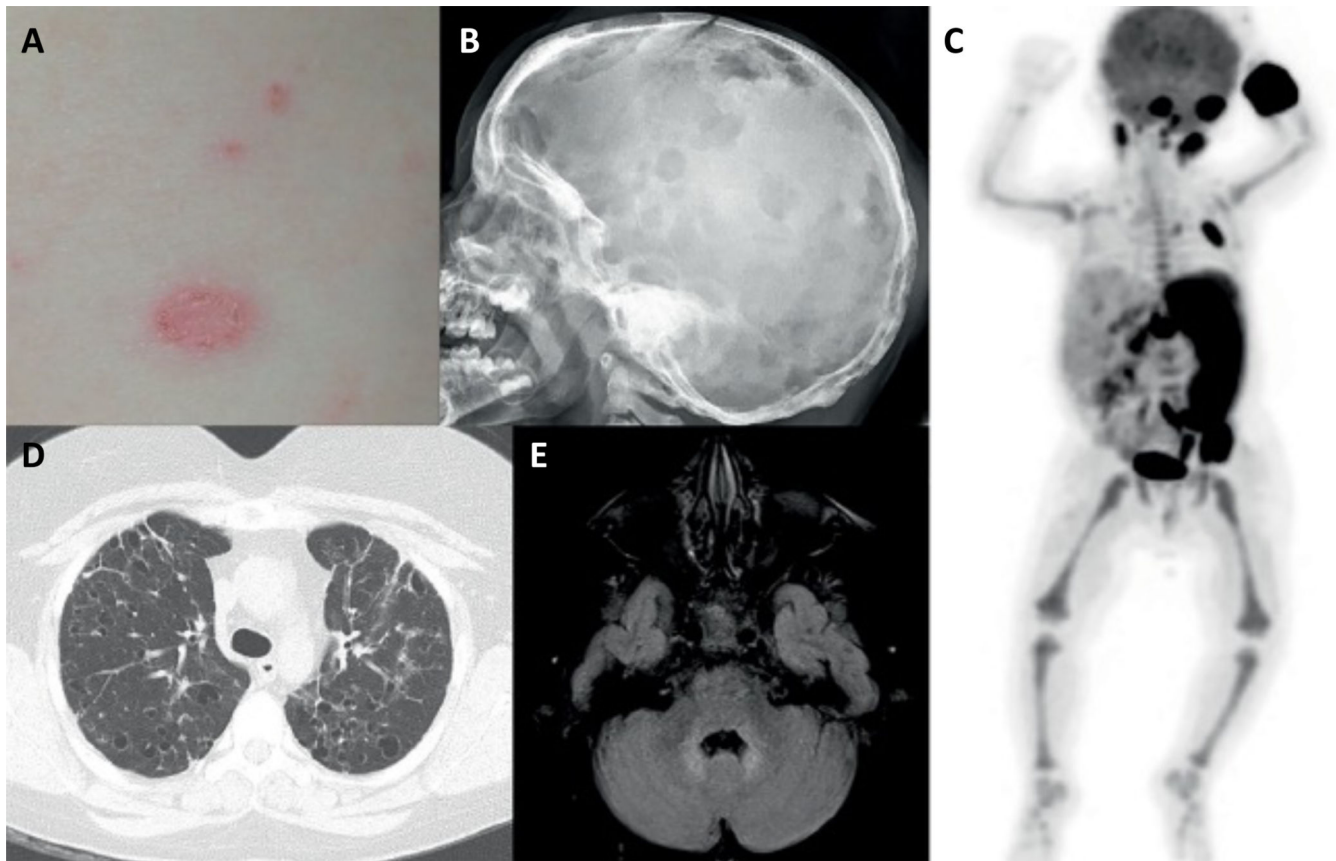
(A): The  $BRAF^{V600E}$  mutation is detected in about 50% of LCH lesions. Interestingly, this mutation is also detectable in pluripotent  $CD34^+$  hematopoietic progenitor cells (HPC) in the bone marrow and most particularly in CMP and GMP that are the bone marrow precursors of dendritic cells and monocytes. In the blood from LCH patients, the mutation can be mostly detectable in monocytes and dendritic cells but not in lymphocytes and neutrophils. This myeloid skewing has been further explored using mouse models. The  $BRAF^{V600E}$  mutation was recently expressed in murine haematopoietic stem cells. Strikingly, the mice developed a LCH disease, suggesting that the expression of the  $BRAF^{V600E}$  mutation in multipotent HPC is sufficient to drive to LCH lesion formation. Moreover, the mutated HPC acquired a senescence programme, so called senescence associated secretory phenotype (SASP), that is characterized by cell cycle arrest and secretion of cytokines, in particular IL-1 and IL-6. These cytokines partially participated in an autocrine and paracrine fashion in the myeloid skewing of the HPC observed in LCH. Thus, the myeloid skewing observed in LCH is driven by extrinsic and intrinsic cues. (B): The senescence programme that is started in the bone marrow is conserved in tissues. Mutated mononuclear phagocytes do also secrete a lot of cytokines as part of the SASP. For example, IL-6, IL-8 and IL-1 participate in recruiting inflammatory cells to the tissue, leading to granuloma formation. Matrix metalloproteinases have a role in the tissue destruction observed in LCH lesions. Moreover, mutated

