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REVIEW

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Langerhans cell histiocytosis is a neoplasm and consequently its recurrence is a relapse

In memory of Bob Arceci

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Abstract

Langerhans cell histiocytosis (LCH) remains a poorly understood disorder with heterogeneous clinical presentations characterized by focal or disseminated lesions that contain excessive CD1a+ langerin+ cells with dendritic cell features known as "LCH cells." Two of the major questions investigated over the past century have been (i) the origin of LCH cells and (ii) whether LCH is primarily an immune dysregulatory disorder or a neoplasm. Current opinion is that LCH cells are likely to arise from hematopoietic precursor cells, although the stage of derailment and site of transformation remain unclear and may vary in patients with different extent of disease. Over the years, evidence has provided the view that LCH is a neoplasm. The demonstration of clonality of LCH cells, insufficient evidence alone for neoplasia, is now bolstered by finding driver somatic mutations in *BRAF* in up to 55% of patients with LCH, and activation of the RAS-RAF-MEK-ERK (where MEK and ERK are mitogen-activated protein kinase and extracellular signal-regulated kinase, respectively) pathway in nearly 100% of patients with LCH. Herein, we review the evidence that recurrent genetic abnormalities characterized by activating oncogenic mutations should satisfy prerequisites for LCH to be called a neoplasm. As a consequence, recurrent episodes of LCH should be considered relapsed disease rather than disease reactivation. Mapping the complete genetic landscape of this intriguing disease will provide additional support for the conclusion that LCH is a neoplasm and is likely to provide more potential opportunities for molecularly targeted therapies.

KEYWORDS

Langerhans cell histiocytosis, LCH, neoplasm

1 | INTRODUCTION

For 25 years, basic scientists and clinicians have met in May at an interactive "think tank" known as the Nikolas Symposium to discuss the biology, pathophysiology, and clinical features of Langerhans cell histiocytosis (LCH).[1] This annual scientific symposium is sponsored by Paul and Elizabeth Kontoyannis in honor of their son Nikolas, a long-term survivor of LCH, who suffered from severe multisystem disease in addition to experiencing significant late effects. Together with research-minded physicians, they organized the first Nikolas Symposium, now some 30 years ago. The overall mission of the Nikolas Symposium is to find a rational cure for LCH, through the understanding of the disease rather than therapy on its own. These annual "think-tank" Symposia—25 to date—have continued to provide a forum to bring together

clinician scientists and a diversity of scientific experts (each year some 25 individuals) to discuss the problems of LCH, pinpoint research questions, and carry out the research. As a result, several participants worldwide have interacted, often in collaboration with the Histiocyte Society.

Topics discussed over the years include (i) the biology and origin of the Langerhans cell (LC) within the disease versus the physiologic LC, (ii) clonality and what would this mean in LCH, (iii) the role of cytokines and chemokines in LCH, and (iv) whether LCH is an immune dysregulation or a neoplasm. The Nikolas Symposium has been a catalyst on a lot of research in LCH, but clearly much research has also been performed completely outside the scope of these Symposia. Although LCH is a rare disease, the past 25 years have witnessed a dramatically increased understanding of the biology and treatment possibilities for patients with LCH as well as insights into dendritic cell (DC) physiology.

Since the discovery of LCs during the last part of the 19th century,[2,3] these cells have generated great interest for researchers and clinicians. Besides their physiologic roles in the immune system and in tumor surveillance, LCs are also thought to be the key pathological cells in the spectrum of disorders collectively referred to as LCH. Already in 2001, the World Health Organization (WHO) classified LCH as a neoplastic proliferation of LCs.[4] Despite this WHO definition, and reiteration by the WHO over the last decade,[5] within the LCH community until recently there has been some doubt.

