Histiocyte Society blueprint for Langerhans cell histiocytosis research: from cell-of-origin to a more comprehensive cure

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Abstract

Langerhans cell histiocytosis (LCH) is a rare myeloid neoplastic disorder driven by tissue-accumulating histiocytes expressing somatic mutations mostly in genes encoding components of the intracellular mitogen-activated protein kinase (MAPK) pathway. Its clinical presentation is highly variable, ranging from self-limiting unifocal lesions to severe, multisystem disease. As a result of new therapeutic strategies, the outcomes of patients with LCH have improved significantly during the past decade. Although mortality risk is minimal nowadays, a substantial proportion of patients experience significant morbidity due to (recurrent) disease reactivations or the development of (late) complications such as organ dysfunction – including neurodegeneration – or second hematologic cancers. To date, there are no prognostic tools that adequately predict who is at risk of developing such late sequelae. Given that all major clinical advances in the field of LCH have been driven by pivotal discoveries regarding its pathophysiology, we advocate that a deeper understanding of the cell-of-origin that gives rise to pathology-inducing cells found in the patients' blood and tissues before treatment initiation and occasionally even after treatment completion is an important missing piece of evidence with respect to understanding the pathophysiology of LCH and its late effects. Emerging techniques, such as longitudinal monitoring of driver mutations in the blood, now offer the potential to unveil the biological dynamics of the disease over its natural history. It is hoped that the much needed information, obtainable only through international collaboration and sustained long-term research projects, will bridge current gaps in clinical decision-making with the ultimate goal of improving outcomes of LCH patients worldwide.

Introduction

Langerhans cell histiocytosis (LCH) is a rare clonal disorder characterized by the accumulation of pathological CD1a$^+$ and CD207$^+$ cells in one or more organ systems.[1] Once called histiocytosis X (X standing for "unknown"), it was renamed LCH after the discovery of langerin expression in biopsies from subjects with the disorder.[2,3] Langerin is a protein encoded by the *CD207* gene which is essential for forming Birbeck granules, the zipper-like organelles that are unique to normal Langerhans cells (LC). Transcriptomic profiling of LCH biopsies performed several decades later revealed, however, that pathological histiocytes accumulating in LCH lesions have a unique transcriptomic profile, which minimally overlaps transcriptomic activity of resident tissue LC.[4,5] Lesional CD1a$^+$/CD207$^+$ histiocytes often co-express myeloid lineage markers (CD33, CD14 and CD163) typically expressed by circulating monocytes and myeloid dendritic cells.[6] This fits with the reported potential of the latter cells to differentiate into CD1a$^+$/CD207$^+$ cells when exposed to certain growth factors.[4,7,8] These more recent findings suggest that monocytes and myeloid dendritic cells are

the immediate precursor cells of LC 'look-alike' histiocytes found in LCH lesions.

LCH lesions additionally comprise lymphocytes and other myeloid cells such as granulocytes, macrophages, and osteoclast-like multinucleated giant cells.[1,9] Collectively, these cells induce inflammation and structural damage to infiltrated organs.[10] Due to its clonal nature and expression of gain-of-function somatic mutations, LCH is best described as a clonal myeloid neoplastic disorder with inflammatory features. Accordingly, LCH is part of a combined group of histiocytic/dendritic cell-driven neoplastic disorders recognized by the International Consensus Classification and the World Health Organization classification of hematolymphoid tumors.[11,12] As our biological understanding of LCH has progressed over the past decades, so too has our ability to treat the disease. The Histiocyte Society has played a major role in several international clinical trials and death from LCH is now incredibly rare due to well-established frontline therapy. However, relapses and long-term morbidities, such as devastating neurodegeneration, remain clinical challenges.

The aim of this paper is to review the major clinical and pathobiological milestones in LCH reached in the last two decades. We also spotlight critical, unanswered questions that need to be addressed to further improve the clinical management and outcomes of patients with LCH.

Clinical aspects and pathognomonic features

The incidence of LCH is estimated at three to six cases per million children under 15 years old per year, with a median age at diagnosis of 3 years,[13,14] and one case per million adults over 15 years old.[15] Its clinical presentation varies widely as it potentially involves any organ system, with the bone, skin, and pituitary gland being most frequently affected (Figure 1).[10] The disease can manifest as a single-system disease, often only needing local treatment, as a multifocal disorder affecting one organ system, or as a multisystem disease presenting in two or more organ systems. Multisystem (MS)-LCH with liver, spleen, and bone marrow involvement, mostly seen in infants, is considered risk organ-positive (RO^+), with an increased probability of