mononuclear phagocytes are trapped in the tissue because of CCR7 down-regulation that impairs their migration and because of BCL-2 and BCL-xL up-regulation that make them resistant to apoptosis. The MAPK pathway is an evolutionarily conserved signaling cascade that transmits signals from cell surface receptors that normally execute programs related to cell cycle progression, differentiation, protein translation and resistance to cell death. Mutations such as the *BRAFV600E* lead to constitutive activation of the pathway and uncontrolled cell growth as well as other effect mentioned above. HSC: hematopoietic stem cell; MPP, multipotent progenitors; Mep, megakaryocyte erythroid progenitors; Cmp, common myeloid progenitors; Gmp, granulocyte macrophage progenitors; Clp, common lymphoid progenitors. References:<sup>7, 61, 73</sup>

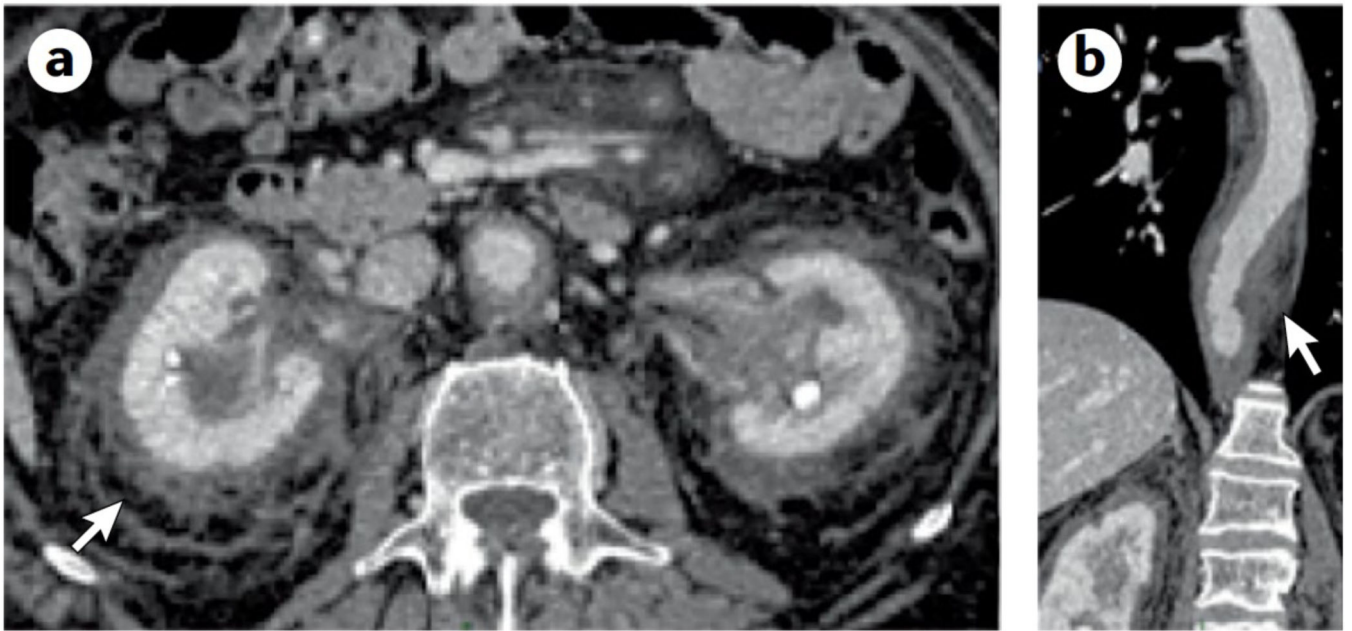




**Figure 3. Clinical presentations of LCH, ECD, JXG and RDD**  
 The organ systems known to be involved in histiocytic disorders. Percentages of patients reporting a specific manifestation are derived from large series studies for **LCH**:<sup>17</sup>; **ECD**: 28, 84, 151; **JXG**: 29, 30; **RDD** 33, 34

**Figure 4. Manifestations of LCH**

A. Erythematous, scaly LCH rash; B. Multiple lytic skull lesions (arrows) in a patient with LCH; C. PET scan showing involvement of skull base, occiput, left fifth rib, left iliac bone, and left hemisacrum. Although the intense uptake in long bones is physiological, the scan shows hypermetabolic splenomegaly and bilateral cervical/upper abdominal lymphadenopathy.; D Pulmonary cysts and nodules (arrows) from LCH; E. Brain MRI with intense T2 signal in the dentate nuclei characteristic of neurodegenerative LCH (arrow).



**Figure 5. ECD clinical presentations.**

A. The classical ECD finding of “hairy kidney” (a ring of ECD tissue around the kidneys seen on CT scan of the abdomen with contrast; arrow). B. Typical sheathing of the aorta (arrow) illustrated on this contrast-enhanced CT scan of the chest.