The recently published revised classification of histiocytosis and neoplasms of the macrophage- DC lineages consists of five groups of diseases, in which LCH is grouped in the class "Langerhans-related."[6] Important shortcomings in LCH research are in part due to the fact that the vast majority of studies involve small numbers of patients and often examine tissue only from easily accessible anatomic sites, for example, bone or skin lesions, using low-resolution and low dimensionality techniques. Furthermore, most analyses are retrospective. Results that may seem highly relevant to pathogenesis or treatment are often drawn either from a single case study or from animal models with limited applicability to the human disease. However, in a rare, heterogeneous disease such as LCH, in which cell lines and true *in vivo* models are lacking, these studies may provide what is often the best available evidence.

2 | THE LCH CELL OF ORIGIN

The question regarding cell of origin for this intriguing disorder has been intensively pursued. Initial description of LCs by Paul Langerhans suggested that these cells were related to neurons and were intraepidermal nerve endings, owing to their impregnation with gold chloride, which was thought to be specific for neurons.[2,3] The cells remained an enigma to scientists for decades[7] and it took more than 100 years to establish their hematopoietic origin, similarities with DCs, and function in the immune system,[8] a discovery that was recognized in part, by awarding the Nobel Prize in Physiology and Medicine in 2011 to Dr. Ralph M. Steinman (www.nobelprize.org/nobel_prizes/medicine/laureates/2011/), who for years served on the Steering Committee of the Nikolas Symposium. In addition, among several similarities between LC and LCH cells (see next), expression of prototypical LC markers such as CD1a by LCH cells, and their localization at the dermoepidermal junction suggested that the LCH-initiating cells were related to LCs.[9] However, the first decade of the 21st century has seen a revision of the notion that LCs are prototypical migratory tissue DCs because of a number of unique aspects regarding their ontogeny and function. As a consequence, it is now evident that not all LCH cells are related to *bona fide* LC, but are rather related to DC.

LCs, in contrast to other tissue DCs, are continually self-renewing in the steady state and during low-grade inflammation.[10,11] This renders them independent of bone marrow derived precursors under normal physiological conditions, although when the epidermis is breached, they can be replaced by blood-borne myeloid cells.[7,12] The LC population is in fact established before birth independently of adult bone marrow hematopoiesis.[13,14] In mice, the embryonic origin of LCs even predates the onset of definitive hematopoiesis, with most of their precursors arising from fetal liver monocytes and a minority from yolk sac macrophages.[14,15] Importantly, LC differentiation and homeostasis is regulated by colony stimulating factor (CSF)-1 receptor signaling, rather than Flt-3 as for DCs.[16] Although LCs are able to differentiate fully into afferent lymphatic DCs, their steady-state gene expression profile overlaps with macrophages, leading one of us (FG) to comment that they have a "macrophage history but a dendritic cell future."[17]

Although equivalent studies cannot be carried out in humans, several reports suggest that human LCs share similar properties. In humans, the infiltration of embryonic dermis with LC precursors can be observed at early time points.[18] Proliferating LCs were identified in human skin,[19,20] and graft-resident LCs were also found to remain for several years in a transplanted human limb graft.[21] In addition, studies of hematopoietic stem cell recipients showed that LCs become donor-derived when there is inflammation due to graft versus host disease.[22,23] The earlier mentioned plasticity was reiterated in LCH patient material, when in 2005 the Egeler lab studied the multinucleated giant cells (MGCs) in LCH. MGCs in nonostotic lesions, besides expressing characteristic osteoclast markers, also co-expressed CD1a under the influence of osteoclast- and DC-inducing cytokines such as M-CSF and GM-CSF, respectively, in the lesions. Obviously, the osteoclast-derived enzymes play a major role in the tissue destruction in bone in the well-known osteolytic lesions of LCH, thus providing a rationale for antiosteoclast therapy in patients with bone lesions.[24]