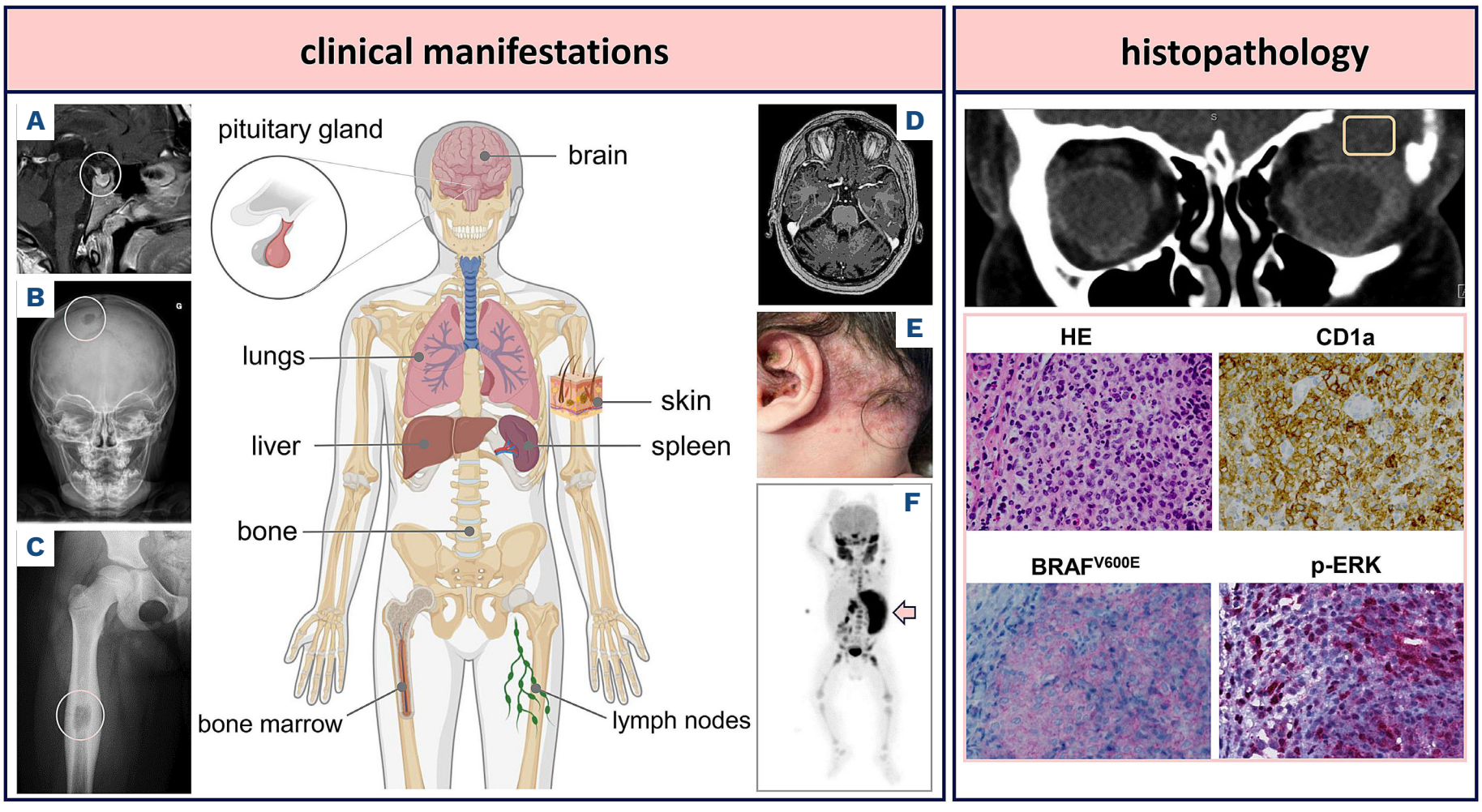


Figure 1. Clinical and histopathological characteristics of Langerhans cell histiocytosis. Left panel. Examples of manifestations of Langerhans cell histiocytosis (LCH). (A) Enlargement of the pituitary stalk in a patient with LCH. (B, C) Typical lytic LCH lesions in the skull (B) and femur (C). (D) Axial T1-magnetic resonance image of the brain of an adult with advanced neurodegenerative LCH showing cerebellar volume loss. (E) Cutaneous LCH presenting as a scalp rash. (F) Positron emission tomography-computed tomography scan of an infant with disseminated LCH with significant splenomegaly, among other manifestations, indicating splenic involvement. Right panel. Histological findings of an orbital LCH lesion (indicated by the light pink insert) showing large histiocytes with pale cytoplasm and reniform, often coffee bean-shaped nuclei intermixed with some eosinophilic granulocytes visualized by hematoxylin-eosin (HE) staining. The pathognomonic histiocytes in this orbital lesion stained positive for CD1a (brown staining), $BRAF^{V600E}$ protein (VE1), and phosphorylated-ERK (p-ERK); the latter two are stained in pink. Histological images are shown at 400x magnification.

treatment failure, reactivation, and death if untreated. LCH lesions in craniofacial bones are considered 'central nervous system (CNS)-risk lesions', as such lesions are associated with an increased probability of late CNS complications affecting the brain, including central diabetes insipidus (DI) and neurodegenerative (ND)-LCH.[16,17] Consequently, even solitary CNS-risk lesions are nowadays treated systemically to minimize the chance of late complications.

The majority of LCH patients present with single-system disease,[18] and among the remaining cases, 15% present with MS-RO$^+$ disease.[14] The initial clinical evaluation should include biopsies taken from affected tissues, total-body imaging for disease staging and laboratory tests to assess organ dysfunction. As LCH shares some histopathological and molecular features with other histiocytic disorders, such as indeterminate cell histiocytosis and Erdheim-Chester disease, experienced pathologists are required to aid in interpreting the diagnosis.[19]

Historically, LCH was considered a reactive inflammatory condition, but evidence of a clonal neoplastic origin has challenged this paradigm since the early 1990s.[20] The identification of a recurrent, ERK-activating driver mutation in exon 15 of *BRAF* (*BRAF*^{V600E}) in over 50% of LCH lesions marked a transformative era in understanding LCH pathophysiology and its clinical management (Figure 2A).[21] Additional mutations have since been identified in *BRAF*^{V600E}-negative, phosphoERK$^+$ LCH lesions. The majority of these molecular alterations also affect cytoplasmic mitogen-activating protein kinase (MAPK) pathway signaling and include point mutations, deletions, or indels in *KRAS, NRAS, ARAF, BRAF* (exon 12), and *MAP2K1*, and fusions involving *BRAF*.[18,22] Rare mutations in *ERBB3, CSF1R, MAP3K1*, and *PIK3CA* have also been reported.[19] Despite advances in unraveling the molecular landscape of LCH, its pathogenesis still remains poorly understood, with the most debated issue being its cell-of-origin.

Biological achievements

Disease pathogenesis

Oncogenic driver mutations in genes such as *BRAF* underscore the neoplastic nature of LCH, and this discovery opened new avenues for disease modeling. Various mouse

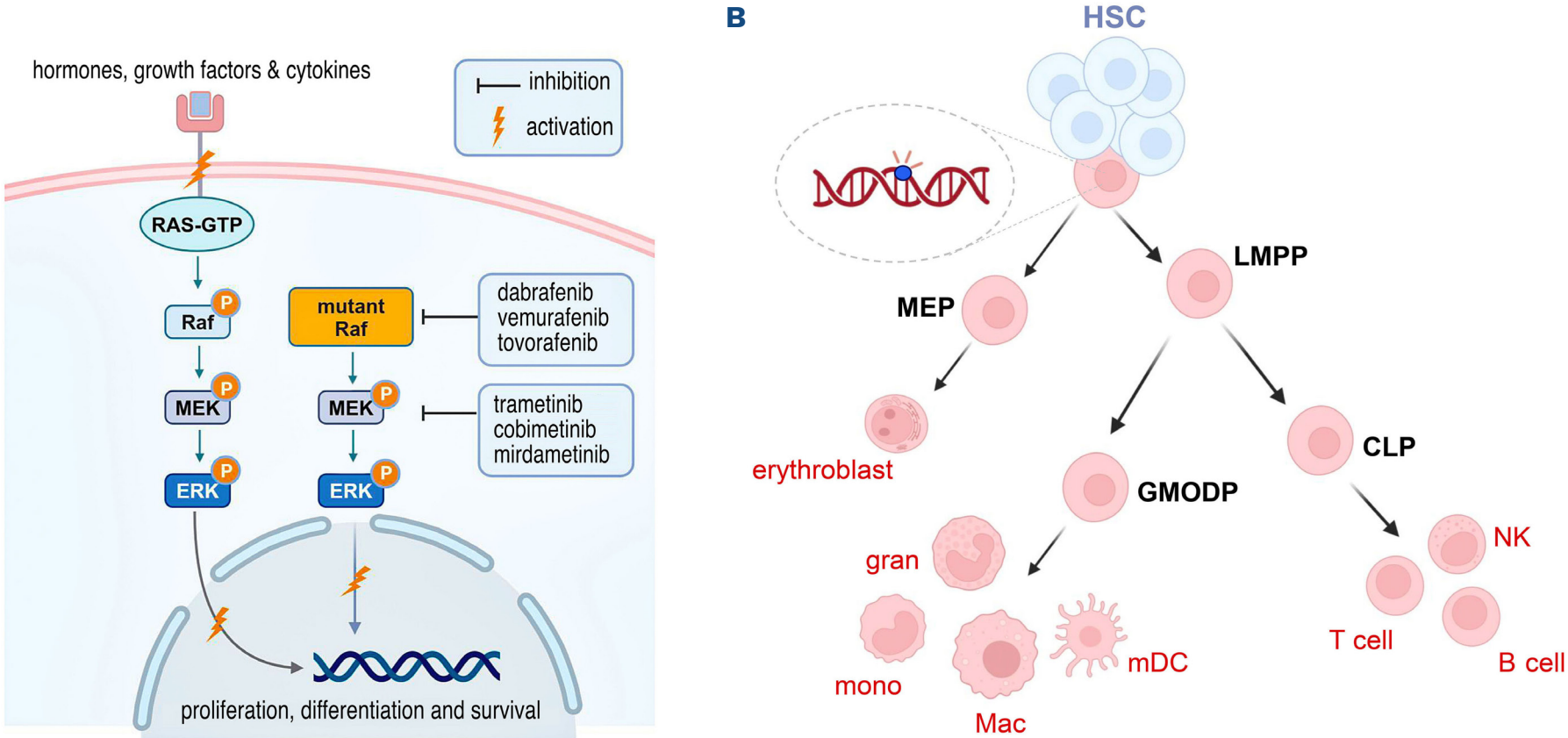


Figure 2. MAPK pathway signaling, driver mutation distribution among hematopoietic cells and the various drugs that can target mutated hematopoietic cells. (A) Cartoon depicting the main components of the mitogen-activated protein kinase (MAPK) pathway, its normal activation via cell surface receptors binding hormones, growth factors or cytokines, *versus* aberrant activation via mutated proteins such as BRAF^{V600E}. The drugs indicated on the right target a specific component of this pathway. Note that mutations in other Raf family members (a-Raf and c-Raf/RAF1) are found far more rarely than BRAF^{V600E} or other b-Raf mutations in Langerhans cell histiocytosis lesions. (B) Overview of hematopoietic progenitor-like multipotent hematopoietic stem cells – megakaryocyte-erythrocyte progenitors, lymphoid-primed multipotent progenitors, common lymphoid progenitors and myeloid committed progenitors with granulocyte, macrophage, osteoclast and dendritic cell differentiation potential – and lineage cells derived from these progenitor cells. Lineage cells depicted in red reportedly express mutated MAPK pathway-associated proteins. HSC: hematopoietic stem cell; MEP: megakaryocyte-erythrocyte progenitor; LMPP: lymphoid-primed multipotent progenitor; CLP: common lymphoid progenitor; GMODP: myeloid committed progenitor with granulocyte, macrophage, osteoclast and dendritic cell differentiation potential; gran: granulocyte; mono: monocyte; Mac: macrophage; mDC: myeloid dendritic cell; NK: natural killer cell.