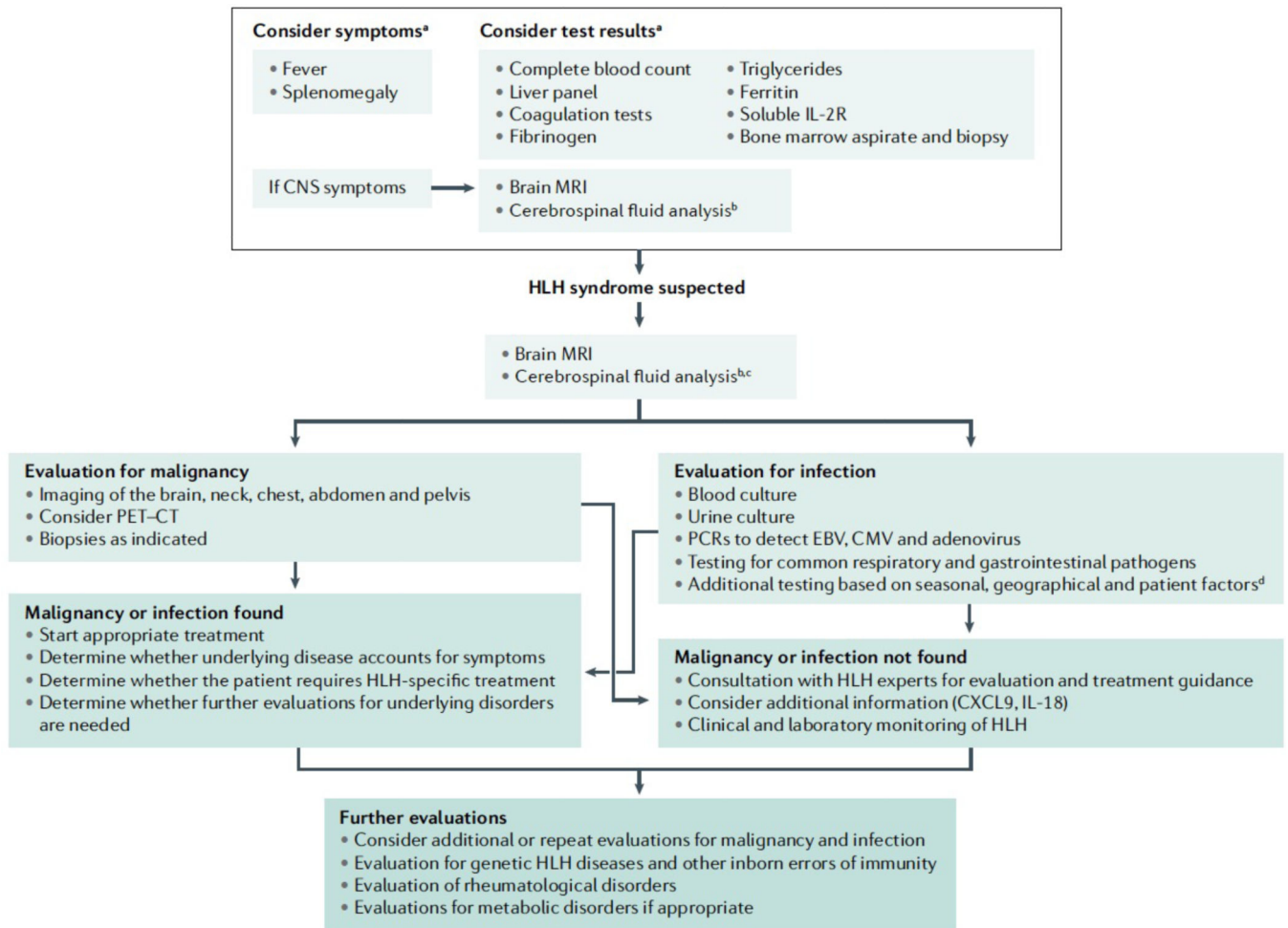


**Figure 6. JXG Rash**

The rashes of JXG patients are typically yellowish, orange, or purplish papules, which can appear anywhere on the body. Most patients have fewer than a dozen scattered lesions, but some can have many more, like this patient. JXG involves the visceral organs (lung, liver, kidney, spleen, brain, pancreas, adrenal glands, or intestines) in <5% of patients and causes bone lesions in <3%.

