The coming of age of Langerhans cell histiocytosis

Progress in understanding the rare disease Langerhans cell histiocytosis has stimulated immersive meetings occurring annually over a 30-year period that bring together clinicians, scientists and patients in a unique collaboration.

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The enigmas of rare diseases have often provoked major conceptual advances in biology and medicine. Langerhans cell histiocytosis (LCH) is one example, as recurrent somatic mutation in the same signaling pathway in myeloid progenitor cells results in a striking diversity of phenotypes ranging from subtle skin lesions to fatal disseminated disease. LCH is one of the histiocytoses, rare diseases of 'histiocytes', a historical term describing tissue-resident myeloid cells. The disease has an incidence of ~1 in 20,000 per year in childhood, and the incidence is probably similar in adults, but because these patients are treated by many different specialists, epidemiological data are scarce. Non-LCH forms of histiocytosis, including Erdheim Chester disease (ECD), juvenile xanthogranuloma and Rosai Dorfman disease (RDD), are even rarer, so progress in understanding and treating these diseases is difficult. For this reason, the publication of a first classification of histiocytoses in 1987 was an important step[1]. Two major categories of histiocytoses were proposed: those that clearly behaved like hematopoietic malignancies and those of "varied biological behavior" — LCH, ECD, juvenile xanthogranuloma and Rosai Dorfman disease.

In 1981, a baby boy named Nikolas Kontoyannis was diagnosed with LCH and taken by his parents to the late John Pritchard, a pediatric oncologist at Great Ormond Street Hospital in London. Nikolas achieved remission, and he has lived for more than 38 years, but has suffered from the disabling neuro-degenerative disease that, rarely, accompanies overt LCH[2]. Grateful for Pritchard's efforts and eager to advance understanding and treatment of LCH, Nikolas's parents, Paul and Elizabeth, asked Pritchard for advice. Emphasizing that almost nothing was known about LCH but that knowledge would be required to develop a rational basis for treatment, Pritchard offered them some of the best advice ever given to potential philanthropists: sponsor a collaborative meeting of scientists and clinicians, many of whom would have had no prior exposure to the disease.

The first meeting, organized in 1989 by Pritchard, became a series, The Nikolas Symposia, held annually and funded and inspired by the Kontoyannis family (Table 1). The aim of these symposia is the discovery of a rational cure for LCH by promoting collaboration among scientists and clinicians from the field of histiocytosis and other diverse disciplines. The family well understood that progress might be slow. The meetings are immersive events with much scientific discussion and informal social events, making these meetings true symposia. So that non-clinicians can appreciate the devastating effects of a chronic and unpredictable disease, the meetings included in-person sessions with families affected by LCH. To bring young investigators into the field, junior faculty are sponsored as 'Artemis' or 'Pritchard' Fellows (Artemis was a young Greek patient who died of LCH). It was hoped that attendees, inspired by the meetings, might work on LCH, and this hope has frequently been realized. The enormous impact of these symposia is demonstrated by the attendance of so many thought leaders in immunology and cancer biology, who have catalyzed many of the

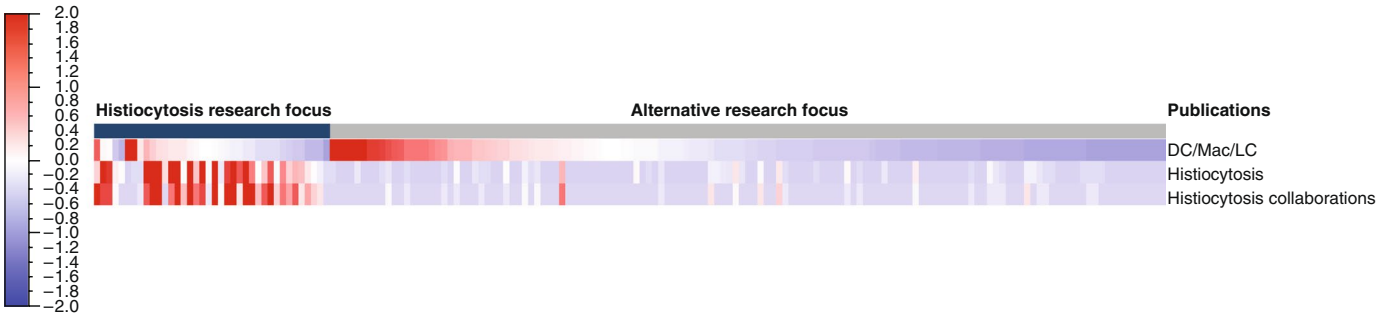

Fig. 1 | Publications of Nikolas Symposium participants 2000–2019. Heat map demonstrates (top row) participants ($n = 173$) with (blue) or without (gray) research focus in histiocytic disorders before attending a Nikolas Symposium. Subsequent rows reflect (1) number of publications on "dendritic cells, macrophages or Langerhans cells" (DC/Mac/LC; range: 0–418); (2) number of publications on "histiocytosis or LCH" (range: 0–96); and (3) collaborative publications from no. 2 including at least two Nikolas symposium participants from different research groups (range: 0–36) as of July 2019. Values were normalized for each group and represented by log values relative to each row.