models collectively make use of (inducible) transgenic expression of *BRAF*^{V600E} under the control of promotors active in distinct hematopoietic cell types. For example, expression of *BRAF*^{V600E} under the murine CD207 promoter led to mild inflammation restricted to the liver and lungs,[23] but mice expressing the same transgene under the human CD207 promotor did not display tissue inflammation, unless expression of *PTEN* was simultaneously disabled.[24] Restricted expression of *BRAF*^{V600E} in common dendritic cell progenitors and mature circulating CD11c^{+} myeloid cells induced inflammatory lesions in the liver, lungs, skin, and spleen.[25] In contrast, temporarily induced expression of *BRAF*^{V600E} in hematopoietic, not yet lineage-committed, stem/progenitor cells (HSC)[25] led to a selective expansion of transgene-expressing monocytes, macrophages, and dendritic cells. Tissue biopsies taken from the livers and lungs of these mice contained CD207^{+} histiocytes and multinucleated giant cells, reminiscent of biopsies taken from children with LCH.[26] These mice also developed organomegaly.[23] In line with observations made in murine LCH disease models, *BRAF*^{V600E} was detected in blood-derived CD11c^{+} myeloid cells such as myeloid dendritic cells[9,27,28] and CD14^{high} monocytes[8,9,29] as well as in LCH biopsy-derived myeloid cells collected from LCH patients.[5,9] Furthermore, transplantation of either human cord blood-derived CD34^{+} HSC[30] with enforced *BRAF*^{V600E} or *MAP2K1* deletion or T-cell-depleted bone marrow cells from a LCH patient with *BRAF*^{V600E} LCH[31] into immunocompromised mice resulted in bone marrow, spleen, liver and lung infiltration by mutated cells co-expressing CD1a and CD207. These studies provided the first evidence that pathological histiocytes in LCH are most likely part of the myeloid-skewed progeny generated by a CD34^{+} HSC clone expressing a MAPK pathway-activating mutation.[32]

Additionally, LCH patient-derived CD34^{+} cells were found to contain various types of *BRAF* mutated stem/progenitor cells, including myeloid lineage committed progenitor cells. These myeloid progenitors gave rise to different *BRAF*^{V600E}-expressing types of myeloid cells, including LC.[9] Targeted sequencing of hematopoietic cells isolated by fluorescence-activated cell sorting from blood and bone marrow of LCH patients revealed *BRAF*-mutant cells from both the myeloid and lymphoid lineage.[9,23,32] These studies highlight that murine LCH models recapitulate only some aspects of the disease in humans, as all published LCH models lack *BRAF*^{V600E}-expressing lymphoid cells found in the majority of children with disseminated LCH (Figure 2B).[9,23,28,33,34] Mutation burden seems highest among natural killer (NK) cells followed by B cells; mutant T cells are rarely found in patients with new-onset disease[35] (van Halteren *et al*. unpublished data). It is unknown whether cases of adult LCH display a comparable mutation distribution pattern as two of three published studies did not investigate *BRAF* mutation frequencies in circulating NK cells.[8,33,36] Furthermore, while osteolytic bone lesions are very often seen in LCH patients (Figure 1), such pathological lesions are not formed in either xenografted mice or in mice with (transiently) *BRAF*^{V600E}-expressing HSC.

BRAF^{V600E}-expressing histiocytes – both in the presence and absence of *RAS*-mutated cells – have also been found in pulmonary LCH lesions.[37] The cell-of-origin that gives rise to these cells remains elusive, as there are no publications to date reporting mutational loads in the blood of such patients. Data from a mouse model, in which *KRAS*^{G12D} is conditionally expressed in lung-resident myeloid cells,[38] provided evidence in favor of lung-resident myeloid precursor cells. Proliferation and differentiation of these cells into pathological CD207^{+} histiocytes is facilitated by *KRAS*^{G12D}-induced constitutive activation of the intracellular phosphatidylinositol 3-kinase (PI3K) signaling pathway. PI3K activation leads to increased levels of phosphorylated AKT which stimulates the formation of pathological myeloid cell clusters in the lungs of these mice. Intriguingly, a similar model with conditional expression of *BRAF*^{V600E} did not induce lung pathology. This finding could explain why *BRAF*^{V600E} was found to be more prevalent in pulmonary LCH biopsies of adults presenting with both pulmonary and extrapulmonary LCH lesions[39] than in patients with isolated pulmonary LCH.

Building on the first insights from mouse LCH models, a "misguided myeloid differentiation" model for LCH ontogeny was proposed. In this model, the differentiation stage of the first cell acquiring a disease-driving mutation determines disease extent. According to this model, mutations occurring in HSC prior to lineage commitment cause MS-RO^{+} LCH. In contrast, mutations in more differentiated myeloid cells lead to more limited disease presentation.[40] Halbritter *et al*. challenged this concept, identifying two distinct cell populations among *BRAF*-mutant CD1a^{+} lesional cells expressing high levels of Ki67 and aurora kinase RNA transcripts, two genes associated with cell proliferation. These progenitor-like cells were also found in LCH lesions of patients with limited disease extent.[41] Furthermore, the concomitant presence of *BRAF*- or *KRAS*-mutant myeloid and lymphoid cells is not limited to patients with disseminated histiocytosis, suggesting that acquisition of such driver mutations by lymphoid primed multipotent progenitors or even multipotent HSC may be a common event in both LCH and other non-Langerhans cell histiocytoses.[36]

Neurodegeneration

One of the most feared complications of LCH is neurodegeneration, which may arise decades after LCH presentation even in non-CNS-risk tissues. The biology of this devastating condition remains poorly understood, as autopsy samples from patients who died of ND-LCH are rare. ND-LCH was initially presumed to be an autoimmune or paraneoplastic phenomenon based on CD8^{+} T-cell infiltration in brain biopsies lacking CD1a^{+}/CD207^{+} histiocytes.[42] However, two recent studies on human autopsy material showed peri-

vascular localizations of *BRAF*^{V600E} hematopoietic cells with myeloid features in different parts of the brain.[43,44]

Two distinct mouse models revealed that the underlying mechanisms of ND-LCH are far more complex than previously thought. In the first model, transient mosaic expression of *BRAF*^{V600E} in yolk sac erythromyeloid progenitors resulted in the generation and distribution of mutant and non-mutant myeloid cells to different organ systems, including the brain, during fetal development. After birth, *BRAF*-mutated macrophages in the brain became ERK-activated amoeboid microglia, causing pathological inflammation followed by neurological dysfunction. Providing a *BRAF*-targeting drug early after birth prevented the development of neurological symptoms in these mice.[45] Surprisingly, PU.1^{+} nuclei isolated from brain samples derived from histiocytosis patients who died with or without clinically overt neurodegeneration showed *BRAF*^{V600E} myeloid cells (mostly microglia cells) in the brains of all patients, but their frequency was higher in patients with neurodegeneration. Paired analysis of blood/bone marrow cells and brain tissue cells from eight patients showed that only one patient displayed BRAF-mutant cells from both sources, suggesting that *BRAF*^{V600E} cells in the brain do not necessarily arise from circulating myeloid precursor cells.[44] Wilk *et al*. explored brain-invading myeloid cells generated after tamoxifen-induced expression of *BRAF*^{V600E} transgene in HSC of young mice. Their data suggested that circulating *BRAF*^{V600E}-mutated myeloid cells alter the blood-brain barrier, which increases myeloid cell influx of additional bone marrow-derived *BRAF*^{V600E} cells that locally differentiate into inflammation-promoting CD11a^{+} macrophages in the brain stem and cerebellum. This ultimately leads to cognitive and behavioral abnormalities over time.[46] Based on the efficacy of MAPK pathway inhibition combined with a senolytic drug specifically targeting the hyperinflammatory state of CNS-infiltrating myeloid cells (navitoclax), this model suggests that early treatment blocks the accumulation, over time, of circulating myeloid cells in the brain. Additional support in favor of a bone marrow HSC being the cell-of-origin in ND-LCH is supported by recent data showing that *BRAF*^{V600E}-expressing monocytes generated from human induced pluripotent stem cells (iPSC) can form microglia-like cells that induce neuronal damage when co-cultured *in vitro* with iPSC-derived neurons with wild-type *BRAF*.[47]