LCH cells and LCs share a number of phenotypic characteristics. For example, Birbeck granules are observed in both normal LCs and LCH cells. Histopathology links the two cell types further by the high expression of CD1a and langerin. However, the straightforward inference that LCH cells are derived from mature LCs that have been "transformed" has recently been challenged by gene expression studies. Comparisons of gene expression between LCH cells and LCs indicate that LCH cells are considerable less mature than LCs and are as close to myeloid DCs as they are to LCs.[25,26] Earlier chemokine receptor expression and chemokine production studies already indicated the immaturity of the LCH cells, and as confocal studies showed, CD1a-

positive LCH cells predominantly co-express CCR6, the immature DC marker.[27] Furthermore, the finding of *BRAF* mutations in circulating myeloid precursor cells also points to an early myeloid cell as an LCH precursor in some cases.[28] In summary, the pathognomonic cell in LCH arises from the hematopoietic precursor cell, probably the myeloid DC precursor cell, and has LC features.

3 | THE QUESTION OF NEOPLASM

For pediatric oncologists and immunologists, a major question has been whether LCH is fundamentally an inflammatory disorder of immune dysregulation or a neoplasm.[9,29–31] The points favoring LCH to be a reactive disorder were the indolent nature of many cases of LCH with documented occurrence of spontaneous remission; remissions with anti-inflammatory treatment; infection-associated flare-ups; evidence that inflammatory lesions can show immature LCs; nonclonality in pulmonary LCH; sporadic disease in the vast majority of cases; low-grade cytological appearance with a low proliferation index; prominent inflammatory infiltration of T cells, eosinophils, and other "accessory" cell types; absence (until recently) of recurrent genetic abnormalities; and rare mutations in TP53 despite common overexpression of the protein.[32–41]

The spontaneous regression of LCH lesions, particularly single-system lesions, which has often been cited as supporting evidence that LCH is an inflammatory disorder, needs further evaluation. In a study of 49 pediatric patients with LCH, the *FAS*/*FAS*-ligand pathway was shown to be active in LCH and may be a reason for the spontaneous regression of lesions in some cases of single-system LCH.[38] The same group demonstrated that the pathologic LCs in patients with LCH express all three *FAS*-related proteins, that is, FADD and FLICE (both pro-apoptotic) along with FLIP (anti-apoptotic), and the net outcome depends on the balance of these expressed proteins.[42] In patients with multisystem disease, this delicate balance (death vs. survival) may be altered causing the LCH cells to survive rather undergo apoptosis. Although a relationship between these expressed proteins and clinical outcome could not be established, the *FAS* signaling pathway may be involved in the pathogenesis of LCH.

In contrast, the points favoring LCH as neoplastic are the clonality of LCH cells, the presence of somatic genetic abnormalities, rare cases of familial clustering with high concordance between monozygotic twins, and evidence of apparent maturation arrest of LCH cells *in vivo*.[27,43–47] Furthermore, short telomeres[48] and even different telomere lengths in single versus multisystem LCH[49] have been reported in the LCH cells, but not in normal DCs or lymphocytes from the same patients, as seen in myelodysplastic syndromes.

From a scientific point of view, as well as a practical view with implications as to how treatments are developed and tested, several key, minimal criteria are helpful to establish a disorder as neoplastic. These include (i) evidence for clonality among the cells driving the disease pathophysiology; this acknowledges that neoplasms may display clonal heterogeneity at the molecular level and (ii) evidence for one or more mutations or molecular alterations that converge on common, key cellular pathways that drive neoplasia.

3.1 | Clonality and LCH

Clonal expansion and evolution are considered evidence of neoplastic natural selection.[50,51] In 1994, Willman et al. studied nonsorted cells of LCH lesions for human androgen receptor polymorphisms and T-cell receptor rearrangements and reported evidence for clonality among pathological LCs but not T cells.[43] In the same year, Yu et al. used the same technique and reported the clonal nature of sorted LCH cells from lesions, in contrast to T cells of the same patients.[32] Of note, subsequent data demonstrated that the pulmonary LCH, found primarily in adults and strongly associated with smoking, proved to be polyclonal in a significant number of cases.[44]