Table 1 | The Nikolas Symposia

Symposium no.	Year	Symposium topic
1	1989	LCH—an immunological disease?
2	1990	Viruses as a possible trigger
3	1991	The role of cytokines in LCH—developing a strategy
4	1992	The neuropathology and pharmacology of LCH
5	1993	Langerhans cell histiocytosis therapeutics past and present
6	1995	Cell biology and molecular biology in LCH[16–18]
7	1996	Apoptosis in LCH
8	1997	Migration and in vivo interactions of immune cells
9	1998	Genetics and animal models of Langerhans cell histiocytosis[13]
10	1999	Dendritic cells and the brain in LCH
	2000	The molecular basis of LCH and the meaning of clonality (joint meeting with the Histiocyte Society)[5]
11	2001	Acute and chronic cytokine networks leading to tissue damage[45]
12	2002	Dendritic cell differentiation: signals, signalling and functional consequences. Clues to possible therapy[12]
13	2003	Langerhans cell histiocytosis: neoplasia or immune dysregulation?[9]
14	2004	Langerhans cell histiocytosis: bystander cells, interactions, pathophysiology
15	2005	Dendritic cell plasticity[2]
16	2006	Langerhans cell histiocytosis: a hematopoietic stem cell disorder?[32]
17	2007	Langerhans cell function: implications for LCH?[11,29]
18	2008	The tolerogenic nature of tumor-associated inflammation: relevance for LCH?[25,28,46]
19	2009	Viral, autoimmune and neoplastic mechanisms of granuloma formation: possible relevance to LCH
20	2010	Mechanisms and therapeutic targets of inflammatory disease of the CNS[4,8,10,19]
21	2011	Genomics and metabolomics in dendritic cells: are there clues for LCH causes and cures?[44]
22	2012	Cell signaling and responses: pathways to cure?[21,33]
23	2013	Cellular origins of dendritic cells: implications for LCH[3,30,48]
24	2014	Harnessing immunology and inflammation in neoplasm: relevance to LCH and histiocytic disorders[22,25,34]
25	2015	Mechanisms and long-term consequences of neuro-inflammatory disease[6,7,15,31,38,40]
26	2016	Beyond BRAF: mechanisms of resistance and therapeutic development[23,27,47]
27	2017	LCH: the cell of origin and a pathway to a rational cure[35,36,39,49,52]
28	2018	Myeloid cell programming and differentiation[37,55]
29	2019	Immune strategies in the histiocytoses[50,51]

Each reference cited in the table includes at least one author who has attended a Nikolas Symposium.

seminal studies of LCH biology and made rational therapeutic strategies for patients with LCH possible (Fig. 1 and Table 1).

Here we review the intertwined histories of LCH and the symposia, illustrating the transformative impact of nearly 30 years of engagement between clinicians and a diverse cast of basic scientists. The coming of age of LCH is marked by the development of molecular diagnosis and targeted therapy; however, the disconnect between a patient's genotype and phenotype remains a profound puzzle. Solving this problem is likely to provide insights into relationships between myeloid cells and host tissues and will be essential to achieving a rational cure for the most severely affected individuals.

Langerhans cell histiocytosis: the disease

LCH tissue lesions contain the hallmark pathological LCH cells and many leukocyte types associated with chronic inflammation. The disease is heterogeneous, varying from single bone lesions to a multi-system disease with organ failure and substantial mortality, often due to concurrent development of hemophagocytic lymphohistiocytosis. Liver, bone marrow and/or splenic ('risk organ') involvement imparts a worse prognosis, as does a failure to respond to first-line therapy[3]. Pituitary involvement is common and central nervous system (CNS) space-occupying lesions occur, as well as severe degenerative CNS disease, often late and even after treatment has ceased[4].