Clinical achievements

Frontline therapy

LCH treatment has evolved significantly over the last 10-15 years. Historically, the Histiocyte Society has been the leading international consortium for clinical trials focused on pediatric LCH. To this day, vinblastine plus prednisone is considered the gold standard frontline therapy for pediatric patients with disseminated LCH.[48,49] Two consecutive trials (LCH-I and LCH-II) established that prednisone- and vinblastine-based chemotherapy backbones were well-tolerated and effective if patients responded within the first 6 weeks of therapy (Table 1).[50,51] Prolongation of consolidation therapy applied in LCH-III was subsequently shown to lower disease recurrence rates.[52] Further reduction of reactivation rates through prolongation and intensification of continuation therapy is the expected outcome of the currently recruiting LCH-IV trial (Table 2). How to select patients who can be cured with minimal chemotherapy does, however, remain an unsolved issue.

In 2022, Histiocyte Society experts published guidelines for diagnosing and treating adult LCH.[6] Treatment strategies are predominantly based on results from pediatric studies, although adult therapies differ in key aspects. First-line chemotherapy for multisystem disease typically includes nucleoside analogs such as cladribine or cytarabine, rather than vinca alkaloids and steroids, because of the higher risk of neurotoxicity in adults. Of note, adults with mixed histiocytosis displaying an LCH component do not respond to chemotherapy and require alternative frontline treatment as explained in the next paragraph.[53] Patients with isolated bone or skin involvement may respond to less aggressive treatments, such as bisphosphonates, methotrexate, hydroxyurea, thalidomide, or lenalidomide. For adults presenting with isolated pulmonary LCH, which is nearly always associated with a history of smoking, cessation of smoking alone is often sufficient to cure the disease.

Targeted treatments

MAPK pathway inhibitors (MAPKi) (Figure 2A) represent the new horizon of histiocytosis treatment as explored by different research groups (Table 3). Vemurafenib, a BRAF^{V600E}-specific inhibitor, produced good responses in adult and pediatric patients with LCH in independent studies. Dabrafenib, another BRAF^{V600E} inhibitor, and trametinib, a MEK1/2 inhibitor, used as monotherapy or in combination, have both been explored as off-label, front-line or salvage therapy producing favorable responses in refractory pediatric LCH.[57,58] In addition cobimetinib monotherapy was found to effectively induce clinical remission in adults.[59,60] Of note, the BRAF inhibitors vemurafenib and dabrafenib can only be used in patients who harbor the *BRAF*^{V600E} mutation, while MEK inhibitors can be effective regardless of the underlying MAPK pathway mutation. Three other inhibitors are currently under investigation in prospective clinical trials enrolling patients with relapsed/refractory LCH. One study is addressing the clinical efficacy of cobimetinib, a MEK1/2 inhibitor that has been approved by the Food and Drug Administration for adults with histiocytosis.[60] The second study is exploring the pan-RAF inhibitor tovorafenib, which showed promising results in pediatric *BRAF*^{V600E}-expressing low-grade glioma.[61] In a third study, mirdametinib, a MEK1/2 inhibitor with recent successes in treating plexiform neurofibromas in patients with neuro-

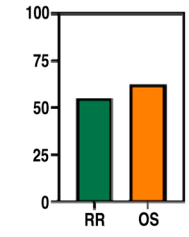
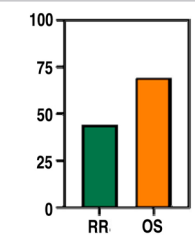
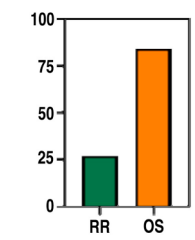
fibromatosis type 1,[62,63] is under investigation as either a frontline or salvage agent (Table 3).

Although these drugs are effective at inducing clinical remission, a large proportion of both adults and children relapse after discontinuation of the treatment.[64] This suggests that inhibitors are incapable of eradicating mutated HSC clones that give rise to LCH precursor cells. ND-LCH was shown to develop in some patients treated with vemurafenib, particularly in infants initially diagnosed with MS-RO^{+} disease.[65] Detectable levels of *BRAF*^{V600E} cells in the blood and bone marrow of patients in remission after dabrafenib[31] support the hypothesis that targeted therapy does not eradicate driver mutation-expressing hematopoietic stem/progenitor cells. Further support for this hypothesis comes from studies involving longitudinal mutation monitoring in blood cells isolated from several LCH patients who had been on targeted therapy.[27,31,66] An important topic of LCH research is to address whether myeloid cells associated with a relapse or ND-LCH develop from aberrant HSC harboring persistent pathological driver mutations. If so, the focus of treatment will likely shift from MAPKi-induced rapid disease control to chemotherapy-based eradication of the pathological clone.[35,67,68]

Neurodegeneration and other late sequelae

ND-LCH is perhaps the most challenging complication of LCH, with a cumulative incidence of 6% in the largest published pediatric LCH cohort[69] and between 1-10% in other cohorts.[18] Radiographic features (i.e., signal abnormalities mostly involving the cerebellum, the basal ganglia, and the pons, sometimes leading to atrophy) are well described,[70] but there are limited data clarifying its natural history. The term “neurodegenerative-LCH” was recently proposed to be replaced by two different entities, i.e., “LCH-associated abnormal CNS imaging” (LACI) for patients with radiologic findings alone, and “LCH-associated abnormal CNS

Table 1. Summary of the Histiocyte Society LCH-I, -II, and -III trials.

Trial name	Years	Design	Key findings	
Frontline therapy trials				
LCH-I[50]	1991-1995	Vinblastine or etoposide (24 weeks)*	Vinblastine and etoposide produced similar outcomes Best chance of cure when active disease better or non-active disease at 6 weeks	
LCH-II[51]	1996-2001	Vinblastine plus prednisone (6 weeks) followed by continuation with vinblastine, prednisone, 6-MP ± etoposide (24 weeks)	Intensified therapy resulted in better early response rates Improved 5-year overall survival rates compared to those in LCH-I Similar reactivation rates at the end of therapy compared to those in LCH-I	
LCH-III[52]	2001-2008	Vinblastine plus prednisone ± MTX (6-12 weeks) followed by continuation with vinblastine, prednisone ± 6-MP (6 or 12 months)**	Prolongation of continuation therapy decreased reactivation rates Improved survival rates compared to those in LCH-I and -II Addition of MTX did not affect outcome	
Salvage therapy trials				
LCH-I-S[106]	1994-1998	CsA ± other LCH-directed therapies***	Only 1/26 patients enrolled responded to cyclosporine	
LCH-S-98[107]	1999-2004	2-CdA (6 cycles)***	2-CdA was active against relapsed/refractory LCH Patients without risk organ involvement and longer time to first relapse had better outcomes	
LCH-S-2005[108]	2005-2010	Ara-C plus 2-CdA (≥2 cycles) followed by 2-CdA, vinblastine, prednisone, 6-MP, and MTX***	92% of patients responded to the initial Ara-C/2-CdA cycles 5-year overall survival was 85% High incidence of severe neutropenia and infection	

*Inclusion of patients with multi-system Langerhans cell histiocytosis (LCH). **Randomization based on risk organ status; ***Inclusion of relapsed/refractory LCH cases only. Graphs illustrate the proportional relapse rates and overall survival rates for the three consecutive trials. RR: relapse rate; OS: overall survival; 6-MP: mercaptopurine; MTX: methotrexate; CsA: cyclosporine; 2-CdA: 2-chlorodeoxyadenosine; Ara-C: cytosine arabinoside/cytarabine.

symptoms" (LACS) for patients with abnormal neurological (mostly cerebellar), cognitive and psychological findings.[71] There are no studies showing whether mutated cells are in the blood or cerebrospinal fluid (CSF) of patients displaying LACI or LACS. Héritier *et al*. identified pituitary gland involvement and the *BRAF*^{V600E} mutation as major risk factors for ND-LCH. Patients with pituitary gland, skull base or orbital bone involvement had a 10-year risk of neurode-

Table 2. Overview of the Histiocyte Society LCH-IV trial.

