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## Introduction

Our understanding and treatment of the diseases known as the histiocytoses have undergone revolutionary changes since the turn of the century. While still recognized as a clinically heterogeneous collection of disorders having a somewhat arcane taxonomy, advances in molecular analyses have revealed pathway abnormalities shared by several of these entities. Other advances in stem cell and lineage analyses have shed light on the biological differences that individuate clinically distinct presentations. Most importantly, these insights have led to new therapeutic opportunities that have so far shown tremendous clinical promise.

The histiocytoses are characterized by the accumulation of cells having morphologic characteristics that are reminiscent of histiocytes. Although histiocytes are technically defined as tissue resident macrophages, the abnormal cells of the histiocytoses are thought to derive both from macrophage and dendritic cell lineages. The clinical and pathological characteristics of histiocytoses are varied, and several attempts have been made over the years to create a rational taxonomy [1, 2]. Thanks to a number of recent

discoveries, some of which are described below, a new classification scheme has been proposed [1] in which histiocytic disorders are grouped into five categories: L Group (Langerhans cell histiocytosis, Erdheim-Chester disease, intermediate cell histiocytosis), C Group (cutaneous non-LCH diseases such as juvenile xanthogranuloma, cutaneous Rosai-Dorfman disease, and others), R Group (non-cutaneous Rosai-Dorfman disease in its many manifestations), M Group (malignant histiocytoses), and H Group (hemophagocytic lymphohistiocytosis). This chapter will address the biology and genomics of the diseases in the L Group.

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## Pathobiology

*Langerhans Cell Histiocytosis* The histiocytes in Langerhans cell histiocytosis (LCH) share several features with normal Langerhans cells (LCs) including the expression of CD1a and CD207 (or Langerin) and the presence of cytoplasmic organelles known as Birbeck granules. Some of these characteristics are actually pathognomonic for LCH. As a result, normal LCs have been thought to be the cell of origin for LCH histiocytes [3, 4]. Nonetheless, as discussed below, the presence of morphologic features characteristic of a specific normal cell type does not necessarily prove that the abnormal histiocyte is derived from that cell type.

As described in detail elsewhere in this volume, LCH is predominantly, although not

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exclusively, a disease of childhood with a peak incidence between 5 and 10 years of age [5, 6]. Some versions, such as the one known eponymously as Letterer-Siwe disease, can affect neonates or infants and, in its disseminated form involving skin, lymph nodes, spleen, and liver, can be associated with 20% mortality rate [7, 8]. The most common forms of LCH may typically involve the bone, the skin, and the anterior pituitary with concomitant diabetes insipidus. These forms of LCH are rarely seen in adults, but the more common adult presentation is pulmonary LCH, which usually occurs in smokers.

This broad spectrum of clinical behaviors and outcomes formed the basis of a complex nosology in which each clinical version was considered to be a distinct entity (summarized in [9]). This approach was bolstered by differences in treatment responses [10]. Disseminated Letterer-Siwe disease required more aggressive therapy and had worse outcomes than Hand-Schüller-Christian disease or eosinophilic granuloma. The discovery that the histiocytes in all forms of this disease share attributes of normal LCs was a remarkable advance that suggested that they also share a common pathobiology. However, this grand unification has been unable to shed light on pathogenesis or on the mechanistic basis for the disparate clinical behavior of the various subtypes of LCH.

**Erdheim-Chester Disease** Unlike LCH, the histiocytes in Erdheim-Chester disease (ECD) have the morphology of foamy macrophages and express macrophage surface markers such as CD68 and CD163 rather than markers that are characteristic of LCH [11]. In many ways, the clinical presentation of ECD could not be more different than LCH [11]. ECD primarily affects adults with a peak incidence between 50 and 70 years of age and is rarer than LCH. The disease affects the long bones, CNS, skin, heart, aorta, and kidneys, the latter often compromised by retroperitoneal fibrosis. Skin involvement is frequently manifested as xanthelasma, consistent with the appearance of fat-laden histiocytes in involved tissues. Despite this very different clinical presentation and the macrophage-like

characteristics of its histiocytes, mixed ECD and LCH may be seen simultaneously in the same patient. In a large ECD cohort from France, this kind of mixed histiocytic picture was observed in 19% of the patients [12]. This is a remarkably high prevalence given the evidence supporting different cells of origin in the two diseases and their disparate clinical presentations and raises the possibility of a shared early precursor.

**Indeterminate Cell Histiocytosis** Indeterminate cell histiocytosis (ICH) is a rare disease characterized by a generalized cutaneous eruption in adults, although some cases may only involve lymph nodes [13–15]. Based on case reports, ICH appears predominantly to affect women. The lesions consist of a non-epidermotropic infiltration of histiocytes that share some characteristics of LCH histiocytes such as CD1a positivity. In contrast to LCH histiocytes, however, ICH histiocytes do not express CD207 and do not have Birbeck granules. Although this mixed picture had led some investigators to suggest that ICH might be a variant of LCH, recent molecular data, described below, indicates that it is a separate disease entity.