By the 1950s, the various forms of the disease were recognized as a single entity involving pathological histiocytes, and the name histiocytosis X was adopted. In the 1970s, electron microscopy and immunofluorescence revealed the specific cytoplasmic organelles called Birbeck granules and cell surface expression of the lipid-antigen-presenting molecule CD1a. Because normal Langerhans cells (LCs), the primary antigen-presenting cells of skin and mucosa, share these properties, LCH cells were presumed to represent aberrant differentiation of LCs, and the name Langerhans cell histiocytosis was adopted. Although some of these presumptions have proved incorrect, the name persists.

At the time of the first symposium, it was thought that LCH was either an inflammatory immune-driven disease or a low-grade neoplasm. Three relevant questions emerged during the first two symposia: whether the LCH cells were clonal, whether aberrant cytokine production might account for the pathological features of the disease, and whether a viral trigger might underlie an abnormal immune response leading to LCH lesion formation. A fourth question came to preoccupy several symposia (Table 1): namely, the origin and nature of LCH cells, since although there are phenotypic similarities to normal LCs, there are also differences.

LCH lesion formation

The inflammatory appearance of LCH lesions, with many leukocytes present, strongly suggests that cytokines and chemokines must be produced locally (Symposia 1, 3, 11, 14 and 18). Apart from LCH cells, the five most prevalent cells in LCH tissues are T cells (including regulatory T cells), eosinophils, macrophages, osteoclast-like multinucleated giant cells (MGC) and stromal cells (Fig. 2). Inspired

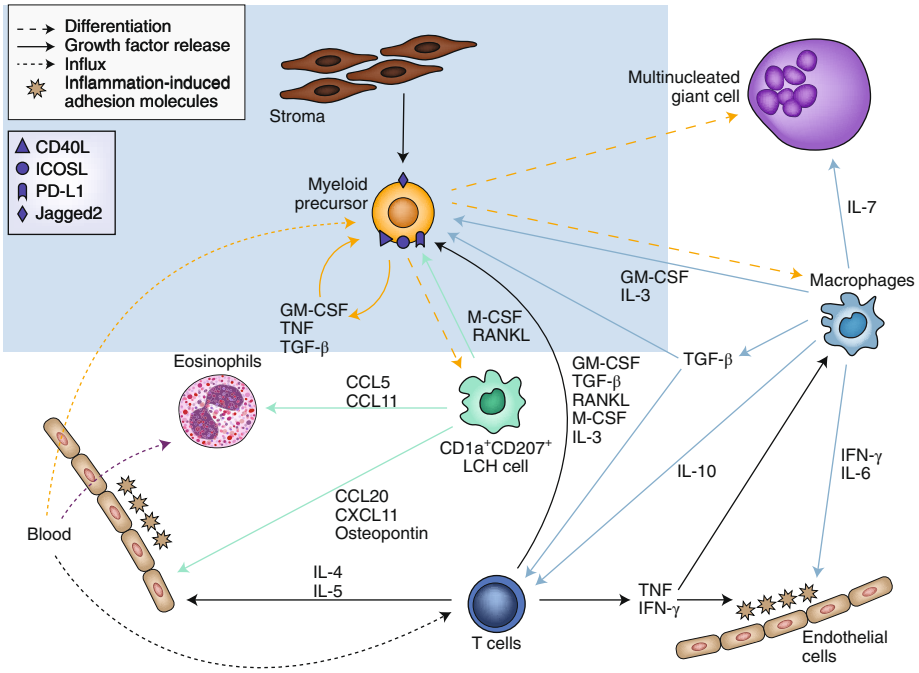

Fig. 2 | Cellular interactions and cytokine and chemokine production in the formation of LCH lesions. Chemokines and cytokines produced by cells in the lesions induce an influx of inflammatory cells, as well as differentiation of mutation-carrying myeloid precursors into LCH cells or MGCs with osteoclast-like function.

by discussions at several meetings, Maarten Egeler and his research team carried out important immunohistological studies showing that costimulatory molecules (CD40–CD40L) important for T cell–dendritic cell (DC) interaction and release of cytokines by activated CD4+ T helper cells are strongly expressed in LCH lesions[5]. This LCH cell–T cell interaction is likely to contribute to the maintenance of the lesions, since isolated LCH cells do not survive in vitro and are difficult to grow in immunocompromised mice. Because the interaction with neighboring cells facilitates the persistence of LCH cells, the lesions become, in effect, sites of chronic inflammation, and other pathways characteristic of continuing inflammation, such as the ICOS–ICOSL costimulatory pathway, known to trigger effector T cells and a subset of regulatory T cells[6] (Symposium 20), and the inhibitory molecules PD-1 and PD-L1, can be detected[7] (Symposium 29).