Arms	Design	Outcome measures
Stratum I	First-line therapy: vinblastine plus prednisone (LCH-III)* Patients with NAD at 6-12 weeks: 12 *vs.* 24 months (Rx) continuation with first-line therapy ± 6-MP (Rx)	Relapse rate and incidence of late sequelae after prolongation of continuation therapy
Stratum II	Patients who fail stratum I: prednisolone, vincristine plus Ara-C If NAD or AD better at 24 weeks: continuation with indomethacin or 6-MP/MTX for 24 months (Rx)	Proportion of stratum I patients with progressive LCH who achieve NAD
Stratum III	MS-RO$^+$ patients who fail stratum I: initial therapy with 2-CdA/ Ara-C for 2 cycles, followed by additional multimodal continuation therapy depending on response to initial therapy	Proportion of patients with MS-RO$^+$ relapsed/progressive LCH who achieve NAD after salvage chemotherapy
Stratum IV	MS-RO$^+$ patients who fail stratum I or III: RIC allogeneic HSCT	OS and DFS after HSCT as salvage therapy for patients with multisystem MS-RO$^+$ LCH
Stratum V	Option 1: Ara-C or IVIG in patients with clinical ND-LCH Option 2: 2-CdA monotherapy in patients with isolated CNS LCH ± extracranial LCH manifestation	Monitoring and treatment of isolated tumorous lesions and neurodegenerative LCH
Stratum VI	Natural history of single-system LCH cases not eligible for stratum I**	Data on natural history of single-system LCH in patients who do not receive systemic therapy (observational arm)
Stratum VII	All patients treated according to any of the strata***	Data on long-term survivorship outcomes including sequelae for all patients enrolled in LCH-IV

*Patients with multisystem Langerhans cell histiocytosis (LCH) (group 1), single-system multifocal bone LCH or central nervous system (CNS)-risk lesions (group 2). **Progression of single-system LCH to multisystem LCH, multifocal bone disease or patients who develop CNS-risk bone lesions should be enrolled in stratum I. ***Patients also enrolled in stratum IV who develop isolated tumorous or neurodegenerative LCH should be enrolled in stratum V. NAD: non-active disease; Rx: randomization; 6-MP mercaptopurine; Ara-C: cytosine arabinoside/cytarabine; AD: active disease; MTX: methotrexate; MS-RO$^+$: multisystem risk-organ-positive; 2-CdA: 2-chlorodeoxyadenosine; RIC: reduced intensity conditioning; HSCT: hematopoietic stem cell transplantation; OS: overall survival; DFS: disease-free survival; IVIG: intravenous immunoglobulin; ND-LCH neurodegenerative LCH.

Table 3. Summary of published and ongoing studies addressing the impact of targeted treatment (mitogen-activated protein kinase pathway inhibitors) in Langerhans cell histiocytosis.

Name	Target	Other considerations	Common adverse effects	Active LCH trials
Vemurafenib[54-56,67]	*BRAF*^{V600E}	Must have *BRAF*^{V600E} mutation*	Rash and skin photosensitivity, QT prolongation, liver enzyme elevations	-
Dabrafenib[57-59,68]	*BRAF*^{V600E}	Must have *BRAF*^{V600E} mutation*	Pyrexia, vomiting, cough, dry skin, elevated creatinine	-
Trametinib[57,59,68]	MEK1/2	Active in patients without *BRAF*^{V600E}	Rash, vomiting	-
Cobimetinib[60,68]	MEK1/2	Active in patients without *BRAF*^{V600E}	Rash, diarrhea, vision changes, decreased heart function	North American Consortium for Histiocytosis (NCT04079179)
Mirdametinib	MEK1/2	Active in patients without *BRAF*^{V600E}	Rash, diarrhea, nausea/vomiting, decreased heart function	Cincinnati Children's Hospital (NCT06153179)
Tovorafenib	RAF**	Active in patients with wild-type BRAF and fusion-positive hyperactivating BRAF mutations; greater CNS penetration***	Rash, anemia, neutropenia, elevated liver enzymes, itching, vomiting	Children's Oncology Group (NCT05828069)

*BRAF inhibition for wild-type or non-V600E mutations may paradoxically hyperactivate BRAF-driven pathways, which would need dual MEK inhibition for *Tovorafenib is a Food and Drug Administration-approved selective type II RAF kinase targeting wild-type and mutant forms of A-RAF, B-RAF and C-RAF. ***No paradoxical BRAF activation. LCH: Langerhans cell histiocytosis; CNS: central nervous system.

generation of 7.8%.[69] This group of patients accounted for approximately one-third of the cohort, and, surprisingly, no patient outside this group developed ND-LCH. Moreover, patients with ND-LCH harbor the *BRAF*^{V600E} mutation in more than 90% of cases.[18,43,69] Historical treatment options for ND-LCH, including cytarabine ± vincristine,[72] cytarabine and intravenous immunoglobulins,[73] intravenous immunoglobulins ± chemotherapy,[74-76] and retinoic acid[77] have failed to stop disease progression or improve symptoms. More recently, MAPKi have shown some promising results in ND-LCH, as single agents[43,59,65,78] or in combination with chemotherapy,[68] but only in limited cases.

DI is the most common permanent consequence of LCH, with a prevalence of up to 25% in pediatric cases.[14,16,79,80] Craniofacial bone lesions (CNS-risk lesions), direct CNS parenchymal involvement, prolonged initial disease activity, and multiple LCH reactivations are considered risk factors for the development of DI, but treatment duration does not have a significant impact on DI incidence.[16,79] DI at presentation of multisystem disease is significantly associated with the *BRAF*^{V600E} mutation.[81] It is hoped that the LCH-IV study is sufficiently powered to perform a multivariate analysis to establish which of these factors independently predicts DI. In rare cases, DI is the sole manifestation of LCH involving the pituitary gland, particularly when accompanied by circulating *BRAF*^{V600E}-positive cells in the blood.[9] In analogue to observations in *BRAF*^{V600E} transgenic mice,[46] we speculate that low frequencies of circulating *BRAF*^{V600E} cells may accumulate over time in specific localizations of the brain, including the pituitary gland, without LCH lesions developing elsewhere.