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## Genomics

### Neoplasia vs Inflammatory Disorder

Each of the diseases in the L Group of histiocytoses is remarkable for being associated with a prominent inflammatory infiltrate. For example, LCH lesions commonly contain an impressive number of eosinophils. In fact, the version of LCH that involves a small number of sites in long bones was known as eosinophilic granuloma. Thus, initial hypotheses about pathogenesis suggested that LCH might be an inflammatory disorder. This notion was supported by documented cases of spontaneous remissions even in advanced forms of Letterer-Siwe disease [16, 17]. Further, even though the lesions in histiocytoses are granulomatoid, decades of searching for possible infectious, autoimmune, or exposure associations have

been fruitless. Reports of EBV, CMV, HHV-6, and Merkel cell polyomavirus in LCH samples have not been confirmed [18–20]. While high plasma levels of cytokines that might be associated with inflammatory conditions, such as GM-CSF, M-CSF, FLT-3L, and IL-17A, have been reported in LCH patients, their role in pathogenesis remains uncertain [(21–27)].

As inflammatory or autoimmune mechanisms were serially hypothesized and then excluded in LCH, it became more reasonable to start looking at this disease and, perhaps, some of the other L Group histiocytoses as being neoplasms. In order for a disease process to be considered neoplastic, two criteria must be fulfilled. First, the abnormal cells that drive the disease must be clonal, and, second, the clonal cells should have evidence for recurrent genetic or epigenetic abnormalities. In 1994, two reports described the use of human androgen receptor gene-based X chromosome inactivation assays (HUMARA assays) to demonstrate that the abnormal histiocytes in LCH are clonal [28, 29]. Thus, one of the conditions for LCH to be classified as a neoplasm was met.

Identifying recurrent genetic or epigenetic abnormalities in LCH has been far more challenging. Until recently, assays for single-nucleotide variants (SNVs), copy number variations (CNVs), translocations, or epigenetic modifiers of DNA have required abundant amounts of fresh frozen tissue. Because the incidence of the histiocytoses is so low and the amount of tissue required to make a clinical diagnosis is so small, frozen samples of LCH or other L Group histiocytoses are rarely available in large numbers. This has led to many reports of various molecular abnormalities, but nearly all were either nonrecurrent or have not been reproduced in subsequent studies. These included nonrecurrent cytogenetic abnormalities [30, 31], loss of heterozygosity at a number of loci [32], and fractional allelic loss in patients with advanced disease [33]. Another study found no significant SNVs or CNVs in a large number of samples [31]. In contrast to these one-off or non-reproducible findings, overexpression of p53, the product of the TP53 gene, is observed

frequently by immunohistochemistry. The basis of this overexpression remains obscure, however, because of the very low frequency of mutations in *TP53* or the genes such as *MDM2* which modulate levels of p53 [31, 34].

Our understanding of the genomic landscape of the histiocytoses was aided by the advent of analytic tools that can identify abnormalities reliably and robustly in formalin-fixed, paraffin-embedded tissue samples. These techniques made available for analysis patient samples in the archives of pathology departments where reasonable numbers of histiocytosis specimens have been stored. One of the first such technologies to be applied to LCH was Sequenom's mass spectrometry-based allelotyping platform [35]. A customized version called OncoMap [36], which tested 983 specific alleles in 115 cancer-related genes, was used to analyze 61 archived LCH cases and demonstrated the presence of the oncogenic mutation encoding the BRAF V600E variant in 57% of the samples [34]. These mutations were confirmed using an orthogonal identification method, namely, pyrosequencing, and a variety of techniques were used to demonstrate that the mutations occurred specifically in the CD1a-positive LCH histiocytes. Thus, LCH cells are clonal, and over half of LCH cases have recurrent oncogenic mutations in *BRAF*, making LCH a neoplastic disease. (This mutation and others are described more fully, below.)

A similar evolution in thinking about the pathogenesis of Erdheim-Chester disease also occurred. As in LCH, HUMARA assays demonstrated clonality although the sample size was very small [37]. While there has been some dispute about the reliability and reproducibility of the clonality assays in ECD [38, 39], the discovery of mutations encoding BRAF V600E in over 50% of ECD lesions [40] suggests that the histiocytes are clonal and demonstrates the recurrent genetic abnormality that classifies ECD as neoplastic.

A definitive assessment of the neoplastic nature of ICH has been much more difficult to ascertain because it is even rarer and more clinically heterogeneous than LCH or ECD.

The literature contains no direct assessment of clonality and only a single case of ICH carrying the mutation encoding BRAF V600E [41]. However, a recent report described a recurrent translocation in three patients (discussed below) which may be interpreted as supporting a clonal and neoplastic origin for ICH [42].