There is considerable literature showing the association of LCH cases with other malignancies.[50,51] The observation that in the majority of the patients with acute lymphoblastic leukemia (ALL) in association with LCH, the pediatric ALL was of T-cell origin initiated discussion of a common precursor cell. More case reports of combined LCH and T-ALL[52,53] supported the view that LCH likely arises from a clonogenic cell at an early stage of differentiation. More genetic and molecular studies on tissue from these fascinating cases should provide more insight into their origin and the plasticity in cells of the monocyte/macrophage and DC lineage, as for other cells of the hematopoietic and lymphoid lineages. In a patient who developed LCH following T-ALL,[54] both tumor tissues harbored the same T-cell receptor gene rearrangement activating NOTCH-1 mutation. The NOTCH signaling pathway is involved in T-cell development,[55] and gain-of-function mutations of this pathway are a commonly acquired genetic lesion in T-ALL.[56] The NOTCH ligand Jagged2 (JAG2) is also expressed in LCH cells.[25] Furthermore, in normal monocyte-derived DCs, NOTCH signaling triggered either by JAG2 or other NOTCH ligands stimulated the expression of specific LC markers.[57] These findings suggest that the LC nature of LCH cells may be induced in aberrant myeloid precursor cells by NOTCH signaling[25] and that blockade of this pathway could be considered as a therapeutic strategy in LCH.[54]

In summary, LCH is a clonal disorder, and in some cases LCH and T-ALL may even occur as clonally related diseases with a common pathogenetic background. These phenomena are consistent with LCH being a neoplasm.

3.2 | Driver mutations and LCH

Driver mutations lead to initiation and progression of malignancies, while modifying mutations may produce various physiological characteristics, such as altered drug resistance, and still other mutations (so-called "passenger mutations") may not contribute to the malignant phenotype.[58,59] While studies have shown that in the majority of cases LCH is a clonal neoplasm, they did not provide a commonly shared alteration in a specific gene or pathway. Earlier studies using multitargeted molecular approaches could not show any consistent genomic aberrations,[37] and the search for cryptic point mutations was on. An activation mutation in the BRAF gene, leading to the production of a BRAF V600E mutant protein, was found in more than half of all LCH

cases suggesting that this is a genuine driver mutation in this disease.[41] The *BRAF* V600E mutation is a driver in several malignancies, with the highest rates in hairy cell leukemia, an indolent chronic leukemia, which has intriguing similarities to LCH in that the bone marrow and blood become populated with tartrate-resistant acid phosphatase (TRAP)-positive CD11c+ "hairy cells" bearing similarities to DCs. Other malignancies with activating BRAF mutations include melanoma, colorectal carcinoma, thyroid carcinoma, and non–small cell lung cancer.

This groundbreaking work used a limited cancer panel of genes for allelotyping and described the oncogenic *BRAF* A1799T point mutation leading to the V600E amino acid change in 57% of patients with LCH.[41] Single TP53 and MET mutations were also found in this cohort. Importantly, activation of the RAS-RAF-MEK-ERK (where MEK and ERK are mitogen-activated protein kinase and extracellular signal-regulated kinase, respectively) pathway, evidenced by phosphorylation events in the pathway, was reported in 100% of the investigated samples, irrespective of the presence of BRAF mutation, suggesting that alternative genes might be mutated and contribute to the activation of this pathway in LCH. Supporting this view, the intensity of MEK-ERK staining did not depend on whether *BRAF* was mutated or not. The method used in this study to detect mutated genes was not exhaustive and it was concluded that with further research, additional mutations or genetic abnormalities are likely to be found.[41] These authors as well as the accompanying editorial suggested that, based on this critical finding, we need to consider clinical trials to evaluate the therapeutic potential of BRAF inhibitors in patients with LCH.[41,60] Furthermore, the possibility was raised to employ mutated BRAF measurement as a means to determine minimal residual disease in mutation-positive patients.[60] Subsequently, additional studies have confirmed the presence of mutated BRAF in similar frequencies in LCH.[61,62]