Sclerosing cholangitis is a rare but severe permanent consequence of LCH, affecting less than 5% of patients, primarily those with initial hepatic involvement. As with other late sequelae of LCH (Figure 3), no significant reduction in its incidence has been observed in recent years.[14] Some patients progress to develop cirrhosis and require liver transplantation. One study reported *BRAF*^{V600E} cells in

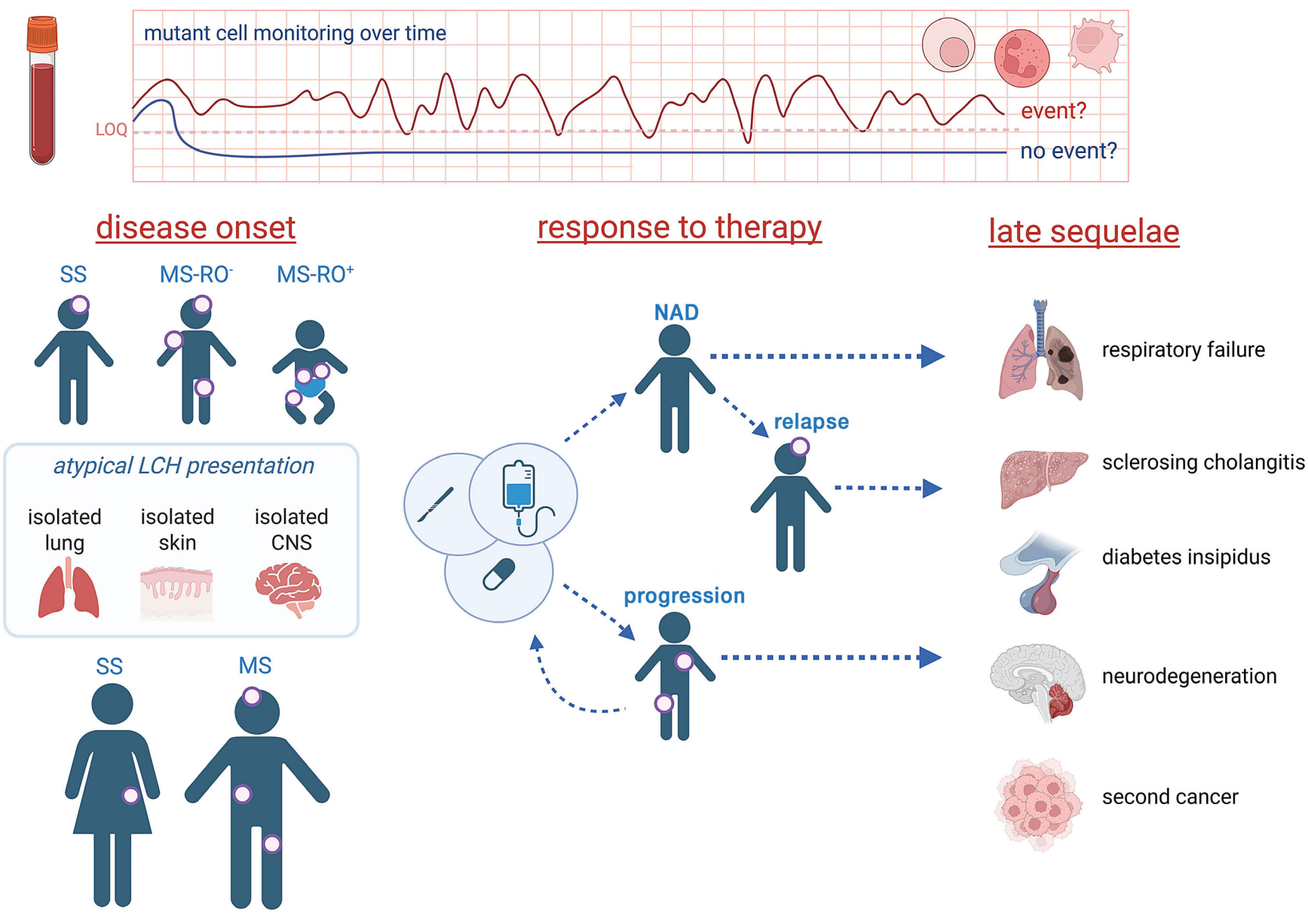


Figure 3. Overview of presentations of Langerhans cell histiocytosis and the different outcomes after treatment in relation to persistence of driver mutation-expressing cells over time. Common and atypical presentations of Langerhans cell histiocytosis and the different outcomes (indicated by dashed lines) after topical (curettage of the lesion) or systemic treatment (chemotherapy, steroids and/or targeted therapy) are shown in relation to persistence of driver mutation-expressing cells over time. Organs potentially affected by late sequelae or (potentially clonally related) second cancers are shown in the right part of the figure. LOQ: limit of quantification; SS: single-system; MS: multisystem; RO: risk organ; LCH: Langerhans cell histiocytosis; CNS: central nervous system; NAD: non-active disease.

liver biopsies derived from three infants with disseminated LCH; liver-accumulating histiocytes expressed CD1a in two out of the three cases.[82] It has not been determined whether *BRAF*^{V600E} liver cells are derived from circulating myeloid cells or tissue-restricted LC.[83] Likewise, it is unknown whether LCH recurrence in transplanted livers is preceded by circulating mutated cells.

Adults and children with LCH also have an associated risk of developing solid and hematologic malignancies, similar to other histiocytoses,[84-87] as well as malignancies manifesting in solid organs.[88] Interestingly, patients with LCH may present with a secondary myeloid or lymphoid malignancy (mostly B-cell lymphoma), or both, despite the myeloid ontogeny of LCH.[13] Clonally related lymphoma cells and CD1a^{+} histiocytes residing in separate tissue localizations may express the same rearranged B- or T-cell receptor-encoding DNA sequence, suggesting that mature lymphocytes can transdifferentiate into myeloid cells under certain pathological conditions.[89] Intriguingly, no patients have been reported to have LCH and hairy cell leukemia, a specific B-cell malignancies with recurrent *BRAF*^{V600E}. Adults with LCH who subsequently develop a secondary myeloid malignancy may display myeloid cells with the same clonal hematopoiesis-associated mutation(s) in their LCH biopsy as found in their bone marrow or blood as is often the case in Erdheim-Chester disease.[86] This indicates the need to extend our focus on driver mutations outside of the MAPK pathway.

Altogether, the potential persistence of mutant hematopoietic stem/progenitor cells may give rise to both LCH and other clonally related hematologic malignancies. Whether this situation is associated with an increased risk of morbidity and even mortality as suggested by recent epidemiological data needs to be further investigated.[88]

Unanswered questions and future directions

Reactivation rates remain significant in pediatric LCH, with a 5-year cumulative incidence of 37% in MS-RO^{-} patients and 25-29% in MS-RO^{+} patients treated with the 12-month LCH-III regimen.[52] Relapse rates are even higher in patients who stop targeted therapy.[55,90] Moreover, the incidence of long-term sequelae, such as DI and ND-LCH, has not decreased in patients treated with conventional chemotherapy.[14] It is unknown whether this is also the case in patients on MAPKi therapy. Treatment decisions are still stratified by disease localization(s) due to the lack of consensus biomarkers associated with poor LCH outcome. A deeper understanding of pathological cells driving LCH complications will aid in developing tools that can identify patients at risk of clinical or radiological abnormalities earlier. This process could benefit from the integration of health-related quality of life measures, ultimately advancing a more patient-centered approach to survivorship (Figure 4).

Early identification of patients at high risk of relapse

Polymerase chain reaction-based driver mutation detection in blood samples using cell-free DNA techniques seems a promising non-invasive option for monitoring pathological HSC activity. Several studies have shown that measuring *BRAF*^{V600E} cell-free DNA levels can confirm the diagnosis of LCH and improve response assessment.[66,81,91-93] The presence of cell-free *BRAF*^{V600E} at LCH diagnosis is an independent risk factor for worse outcomes.[93] Of note, mutations in *BRAF, KRAS* or *MAP2K1* can be found in many types of malignancies. Hence, a thorough clinical work-up, including total-body imaging, is needed to exclude the presence of other malignancies that could shed DNA fragments containing such mutations in the blood. Lin *et al*. developed the LangIndex to score and identify patients at highest risk of failing frontline therapy; the presence of multiple lesions and blood *BRAF*^{V600E} positivity emerged as the main independent risk factors.[94] Alternatively, *BRAF*^{V600E} can be detected in peripheral blood leukocytes, mononuclear cells,[31] whole blood,[66] or in distinct cell types isolated by flow cytometry.[8,9,23] Measuring mutant *BRAF* or other driver mutations in fractionated blood cells will provide a deeper insight into precursor cell types associated with LCH onset, disease spread, and relapse. New insights into HSC biology and the cell-of-origin could be further investigated by fractionating blood leukocytes into various subtypes, such as granulocytes[35] and nucleated erythroblasts.[36] Milne *et al*. found that inhibitor therapy leads to a significant reduction of *BRAF*^{V600E} myeloid cells, but a subsequent increase in *BRAF*^{V600E} T cells in the early years of therapy.[95] This "lineage switch" may result from reversible suppression of myeloid, B-, and NK-cell differentiation from a not yet lineage committed mutated stem/progenitor cell. This also mechanistically aligns with LCH relapse after discontinuation of targeted therapy.[34,35,73]

A recent iPSC-based *in vitro* model of differentiation showed that MAPKi therapy specifically affects functional features of mature *BRAF*-mutant monocytic cells, but not of iPSC-derived stem/progenitor cells.[47] We therefore advocate the importance of longitudinal sampling and biobanking of peripheral blood from LCH patients receiving multiple tiers of therapy, as this will provide valuable insights into the natural disease course and long-term fibro-inflammatory consequences of LCH.[35] It is hoped that this much needed information will answer the key question of whether persistence of a single *BRAF*-mutated HSC clone also drives the onset of late sequelae. Whether the same scenario also applies to the 40% of non-*BRAF*^{V600E}-mutated cases still needs to be addressed. Ultimately, the outcomes of prospective monitoring studies will help to design tailored approaches to treatment intensification for patients with persistent HSC activity to prevent frontline therapy resistance, relapses and late sequelae.