The three major cytokine-producing lesional cells are T cells, macrophages and LCH cells (reviewed in[8]). LCH cells release the chemokines CCL20 and CXCL11, which promote T cell accumulation by binding to CCR6 and CXCR3, chemokine receptors expressed by antigen-experienced CD4+ T cells[9]. Gene expression analyses confirmed that LCH cells produce additional chemotactic factors, including osteopontin[10]. Lesional CD4+ T cells release many cytokines and chemokines (Symposium 28). Tumor necrosis factor (TNF) and interferon-γ act on endothelial cells and increase T cell accumulation in the lesions, while interleukins 4 and 5 (IL-4 and IL-5) also recruit macrophages, eosinophils and myeloid precursors.

In the lesional tissue, tissue-resident macrophages and stromal cells release cytokines, including transforming growth factor-β (TGF-β), that normally control tissue inflammation by recruiting regulatory T cells to induce wound healing and tissue remodeling[6,11]. In conjunction with granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF and Notch, TGF-β also drives the differentiation of myeloid precursor cells into CD1a+ CD207+ LCH cells, and the cytokines M-CSF, RANK-L and IL-7 stimulate the formation of osteoclast-like MGCs (Symposium 28), another typical feature of LCH lesions. These MGCs express the matrix metalloproteinase MMP9, cathepsin K and other molecules characteristic of prototypic bone-resorbing osteoclasts found in healthy bone. M-CSF also supports local macrophage differentiation. This cytokine microenvironment drives the differentiation pathway(s) of progenitor or precursor cells recruited into the lesion. While this may result in substantial heterogeneity among the cells that express CD1a, LCH lesions are remarkably uninfluenced by disease extent[12].

The variable proportion of LCH cells expressing Ki67, a marker of cell cycle progression, suggests a reactive process, but overexpression of p53 and other pro-survival gene products (MDM2, Rb and Bcl-2) supports a neoplastic origin[13,14]. Overexpression of Fas and FasL apoptosis-inducing proteins indicates a delicate balance between cell survival and death in LCH, perhaps accounting for the spontaneous regression of some single-system-disease lesions[15] (Symposium 7). Although suggestive of a neoplastic process, immunohistological study results could not resolve the issue of LCH etiology. Nevertheless, the cytokine-rich microenvironment of lesions and the anti-apoptotic signature of LCH cells, together with heterogeneous expression of the chemokine receptors CCR7 and CXCR4, both needed for migration to draining lymph nodes[16], likely contribute to the characteristic tissue accumulation and persistence of LCH cells in vivo.

Clonality and activated oncogenes in LCH

At an early symposium, Cheryl Willman (University of New Mexico) was inspired to investigate clonality, and at Symposium 8 she reported non-random X chromosome inactivation at the androgen receptor locus in lesions, correlating with the prevalence of CD1a+ cells, while lesional T cell antigen receptor (TCR) genes were polyclonal[17]. Later, flow-sorted CD1a+ cells were similarly shown to be clonal[18], and in LCH associated with acute lymphoblastic leukemia the same TCR rearrangement was found in both LCH cells and leukemia cells, favoring the view that LCH is a clonal proliferation[19].

While neoplastic diseases are clonal, there should also be evidence of driver genetic abnormalities. Therefore, after attending Symposium 15, Barrett Rollins (Dana-Farber Cancer Institute) with his colleagues searched for mutated oncogenes in archival pathological samples and identified the mutation encoding the oncogenic serine-threonine kinase BRAF V600E in 35 of 61 LCH specimens[20]. This mutation is a driver in tumor types that include malignant melanoma; thyroid, colorectal and non-small cell lung carcinomas; and hairy cell leukemia. The presence of BRAF V600E in ~50% of LCH samples was rapidly confirmed in children and adults (reviewed in ref. [21]). The same mutation was found in some adults with ECD[22], and although LCH

and ECD coexist in some patients, BRAF V600E is found in those with and without histological evidence of LCH[23].

BRAF is part of the extracellular signal-regulated kinase (ERK) or mitogen-activated protein kinase pathway (MAPK) RAS–RAF–MEK–ERK signaling cascade, and MEK and ERK are activated both in BRAF V600E$^+$ and wild-type samples[20], prompting a search for further mutations in this pathway. Mutations in *MAP2K1* (encoding MEK1) were found in 33–50% of *BRAF* wild-type cases and, less frequently, in *ARAF*, *MAP2K1*, *MAP3K1*, *ERBB3 and NRAS*. *BRAF* also exhibits duplications, fusions and in-frame deletions[21], while kinase fusions have been described in *ALK* and *NTRK1*[24,25], as have rare mutations in the phosphatidylinositol-3-OH kinase (PI(3)K)–AKT–mTOR signaling pathway[21]. *BRAF* and *MAP2K1* mutations are mutually exclusive in LCH but not always in ECD[21,26].