Interestingly, the *BRAF* mutation was also found in a significant proportion of patients with Erdheim–Chester disease (ECD), a non-LCH, but not in other histiocytoses.[63] Among these cases were some patients with mixed ECD and LCH. In the "mixed" cases expressing *BRAF V600E*, treatment with a *BRAF* inhibitor produced clinical responses, prima facie evidence that in these specific patients mutant *BRAF* is a driver of LCH. The prevalence of the *BRAF* driver mutation fulfills one of the most important criteria for LCH to be called a neoplasm. Data on *BRAF* mutations in LCH from discovery to driver mutation in LCH are summarized in Table 1.[41,63–82]

The detection of mutated BRAF already shows potential clinical applications. In the original reports, there were no clear correlates of BRAF status with clinical features, but a subsequent study of 100 pediatric patients suggests that *BRAF* V600E+ LCH has a higher rate of relapse.[83] Although earlier reports failed to detect *BRAF* V600E in peripheral blood using next-generation sequencing,[62] Berres et al. report that allele-specific polymerase chain reaction (PCR) was able to detect *BRAF* V600E in active multisystem LCH, but not in single-system or quiescent disease.[28] These findings suggest that assessment of clinical risk and monitoring of response to therapy may both be assisted by the detection of mutated BRAF. Due to the difficulty of detecting the BRAF mutation in LCH lesions, Hyman et al. applied a droplet digital PCR assay in plasma and urine for the quantitative detection of the BRAF mutation in a combined ECD/LCH cohort, which provided reliable results.[84] As only 17% of the patients in this study were diagnosed with LCH, this relatively easy and noninvasive method should be studied in larger LCH cohorts.

The ERK signaling pathway is activated in all pathologic CD1a+ histiocytes in patients with LCH regardless of *BRAF* mutation status, suggesting that other mutations in this pathway might be present. Using whole exome sequencing on DNA from purified CD1a+ LCH cells, a case with mutant ARAF was reported, further linking genes in this ERK signaling pathway.[85] Brown et al., using a targeted next-generation sequencing approach, found that approximately 50% of the BRAF wild-type cases harbored MAP2K1 mutations.[86] Subsequently, others confirmed the presence of *MAP2K1* mutations but at lower frequencies.[83,87] All studies showed that mutations in *BRAF* and *MAP2K1* were mutually exclusive in any given LCH case, which confirmed that BRAF and MAP2K1 are acting in the same transformation pathway resulting in constitutively active ERK. Recently, whole exome and transcriptome sequencing in a combined cohort of LCH and ECD patients identified new kinase fusions involving *BRAF*, *ALK*, and *NTRK1*, which clearly identifies new mechanisms for activating clinically tractable kinase pathways in histiocytoses.[88] Although these fusions were found only in the non-LCH patients, they raise the possibility of important structural genomic changes in LCH.

The inflammatory environment in LCH lesions is now well established and its clinical implications are being evaluated. In LCH lesions, T cells and LCH cells are in a proximity and LCH cells display prominent expression of CD40, while the T cells express CD40 ligand.[36] This interaction activates T cells, which are the main source of hematopoietic growth factors and inflammatory cytokines,[35] leading to increased cytokine production in LCH that is likely responsible for establishing a clinical picture with similarities to an inflammatory disorder. The activation of the RAS-RAF-MEK-ERK pathway may also provide new avenues and explanations for the association of tumor inflammatory responses and disease progression. The concept that tumor cells can elicit an immunologically inflammatory environment to sustain their survival and regulate treatment responses has been well documented.[89] The increased incidence of colorectal carcinoma in patients with inflammatory bowel disease and melanoma in patients with cutaneous inflammatory disorders are examples of cancer and inflammation associations. Many such tumors show activation of the RAF-MEK-MAPK pathway.

Cell-line experiments have shown that the NOTCH pathway is involved in the crosstalk with the ERK pathway, although the exact link remains unknown.[90] One consequence in LCH might be that activation of the ERK pathway leads to increased cellular inflammatory responses through the NOTCH pathway. This in turn leads to the hypothesis that the inflammatory response observed in LCH may be a consequence of the initiating RAF family gene mutation rather than a primary cause of the disease. However, experimentation done thus far cannot rule out an initiating inflammatory event that leads to mutation through, for instance, reactive oxygen species.[90,91] Further research, and, possibly, animal models, may help unravel such key questions.