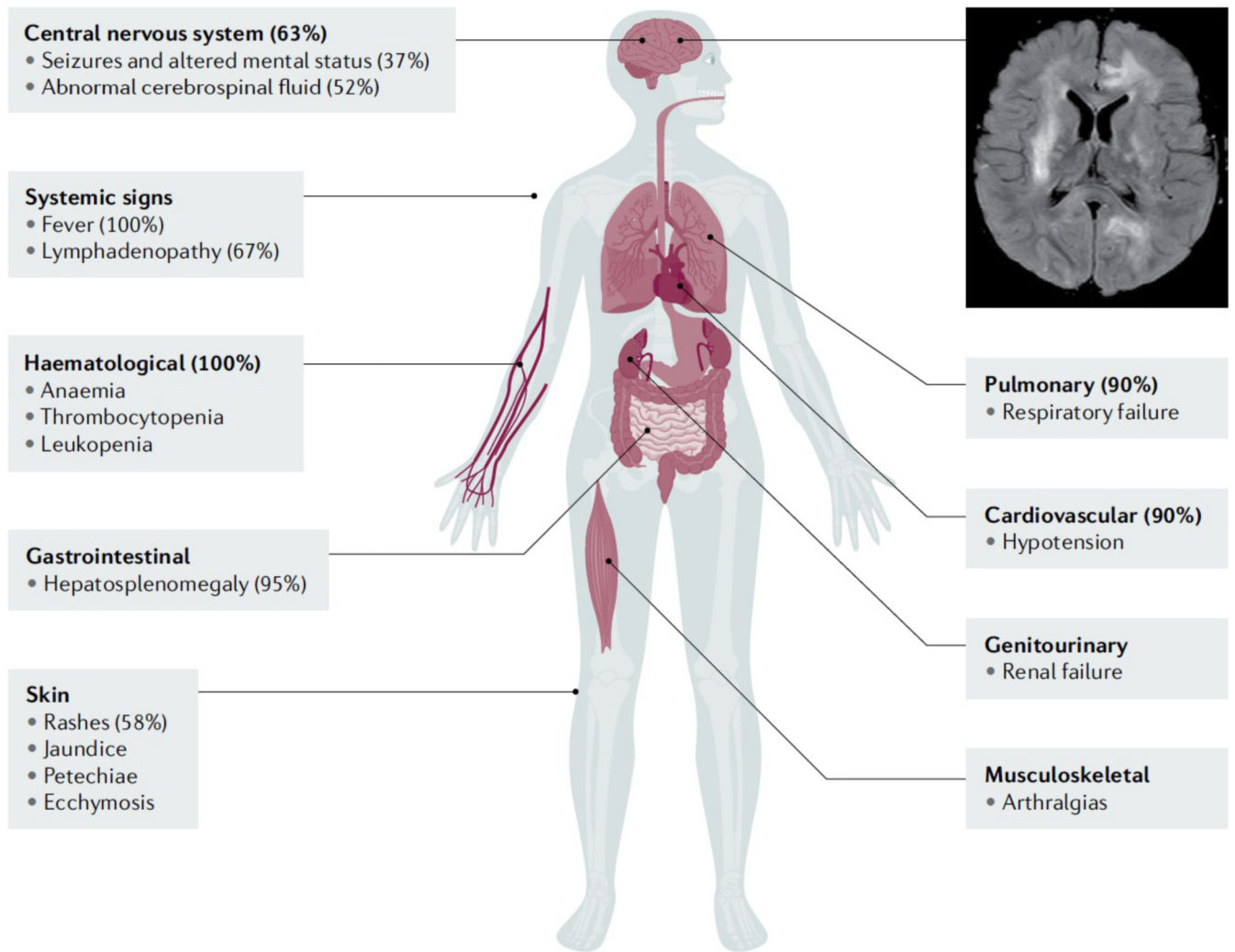


**Figure 7. RDD clinical Presentation**  
Massive cervical adenopathy