In adults with LCH, isolated pulmonary involvement is clearly associated with smoking. In a series of 26 lung samples from such patients, 13 (50%) had the *BRAF* V600E mutation, but 7 also showed oncogenic *NRAS* mutations. Four patients with wild-type *BRAF* exhibited an *NRAS* mutation, and one patient a *KRAS* mutation. Analysis of distinct areas in the lesions suggested that the *BRAF*V600E and *RAS* mutations are in different clones of CD1a$^+$ cells. Thus, isolated pulmonary LCH in adults is, overall, a polyclonal disease but made up of independent LCH clones. Since RAS activation promotes BRAF dimerization and activation, there may be two distinct mechanisms of ERK activation in these pulmonary lesions[27].

It is now clear that the majority of LCH and ECD patients have diverse genetic alterations in signaling pathways that converge on ERK. The development of more sensitive methods to detect genetic alterations, even when the clonal population makes up a very small percentage of lesional cells, is likely to lead to the discovery of abnormalities in further, if not all, patients with LCH and ECD. The presence of clonal abnormalities in these patients has also led to a reappraisal of the nature of LCH and ECD as closely related neoplastic disease entities and to a proposed new classification of histiocytoses[28].

The cellular origin of LCH

After the discovery of LCs by Paul Langerhans, their function remained unknown for over a century. Ralph Steinman (Rockefeller University), who came to several symposia and served on the Symposium Steering Committee, and Niki Romani (Medical University of Innsbruck) then demonstrated the remarkable ability of LCs to migrate from tissues and differentiate into lymphoid-homing DCs. In mice, many tissue-resident myeloid cells, including LCs, are seeded during prenatal life and originate from the yolk sac and fetal liver[29], while most other tissue DCs arise from circulating precursors[30]. LCs are self-renewing in the steady state but can be supplemented or replaced from blood-borne precursors following tissue inflammation[29,31]. The progenitors of these DC precursors are found in the bone marrow and originate from lympho-myeloid trajectories of hematopoiesis[32].

In spite of increasing understanding of DC and LC differentiation (Symposia 6, 10, 12, 15, 17, 23, 27 and 28), definitive determination of the origin of LCH cells has proved difficult, most likely because neoplastic cells may not follow a normal developmental trajectory. Nonetheless, attempts to define the 'cell of origin' have been pursued to define more precisely the nature and potential therapeutic vulnerabilities of LCH.

Initially, the expression of CD1a, langerin (CD207) and Birbeck granules seemed to indicate that LCH cells shared a pathway of differentiation with LCs. However, langerin expression is not restricted to LCs; it may be found on CD8$^+$ DCs in mice (cDC1) and on CD1c$^+$ DCs (cDC2) and CD14$^+$ monocytes in humans[33,34]. In addition, the gene expression profile of LCH cells is substantially different from that of resident LCs, sharing features with myeloid precursors[10,35].

Clonal markers provide a means to search for LCH cell progenitors. Determining which normal cellular precursors can be induced to express an LCH-like phenotype has been combined with identification of subsets of cells in blood, bone marrow and LCH-affected tissues that contain the *BRAF* mutation to identify likely precursors of LCH and ECD (Fig. 3). The mutation encoding BRAF V600E was found in blood CD1c$^+$ DCs and CD14$^+$ monocytes and in the CD34$^+$ stem or progenitor cell compartment in the bone marrow[36,37]. CD34$^+$ *BRAF* mutant cells were shown to have clonal potential in colony forming assays and form histiocytic lesions in immunocompromised mice[38]. Furthermore LCH-like lesions are observed when BRAF V600E expression is imposed in myeloid cells of transgenic mice[36,39].

Because LCH lesions may contain osteoclast-like MGCs, it is also of interest that osteoclasts have been shown recently to develop in the presence of M-CSF and RANKL in vitro from two closely related CD34$^+$ human myeloid progenitors, granulocyte-monocyte-osteoclast-dendritic and monocyte-osteoclast-dendritic cell precursors (Symposium 28)[40].

Puzzlingly, the pathological phenotypes of LCH and ECD are quite distinct but are not always accounted for by a difference in which blood cells carry a mutation[37]. However, co-incident somatic mutation in the bone marrow may play a role, as 10% of patients with ECD have additional clonal mutations commonly found in myeloproliferative neoplasms or myelodysplastic syndrome, including those in *JAK2*, *TET2*, *NRAS*, *ASXL1* and *IDH2*[21]. The much greater prevalence of ECD in older people suggests that clonal hematopoiesis with additional driver mutations may bias the differentiation of myeloid cells with MAPK mutations towards an ECD phenotype. Constitutional genetic background may also play a role as a case–parent trio study has identified candidate genes linked to LCH[41].