TABLE 1 History of *BRAF*: From Oncogene to driver mutation in histiocytosis

Year	Discovery	Significance of discovery	Ref.
1988	Raf-1-related oncogene found in avian retroviruses and named v-Rmil	For the first time *in vivo* the Raf family was reported as oncogenic	64
1988	Human *BRAF* oncogene was identified	*BRAF* oncoprotein is tumorigenic	65
1988–2002	Many studies	RAF proteins seem not to be mutated in human tumors	Reviewed in 66
2002	*BRAF* somatic missense mutations in 66% of malignant melanomas and a lower frequency in various other human neoplasms	It was established that *BRAF* oncogene is associated with chromosomal aberrations in human neoplasms; the most common mutation found was $BRAF^{V600E}$ and the resultant oncoprotein is constitutively active and capable of cell transformation	67
2002–2012	Various studies reported mutated *BRAF* in colorectal neoplasm, papillary thyroid carcinoma, ovarian carcinoma, glioma, lung adenocarcinoma, sarcoma, breast neoplasm, liver neoplasm, hairy cell leukemia	Established *BRAF* mutation as a frequently occurring genetic abnormality in human cancers and precancerous lesions	68–74
	BRAF mutation found in more than 50% of the patients with LCH	First report of a recurrent genetic abnormality in LCH	41
	BRAF mutations in knock-in mice showed the tumorigenic potential of mutated oncoprotein	*BRAF* mutation established as driver mutation in melanoma, colorectal carcinoma, thyroid carcinoma, non–small cell lung neoplasm, and low-grade glioma	75–80
	High prevalence of *BRAF* mutations in patients with Erdheim–Chester disease	First report of a recurrent genetic abnormality in non-LCH histiocytosis	63
2013	Inhibition of *BRAF* as a therapeutic strategy	Reported response in BRAF-driven histiocytic disorders	81,82

The presence of BRAF mutations may also paradoxically explain spontaneous remissions in LCH. Acute expression of a strongly active, dominant oncogene in most normal cells leads to senescence or apoptosis. This response is thought to be a cellular mechanism that protects the organism from cancer. Support for this hypothesis comes from nevi that express BRAF V600E. The benign nature of these lesions and their occasional spontaneous remission has been suggested to be an example of oncogene-induced senescence *in vivo*. It is possible that some LCH cells expressing *BRAF* V600E undergo several rounds of division followed by induction of senescence or apoptosis, which would be interpreted clinically as a spontaneous remission. However, direct evidence in support of this mechanism remains to be found.

In conclusion, up to 75% of all LCH cases have the proof of activation by a driver mutation, which is critical for the transformation of the progenitor cells leading to LCH. The identification of potential cooperating genes remains an area of active research.

3.3 | New treatment considerations

LCH is a clonal disorder now characterized in over 50% of cases by the presence of activating *BRAF* mutations, in 25% by activation of *MAP2K1*, and in the remaining 25% by activation of the RAS-RAF-MEK-ERKAPK pathway through mechanisms that remain unknown (Fig. 1).[90,91] The ERK pathway contributes to cell survival, proliferation, motility, differentiation, and is usually activated by controlled exposure to growth factors or mitogens. Further, inherent in the normal activation of such a pathway is its suppression through homeostatic inhibition pathways. However, when a key component of the pathway, such as BRAF, develops an activating mutation, constitutive signaling occurs, leading to uncontrolled and pathological proliferation and cell survival. Of note, the RAF gene family has three members (*A-RAF*, *BRAF*, and *C-RAF* [*RAF-1*]). The ERK pathway is found to be activated, usually through mutations in *RAF* genes, in 6–8% of human neoplasms, with *BRAF* V600E the most common mutation.[67] RAF family proteins is phosphorylated by activated RAS proteins for which there are also three family members, *H-RAS*, *K-RAS*, and *N-RAS*, with *K-RAS* the most frequently mutated member. Together, *RAS* gene mutations are observed in approximately 20% of all human cancers.[92] In 1990, enhanced expression of c-MYC and H-RAS was reported in patients with LCH.[93] In a related observation, stabilization of MYC in a neuroblastoma cell line was dependent on activation of the RAF-1 (C-RAF) or PI3K pathways, thus making a potentially important association of RAS activation and MYC stabilization.[94] Inhibition of RAS activation with the RAS inhibitor farnesyl thiosalicylic acid in these cell lines resulted in a significant decrease of active RAS, RAF-1, and PI3K, suggesting the possibility of an additional therapeutic target in LCH.