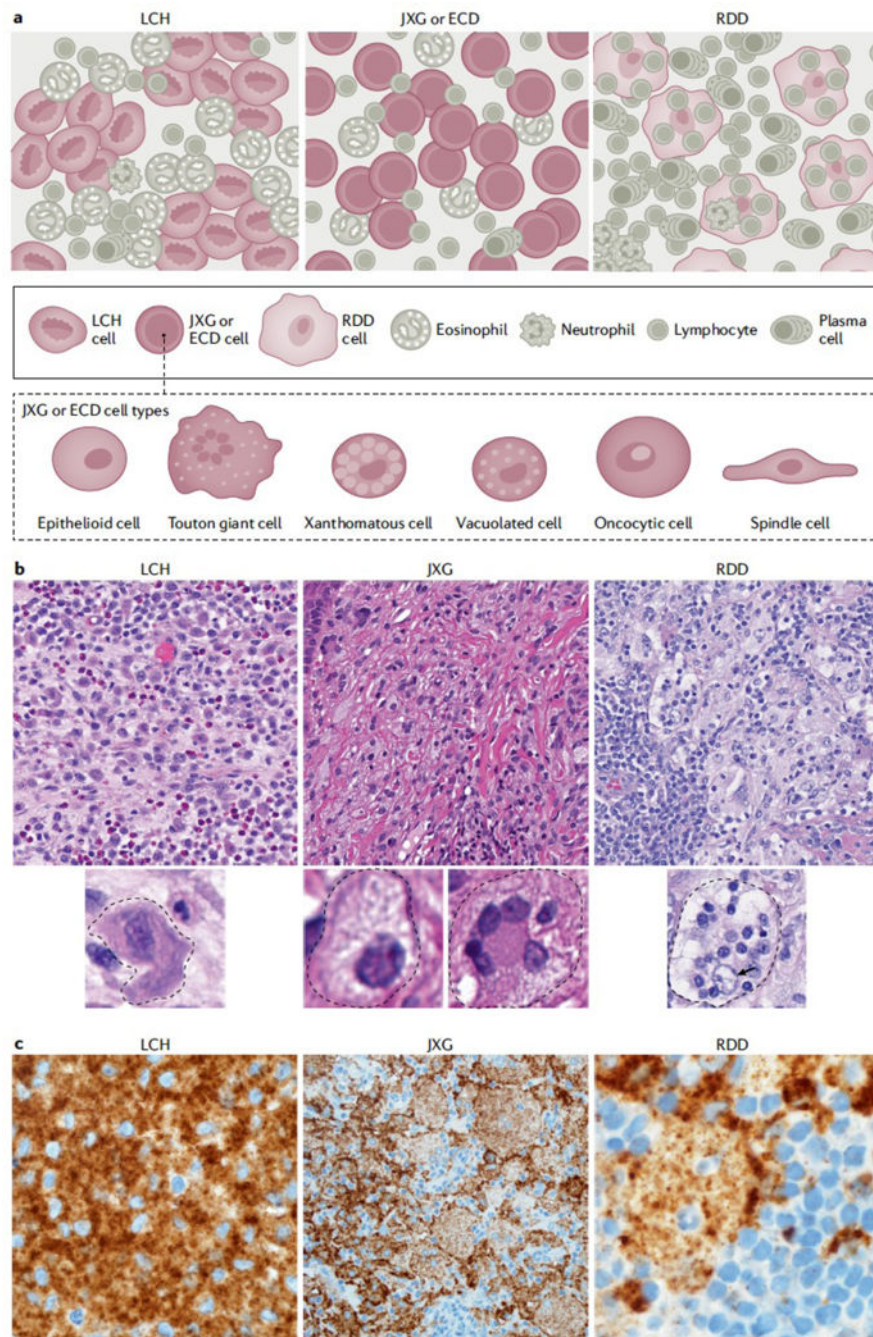
**Figure 8. Diagnostic algorithm for HLH.**