In multi-systemic LCH, circulating cells carrying mutations are found, while in single-system disease the mutation is usually confined to CD207$^+$ lesional cells, suggesting that the range of clinical phenotypes seen in LCH may be linked to the stage of differentiation at which the pathological mutation occurs (the 'misguided myeloid differentiation' model) (Fig. 3)[42]. However, this description may be an oversimplification, since some patients with single-system LCH do have mutation-carrying cells in blood[37], so it might be expected that these precursors would seed other organs. It remains unclear how multi-system disease and relapse occur in some patients where mutated precursor cells are absent from bone marrow or blood. This scenario has led to speculation there could be progenitors in sites other than the bone marrow. Further studies of bone marrow, blood and LCH-draining lymphoid tissue over time in these patients, using the most sensitive techniques for detection of mutations, may resolve these apparent anomalies. However, it is clear that tissue-specific or mutagen-specific (smoking) effects are important in determining the disease phenotype in LCH, as the abundance of *RAS* mutations in pulmonary LCH indicates. Remarkably, *RAS* mutations found in the lungs are not found in other LCH lesions in the same patients, suggesting that LCH cells harboring *NRAS* mutations may have a selective survival advantage in the lung microenvironment (Fig. 3)[27].

In hematopoietic stem cells the RAF–RAS–MEK–ERK pathway is linked to the PI(3)K–AKT–mTOR pathway in a feedback loop that controls exit from the cell cycle[43]. ERK signaling also activates numerous