The clinical experience from treatment of patients with melanoma with BRAF inhibitors provides insight into potential trial design for patients with LCH, as they have the same *BRAF* V600E mutation. In an early phase 3 trial of patients with melanoma, the first RAF inhibitor (Sorafenib, which also inhibits VEGFR and PDGFR) failed to demonstrate any benefit in survival.[95] However, the development of more selective inhibitors of BRAF V600E led to substantial responses and increases in overall survival in patients whose melanomas express mutant BRAF.[96] Unfortunately, essentially all melanoma patients who initially respond to these BRAF inhibitors develop resistance and experience relapse. Resistance results from a variety of mechanisms, including activating mutations in signaling proteins downstream from

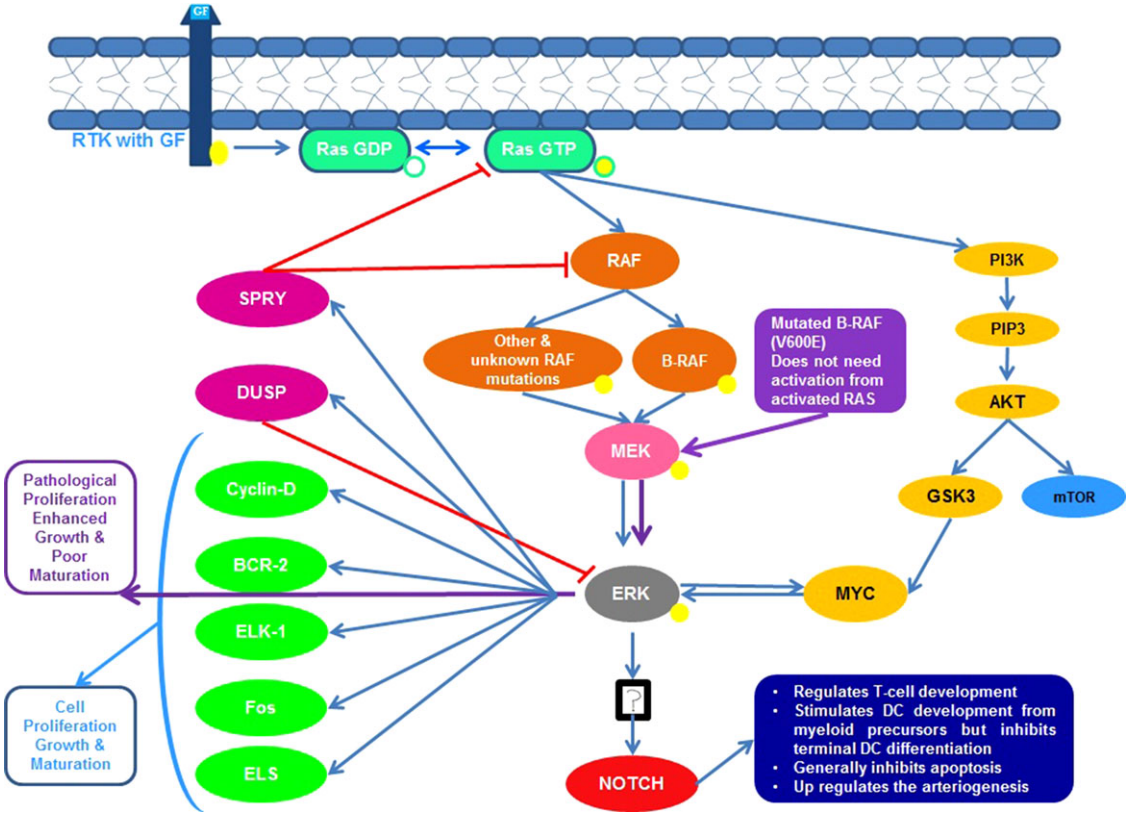

FIGURE 1 Interacting molecular pathways and cellular processes affected by mutant V600E BRAF in LCH cells.
RTK, receptor tyrosine kinase; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinases.
Inhibitory regulators: SPRY, Sprouty protein, has inhibitory effect on RAS and RAF; DUSP, dual-specificity phosphatase, has inhibitory effects primarily on ERK and maybe on MEK.

BRAF, such as MEK1. Relapse is also associated with activation of the *PI3K/AKT* pathway (Fig. 1). A subsequent trial combining BRAF and MEK inhibitors in patients with metastatic melanoma demonstrated a significant improvement in the duration of response. These results also suggest the possible utility of testing combinations of inhibitors of the ERK and AKT pathways. Although the frequency with which resistance to BRAF inhibitors alone appears in LCH is not yet known, there are as yet no reported cases of acquired resistance.

The first report of the use of BRAF V600E inhibitors in patients with histiocytic disorders demonstrated objective responses using vemurafenib in both LCH and ECD.[81] More "proof of principle" followed, but often in single cases[97–99] or in a combined cohort of LCH and ECD, which showed a response rate of 43%, but without indication of whether these were patients with ECD or LCH.[100] Although the follow-up of these patients has been short, the documentation of objective responses demonstrated the need to study such agents in prospective, clinical trials. However, feedback mechanisms in patients with wild-type BRAF exposed to first-generation BRAF inhibitors can lead to up to a 35% incidence of skin cancers, including squamous cell carcinomas and melanoma.