Flow diagram of clinical decision making when confronted with a patient who has signs and symptoms of HLH. <sup>a</sup>Some patients with genetic predisposition to HLH may develop atypical disease presentations; <sup>b</sup>If safe to obtain; <sup>c</sup>If not previously performed; <sup>d</sup>For example, consider HSV, VZV, HHV6, HIV, Parvovirus, leishmaniasis, tick-borne illnesses, mosquito-borne illnesses, histoplasmosis and TB, amongst others.



**Figure 9. HLH clinical presentations**

The organ systems known to be involved in HLH, with percentages of patients reporting a specific manifestation. The image shows brain MRI of an HLH patient with CNS involvement, with T2 signal hyperintensity and micro-cystic encephalomalacia of the cerebral white matter and cerebral cortex.



**Figure 10. Histology characteristics of LCH, JXG and RDD**

A. Although Langerhans cell histiocytosis (LCH), histiocytoses of the xanthogranuloma family (including Erdheim Chester Disease (ECD) and juvenile xanthogranuloma (JXG)), and Rosai Dorfman Disease (RDD) have certain classic morphologies and defining phenotypes, our understanding of histiocytic lesions continues to evolve and may be best conceptualized along a spectrum, sometimes with morphology and phenotypes overlapping in certain instances and often with similar mutation profiles. In fact, ECD and JXG share similar morphological cell types under the microscope, including xanthomatous cells with