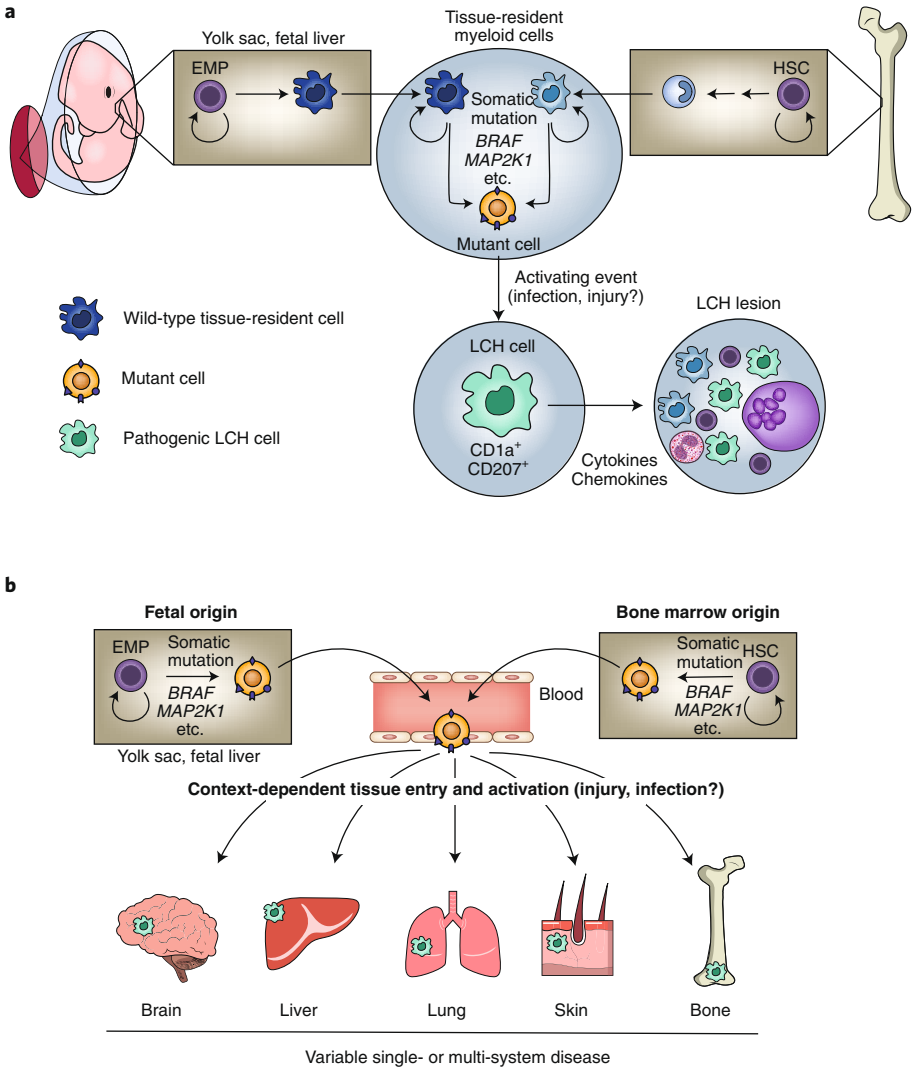

Fig. 3 | The origin of LCH cells. a, A mutation occurring in a fetal hematopoietic or infant bone marrow progenitor cell leads to seeding of a tissue with resident myeloid cells, leaving no reservoir of mutant cells in fetal hematopoietic tissue or bone marrow. A later activating event initiates lesion formation in the tissue. **b**, A mutation occurring in a fetal hematopoietic tissue or infant bone marrow leads to seeding of peripheral tissues, perhaps directed by activating events, but a reservoir of mutation-carrying cells remains in the bone marrow. EMP, erythromyeloid progenitor; HSC, hematopoietic stem cell.

transcription factors involved in growth and differentiation and drives DC maturation[44], but if ERK activation is sustained, DC maturation is inhibited by Toll-like receptor agonists or TNF[45]. Furthermore, when BRAF V600E is expressed in CD11c$^+$ cells in transgenic mice, ERK activation abrogates CCR7 expression and upregulates anti-apoptotic Bcl-xL, trapping cells in lesions and rendering them resistant to cell death[39]. Sustained ERK activation at different stages of myeloid differentiation therefore has complex effects. However, as the proportion of mutant cells in blood and bone marrow is low in LCH, it appears that the single mutations found in LCH precursors do not provide a powerful selective advantage, while in tissue sites, perhaps in the context of local inflammation, accumulation and survival of mutation-carrying LCH cells is favored (Figs. 2 and 3).

Treatment of LCH

Cytotoxic agents alone or in combinations have been the mainstay of treatment[3,46]. Treatment success in trials has been variable, probably due to diversity of the disease, inconsistent disease stratification and inadequate sample size[46]. In 1987, with the foundation of the Histiocyte Society, international prospective trials began (LCH I–III, between 1991 and 2008)[3,47,48]. The overall survival of children with the most severe disease (multi-system LCH with involvement of risk organs) was significantly improved (84% survival at 5 years) and the risk of disease relapse was reduced to 35%[3]. However, non-response to first-line treatment, disease relapses and disease-related permanent consequences, particularly neurodegeneration (ND), remain as challenges[49].

Kinase mutations provide a target for the first rational therapy of LCH and ECD (discussed at Symposia 27–29). Dramatic clinical improvement has been seen in both adults and children using the BRAF inhibitor vemurafenib in patients positive for BRAF V600E and MEK inhibitors in those with a spectrum of other MAPK pathway mutations[24,50–52]. In some patients with BRAF V600E$^+$ LCH-ND, cells have been demonstrated in sites of ND and around blood vessels, while others without systemic symptoms who develop LCH-ND have BRAF V600E$^+$ in peripheral blood cells. Early observations of inhibitor therapy patients suggest that this may improve symptoms in some patients with LCH-ND[51,52], but this remains to be rigorously proven in prospective trials.

Although acquired resistance to these inhibitors is nearly non-existent, it does not appear that MAPK pathway inhibition provides curative treatment, since following cessation of therapy nearly all patients relapse. Furthermore, in patients in which the presence of a mutation can be monitored in peripheral blood mononuclear cells or cell-free DNA, the level does not correlate with the clinical response to therapy, and mutated cells persist in blood and bone marrow[52,53]. It appears therefore that MAPK inhibition has limited cytotoxic potential, instead inhibiting proliferation and differentiation of precursor cells with hyperactive MAPK signaling[39].

The presence of BRAF V600E in ~50% of LCH samples raised the question of whether these patients have a distinct clinical course. Although the original archival study did not show this[20], later series suggested that BRAF V600E$^+$ patients may have higher risk of failure to respond to first-line treatment and higher rates of relapse[49].

Models for LCH

Models are extremely important for advancing understanding of rare diseases, particularly those in children, because of the difficulty of obtaining extensive disease material from such patients. The discovery of the driver mutation in *BRAF* provided a means of modeling LCH, and several

transgenic models have been developed to do so. So-called 'floxed' alleles encoding BRAF V600E have been inserted into the germline of mice by homologous recombination, and these mice have been crossed with mice carrying transgenes in which Cre recombinase expression is controlled by tissue-specific promoters. For example, the Langerin (*CD207*) promoter drives Cre expression in LCs and mature tissue DCs, while the *CD11c* (*ITGAX*) promoter drives expression in DCs and their blood precursors. When either Cre transgenic strain is crossed with mice carrying the floxed *BRAF*V600E allele encoding BRAF V600E, classical LCH-like lesions are induced in both, with the *CD11c*-Cre mice suffering a more severe disease akin to multi-system LCH[36,39].