Integration of novel treatments into standard care

How best to incorporate targeted therapy into standard care remains a topic of debate. The LCH-IV trial does not address this issue because the first investigator-initiated studies exploring MAPKi were being publishing at the time when LCH-IV was initiated across the world. Cobimetinib is now approved for adults with histiocytosis in the USA, but these agents have not yet been cleared by the European Medicines Agency. Consequently, clinical trials investigating the efficacy of particular inhibitors are currently only recruiting in the USA (Table 3). This causes considerable difficulties and delays in providing these drugs to European patients and hampers the implementation of a new international, Histiocyte Society-managed study investigating their efficacy. Moreover, initiating and arranging funding for a local clinical trial is a challenge for rare diseases such as LCH.

Combining MAPKi with chemotherapy could be a better strategy to eradicate tissue-resident histiocytes and their immediate circulating precursors.[67,68] We anticipate that risk stratification using blood mutational burden combined with risk organ and CNS-risk status could better guide individualized treatments and help identify patients who may benefit from targeted therapy provided as upfront therapy.[59] Currently, first-line MAPKi remains controversial as many patients display excellent overall survival with conventional therapy, and targeted therapy has potential side effects that can affect daily life, especially in adults.[18] The duration of MAPKi treatment also remains questioned. Ideally, treatment discontinuation should be investigated within a clinical trial with fixed treatment duration combined with extensive mutation monitoring in blood and bone marrow samples, as was done earlier in patients with Erdheim-Chester disease.[64] Finally, as treatment strategies evolve rapidly, it is crucial to standardize disease monitoring protocols and response assessment criteria to ensure consistency and comparability across different clinical settings (Figure 4). Achieving these goals requires extensive collaborative efforts spearheaded by international consortia involving members of the Histiocyte Society.

Roadmap for future research

Studying the natural history of LCH will allow us to systematically investigate the frequency and clinical predictors of LCH and its complications. A key factor is the harmonization of somatic mutation detection techniques across laboratories (Figure 4), which requires large-scale cohort studies focusing on comprehensive somatic mutation monitoring over time. Current treatment stratification based on LCH-III only incorporates risk-organ status as a

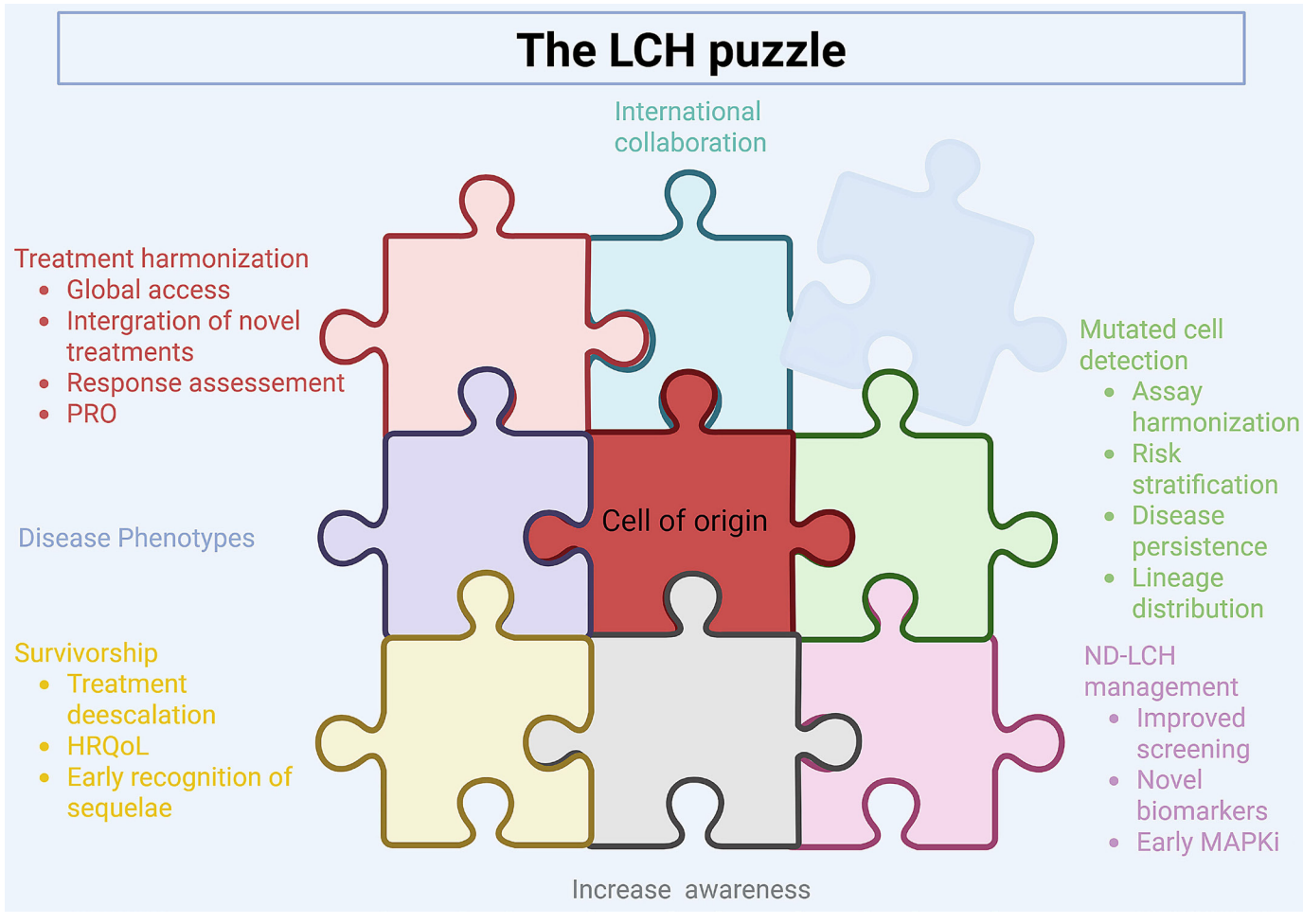


Figure 4. Overview of (pre)clinical research topics that need to be addressed to offer optimal personalized care to future patients worldwide. As unraveling the molecular and biological features of the cell-of-origin is relevant for understanding multiple different unsolved issues, we have placed research on this topic in the center of the puzzle. PRO: patient-report outcomes; HRQoL: health-related quality of life; ND-LCH Langerhans cell histiocytosis-associated neurodegeneration; MAPKi: mitogen-activated protein kinase pathway inhibitors.

means of predicting poor outcomes. However, there is still an unacceptably high reactivation rate.[52] If circulating driver mutation-positive cells need to be completely eradicated to achieve prolonged remission, novel treatment strategies such as combined chemotherapy and targeted therapy should be explored in patients who additionally consent for blood and bone marrow sampling for the purpose of mutation monitoring. We expect that these research efforts will also illuminate why different cell types expressing a pathological driver mutation persist and how they could play distinct roles in disease persistence, reactivation, and late sequelae (Figure 4).