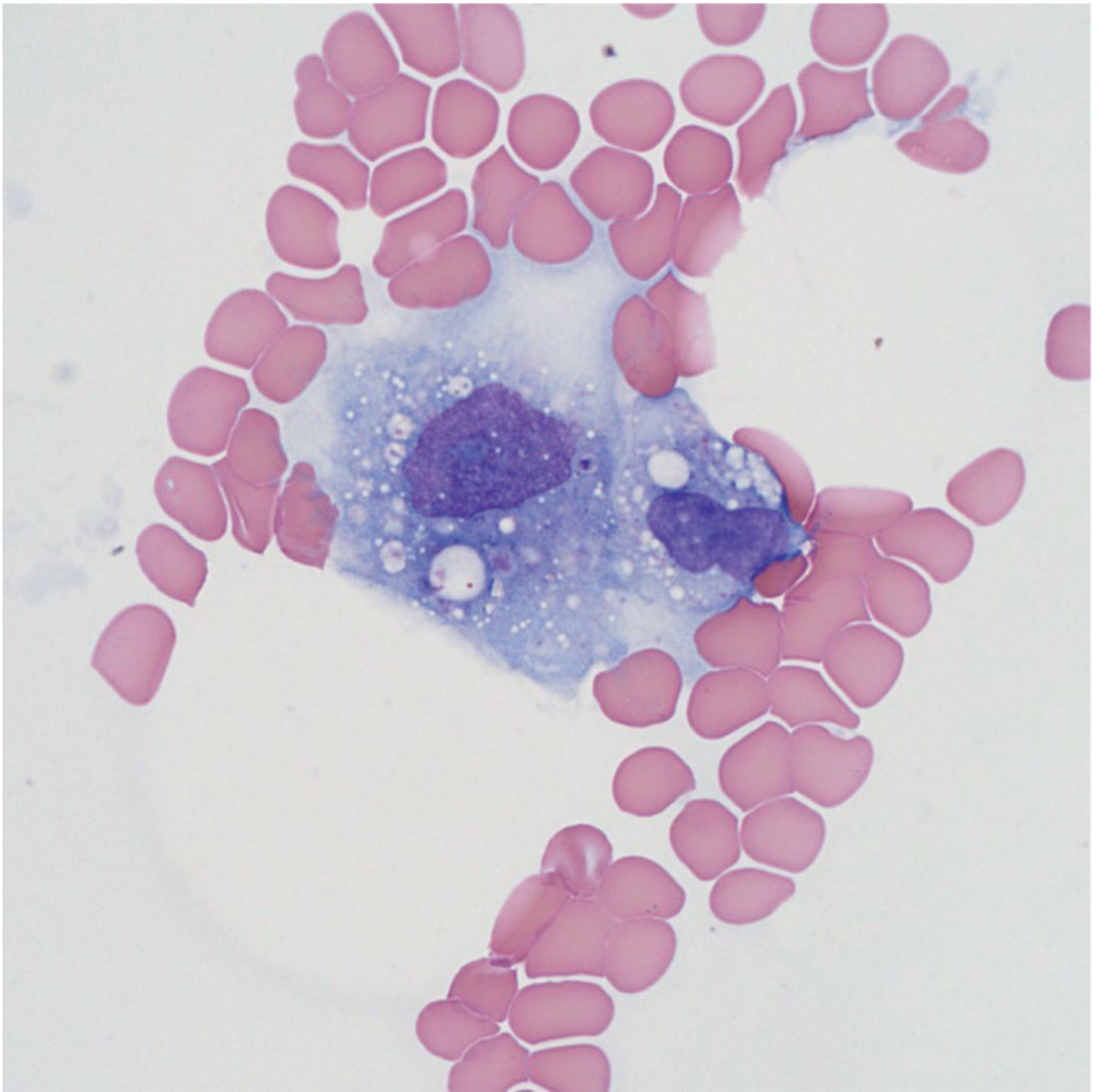
variable admixture of Touton giant cells, vacuolated cells, spindled cells, oncocytic cells, and epithelioid cells. B. H&E images for LCH, xanthogranuloma family, and RDD (original magnification at 400x). C Immunostaining specific for *BRAF*-V600E (known as BRAF-VE1, brown) Although the mutant specific immunohistochemistry for BRAF-VE1 is more commonly expressed in LCH and ECD, with strong granular cytoplasmic staining of the histiocytes, rare examples in pediatric JXG family (limited to the central nervous system of children) and isolated case reports of RDD have been identified to also harbour the mutation and express the mutant protein, which reacts to the immunostaining. Original magnification at 1000x for LCH, 400x for CNS-JXG, and 1000x for RDD. Photos made with assistance of Mr Chris Woods Cincinnati Children's Department of Pathology.

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**Figure 11. Histology of HLH**  
Bone marrow aspirate shows a large activated histiocyte with cytoplasmic phagocytosis.  
(Wright-Giemsa stain, original magnification 1000x).

**Table 1 |**

Treatments for progressive paediatric Langerhans cell histiocytosis

Regimen	Number of patients	Risk group	Outcomes
Vbl/Pred/Etop/Mtx/6-MP266	14	High risk	85% PFS
	121	Low risk	76% PFS
Cladribine198	46	High risk	48% OS
	37	Low risk	97% OS
Vcr/A RA-C/Pred267	10	Low risk	50% PFS
Clofarabine229	4	High risk	33% PFS
	2	Low risk	50% PFS
Clofarabine201	3	High risk	33% PFS
	8	Low risk	100% PFS
Cladribine/cytarabine209	27	High risk	78% PFS
Thalidomide195	6	High risk	0% PFS
	6	Low risk	50% PFS
Vemurafenib/dabrafenib68	13	Neurodegenerative disease	33% PFS
	7	High risk	
	1	Low risk	
Vemurafenib69	44	High risk	5% PFS <sup>a</sup>
	10	Low risk	60% PFS

6-MP, 6-mercaptopurine; ARA-C, cytarabine; CR, complete response; Etop, etoposide; Mtx, methotrexate; OS, overall survival; PFS, progression-free survival; Pred, prednisone; Vbl, vinblastine; Ver, vincristine.

<sup>a</sup>PFS is after stopping vemurafenib. 86% complete response after 6 weeks of treatment.