Two tissues are of particular interest in LCH models: the brain, because LCH-ND is particularly debilitating and difficult to treat[4], and the lung, because adult pulmonary LCH is associated with smoking and *RAS* mutations are common[27]. Transgenic models have addressed both these aspects of LCH. Mosaic expression of BRAF V600E in erythromyeloid progenitors of mice induces clonal expansion of tissue macrophages after birth and spontaneous occurrence of severe progressive ND, associated with accumulation of ERK-activated microglia and neuronal cell death (Fig. 3). Treatment with the BRAF inhibitor PLX4720 delays the development of the disease[54], indicating that early treatment of patients with CNS symptoms with inhibitors might have therapeutic value.

A conditional model for pulmonary LCH has been developed using an adenoviral Cre recombinase vector with a cytomegalovirus promoter to deliver mutant KRAS to the lungs of mice (AdCre/KRAS^{G12D} mice)[55]. LCH-like lesions are induced composed of CD11c$^+$, F4/80$^+$, CD207$^+$ cells. BRAF V600E introduced by the same means fails to induce hematopoietic tumors. Treatment of the AdCre/KRAS^{G12D} mice with a statin ameliorates the disease[55]. These results suggest that site or tissue-specific factors may be important in the development of localized LCH and illustrate the potential of transgenic models to illuminate aspects of LCH biology and test new therapeutic strategies.

Conclusions

At the time of the first Nikolas Symposium, patients with LCH and their families endured the challenges facing those with a rare and poorly understood disease. In the 30 years that have passed, much has changed. Information is readily available for patients and their families through the internet, and international collaboration in research on rare diseases has similarly been greatly facilitated, as has consultation among doctors encountering such rarities as LCH. As well as providing support for patients and their families, patient organizations have made available seed funding for research, and the identification of LCH (and ECD) as neoplastic diseases has meant that they are eligible for cancer research funds. The Nikolas Symposia have contributed to this progress by bringing together patients, doctors and scientists in a forum that has promoted open discussion of the difficult clinical and scientific questions posed by LCH and other histiocytoses and has inspired many individuals to work on these diseases (Fig. 1). The symposia provide a model that could be exploited for other rare diseases, including other rare cancers that in aggregate make up almost 20% of all cancers.

Nevertheless, although LCH may have come of age as an inflammatory hematopoietic neoplasm, many questions remain to be answered. Although it is clear that in most, if not all, cases of LCH and ECD, driver mutations are present, LCH is not a typical malignancy. Present evidence indicates that in most cases, apart from the driver abnormality, there are few other genetic changes or chromosomal abnormalities. This may be in part because LCH occurs most frequently in young children, whereas most other types of tumors occur in older individuals, although the genomes of adult LCH lesions also have almost no additional abnormalities. Data on pulmonary LCH indicates that tissue-specific and environmental factors contribute to the development of the genetic lesions in LCH cells and their survival, a hypothesis supported by the failure of attempts to grow peripheral lesional LCH cells ex vivo or in immunocompromised mice. That LCH survival is so dependent on extrinsic factors may eventually open up new therapeutic approaches.

In the second symposium, the question of viruses as a trigger for an inflammatory process was considered, and recently an association between the Merkel cell polyoma virus and a subgroup of LCH has been described. It is suggested that driver mutations occur in LCH cells in the context of an inflammatory response to the virus[56]. Other viral or inflammatory triggers cannot be ruled out as contributing factors in LCH etiology, perhaps dictating where the formation of lesions occurs (Fig. 3).

Identification of mutations in signaling pathways, and particularly the ubiquity of BRAF V600E and other kinase mutations in both LCH and ECD, has at long last provided a rational target for therapy of these diseases. Early experience is encouraging, in that dramatic clinical responses have been seen with kinase inhibitors in both adults and children, but recurrence occurs when therapy is stopped and mutant cells persist in blood and marrow[52,53]. While kinase inhibitors are a rational therapy and have already saved many lives, durable treatment-free remission remains unexpectedly and frustratingly elusive. Elimination of detectable blood and bone marrow mutant cells may provide a tool to identify new therapies capable of reaching the ultimate goal of the Nikolas Symposia: to provide a rational basis for a cure. ❐

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Competing interests

The authors declare no competing interests.