[79] Such adverse side effects would have significant implications for patients, especially the very young and those with limited stage and non–life threatening LCH. Thus, patients with activating BRAF mutations and severe, progressive disease may provide a more optimal group of patients with LCH in whom such clinical trials can be performed. One can expect continued active research to find more mutations in patients with LCH, and targeting those mutations will hopefully reduce the frequency of late relapse.

3.4 | Relapse rather than reactivation

The rate of disease recurrence in single-system monoostotic LCH is approximately 10%, and can be as high as 25% in polyostotic LCH.[94,101] Furthermore, in multisystem LCH up to 50–70% of cases show recurrent disease after initial remission. Referring to such recurrent disease as reactivation appears disingenuous in light of the molecular data demonstrating that LCH is a neoplasm driven by specific mutations, thus, referring to recurrent disease is more consistent with the language used for describing remission and relapse of a neoplastic disease. Thus when LCH recurs, the concept of disease recurrence, relapse, or progression should replace the concept of reactivation, which, instead, suggests a primary immunoregulatory etiology. While the essence of a disease cannot be completely conveyed by its name or classification alone, getting this part right is fundamentally important in carrying out subsequent investigation and clinical trials in the right direction.

4 | CONCLUSION

LCH is a clonal neoplastic disorder characterized by subtle chromosomal changes and, importantly, an apparently obligatory activation of the RAF-MEK-ERK pathway, most often through mutations of RAF

proteins. This should lead to clinical trials using therapeutic agents based on the current described molecular findings and consequently targeted the eradication of the disease-initiating cell, the myeloid DC precursor with LC features.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CSF	colony stimulating factor
DC	dendritic cell
ECD	Erdheim–Chester disease
ERK	extracellular signal-regulated kinase
LC	Langerhans cell
LCH	LC histiocytosis

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