Early identification of patients at risk of neurodegeneration

Monitoring for driver mutations may also help to predict the risk of ND-LCH. Despite the logistic challenges of biological sample collection, various biomarkers have been investigated for the early detection of ND-LCH, with osteopontin and neurofilament light showing promise. Osteopontin is a protein involved in immune cell recruitment and activation, cancer progression and metastasis, and is overexpressed in several neoplasms, including LCH. McClain *et al*. showed that osteopontin levels measured in CSF were significantly increased in patients with ND-LCH as compared to the levels in other patients with LCH not involving the brain or patients with brain tumors, other neurodegenerative diseases or acute lymphoblastic leukemia. This finding highlights its potential as a biomarker and potential therapeutic target for ND-LCH.[43]

Neurofilament light is a cytoskeletal component of myelinated axons, whose levels in CSF and blood increase upon neuronal injury. Henter *et al*. found that neurofilament light levels in CSF may serve as a valuable biomarker for monitoring treatment response in ND-LCH.[78,96] The predictive potential of neurofilament light, and the feasibility of routine monitoring of its levels in blood or CSF, is currently under investigation. It is uncertain whether these biomarkers can adequately predict the risk of ND-LCH, preferably before its overt clinical manifestation, as this seems the only window of opportunity for successful therapeutic intervention.

Preliminary data reported by McClain *et al*. suggest that LCH patients who develop neurodegeneration may exhibit higher frequencies of *BRAF*^{V600E}-positive cells among peripheral blood mononuclear cells at all stages of treatment.[43] More recently, Lin *et al*. evaluated the prognostic impact of *BRAF*^{V600E}-positive cell frequencies in peripheral blood mononuclear cells collected before treatment initiation. Patients with circulating *BRAF*-mutant cells had the highest risk of developing neurodegeneration.[94] Altogether, these findings could have profound clinical implications and underscore the importance of international collaborative efforts focused on longitudinal driver mutation monitoring to improve early detection of ND-LCH.

Focusing solely on molecular analysis of circulating myeloid cells may not fully elucidate the pathogenesis of ND-LCH. While data from the LCH mouse model developed by Wilk *et al*. suggest that the accumulation of circulating *BRAF*^{V600E}-mutant myeloid cells precedes disruption of the blood-brain barrier and influx of pathological macrophage precursor cells,[46] it may also be worth exploring the potential role of other cell types such as *BRAF*^{V600E} lymphocytes (Figure 2B). Investigating their persistence and potential contribution to pathological processes in the brain could provide deeper insights into the mechanisms underlying the presumed breakdown of the blood-brain barrier, a process with potentially significant clinical implications. Aberrant bone-brain interactions may also contribute to ND-LCH. LCH lesions in specific skull bones located near the brain have been associated with an increased risk of developing overt clinical neurodegeneration.[69] Investigating RNA and protein expression by cells present in these CNS-risk lesions may provide new clues on inflammatory signaling occurring inside the lesions. Unfortunately, the scarcity of fresh biopsies remains a significant obstacle to the advance of this line of research. This issue may be circumvented by performing *in vitro* studies with relevant cell types generated from patient-derived iPSC.[47] Collaborative efforts involving researchers focused on more common neurodegenerative diseases such as multiple sclerosis may help to discover and validate biomarkers associated with disturbed blood-brain barrier function.[97] Such interdisciplinary approaches could offer novel insights and accelerate the development of preventive strategies. Networking initiatives aimed at advancing the LCH field, such as the Nikolas Symposium for the Histiocytoses, are excellent platforms for fostering such crossover studies.[20]

Future management of patients with neurodegeneration or other long-term sequelae

Integrating data on clinical outcomes with profiling studies on LCH patient-derived driver mutation-expressing HSC will aid a better understanding of the mechanisms of ND-LCH. Furthermore, a more thorough screening and assessment approach, incorporating neurocognitive evaluations, plasma biomarkers, specialized clinical scales, and patient-reported outcomes, can provide valuable insights into the natural history of long-term sequelae of LCH (Figure 4). Preliminary data from small patient cohorts treated with MAPKi have shown promising outcomes,[13,68,90] but the efficacy of MAPKi in stabilizing or even improving ND-LCH symptoms requires validation in larger cohorts and standardized response criteria. An ongoing project initiated by the European Consortium for Histiocytosis (ECHO) aims to address this.[98] Optimal monitoring and therapeutic strategies must also be developed for patients presenting with ND-LCH after targeted treatment to ensure durable cure.[99] Novel therapies with preclinical efficacy,

such as CSF1R inhibitors, should also be evaluated.[100,101] Other late complications of LCH, such as DI, sclerosing cholangitis, lung dysfunction, deafness, and orthopedic sequelae, are mostly irreversible and severely impact survivorship. The true incidence of these complications may be underestimated, as some develop many years after the initial LCH diagnosis. Although one report suggests that early-stage DI can resolve with MAPKi treatment,[59] in most cases it remains a chronic consequence requiring lifelong substitutive therapy. While patients with end-organ damage in the liver or lungs can benefit from organ transplantation, histiocytosis treatment must be continued to prevent relapses in the organ allograft.[102-104] Collaborative European projects are currently investigating the efficacy of MAPKi in LCH-associated cholangitis and severe lung involvement.

Fostering international collaboration and improving global access to appropriate genetic testing and medication

Organizing LCH-focused transitional care programs remains another pivotal challenge. Specialized adult care centers can guarantee that young adults are provided with tailored follow-up programs and provide clinicians caring for adults the required experience to manage newly diagnosed patients. The establishment of a network of Histiocyte Society-certified referral centers, as pioneered by the Erdheim-Chester Disease Global Alliance (*https:// www.erdheim-chester.org*), might contribute to such a goal and help raise awareness of the disease. Creating local networks of collaborating clinicians will also help in the collection and biobanking of blood and bone marrow samples for in-depth molecular analyses. Such studies will propel scientific advancements in understanding biological and molecular features of the cell-of-origin in different populations across the world. In this regard, the Histiocyte Society, in close partnership with the Histiocytosis Association, has had a fundamental role in bringing together patients and experienced clinicians and researchers by organizing webinars and annual conferences through which results from clinical and research activities can be disseminated on a global scale.

There is a strong need to harmonize clinical response assessment criteria as well as consensus protocols for measuring driver mutations and other candidate biomarkers in longitudinally collected biological samples. Patient-reported outcomes can best capture data on the impact of LCH treatment on a patient's life, as clinicians often underestimate factors such as pain, fatigue, and emotional distress, alongside daily functioning and social participation. Understanding these factors may also guide therapy decisions in the future (Figure 4).[105] With LCH now formally recognized as a neoplastic disease,[11,12] it is hoped that national funding agencies broadly focused on cancer will fund such studies. As new therapies emerge, disparities between high-income and middle- or low-income countries become increasingly evident and access to new consensus therapies may be lacking in certain parts of the world. To begin addressing these barriers, strategies could include establishing global initiatives to ensure access to clinical trials in middle- or low-income countries, designing resource-adapted therapeutic protocols, and promoting international fellowships to build local capacity and advance global equity in care and research.

Conclusions

While it may be rare today for a patient to die directly from LCH, patients' outcomes are still burdened by significant morbidity, caused by repeated reactivations and long-term sequelae of the disease. To improve outcomes of all patients with LCH, we must gain a better understanding of the underlying mechanisms of disease persistence and the biological determinants of CNS damage. This is essential to optimize preventive and personalized treatment approaches. International collaboration remains an essential priority, as the rarity of LCH makes randomized controlled trials challenging and time-intensive. Conducting regional well-designed prospective studies combined with molecular driver and other candidate biomarker monitoring studies, coordinated by established consortia (COG, ECHO, NACHO) under the umbrella of the Histiocyte Society, would be the way forward to reach these goals. The histiocytosis community should assist in increasing awareness of LCH among relevant healthcare providers and assure patients' access to local centers of expertise in which LCH-dedicated care and research are optimally integrated.

Disclosures

No conflicts of interest to disclose.

Contributions

MK, AGSvH and FP prepared figures. FP, MK, ZDP and AGSvH drafted the manuscript.

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