## Recurrent Genomic Abnormalities

### Langerhans Cell Histiocytosis

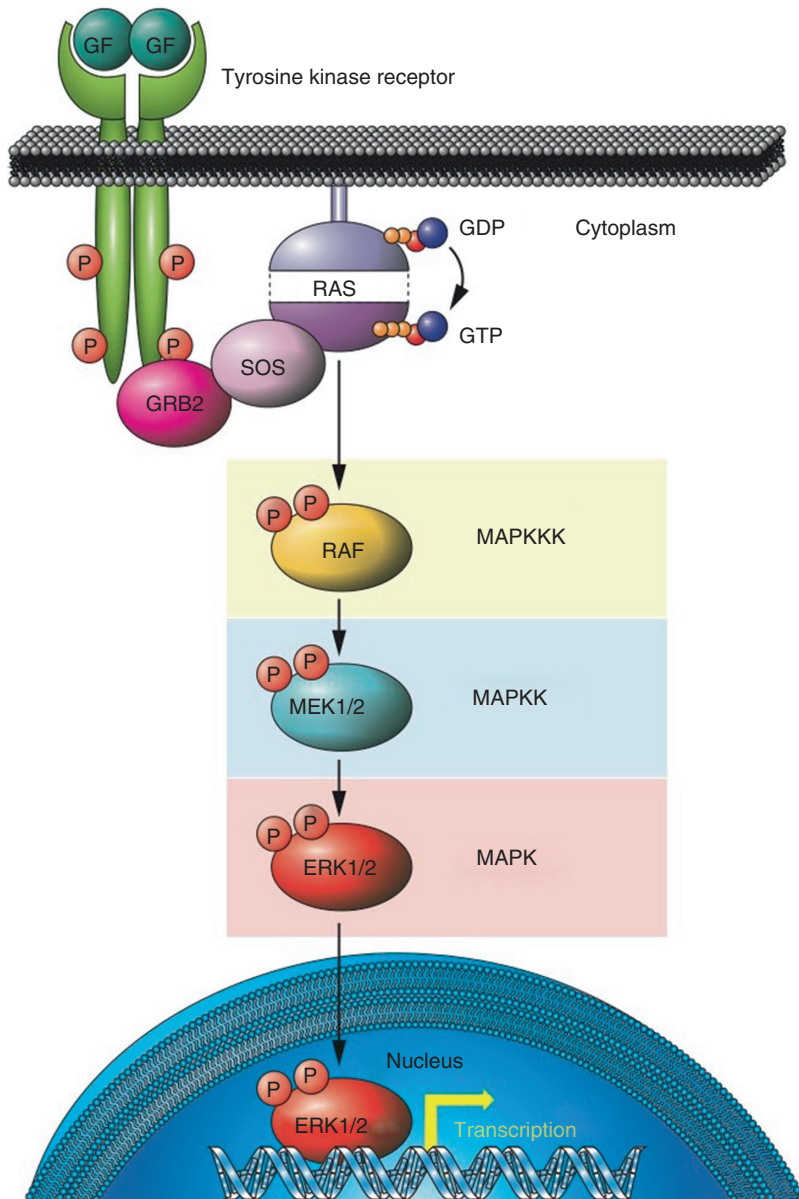
As noted above, the discovery of recurrent *BRAF* mutations contributed to the classification of LCH as a neoplasm. Since then, several groups have used a variety of analytic techniques to interrogate the LCH genome for this and additional abnormalities. Broadly based analyses, such as whole-exome sequencing, reveal a remarkably stable genome having a small number of SNVs compared to most other cancers: an average of six SNVs per patient (0.14/Mb) in the study of Nelson et al. [43] and an average of one SNV per patient (0.03/Mb) in Chakraborty et al. [44]. These mutation prevalences are at the low end of the pan-cancer spectrum, where pediatric tumors such as pilocytic astrocytomas are found, and are lower than prevalences found in acute lymphoblastic leukemia [45]. Nonetheless, a variety of genetic alterations have been reported, and several have important implications for therapy.

**BRAF** BRAF is a component of a multi-step signal transduction pathway that transmits the effects of extracellular stimuli, such as growth factors, to the nucleus where the response to those stimuli is executed by an induced transcriptional program (Fig. 2.1). The final targets of the pathway are the ERK (extracellular signal-regulated kinase) proteins, ERK1 and ERK2, which are MAPKs (mitogen-activated protein kinases). Each step in the pathway consists of a protein kinase which is activated by being phosphorylated by the next proximal kinase. In general terms, the enzyme that phosphorylates a MAP kinase is a MAP kinase kinase, and the next proximal enzyme is a MAP kinase kinase kinase. BRAF, for example, is a MAP kinase kinase

kinase and is one of the three closely related members of the RAF protein family (Figs. 2.1 and 2.2). When extracellular messengers such as growth factors bind to their receptors, the intrinsic tyrosine kinase activity of the receptor is activated. This results in the activation of a RAS protein family member. Activated RAS proteins activate RAF family members which phosphorylate MEK family members, and this culminates in the phosphorylation of ERKs. Phosphorylated ERK then translocates to the nucleus to stimulate transcription of specific genes. The substitution of glutamate for valine at position 600 of BRAF creates a protein with inherent MEK kinase activity, which is not dependent on upstream activation by RAS. This is the basis for clonal neoplastic proliferation or accumulation of cells carrying the BRAF V600E variant. Mutations in other members of this cascade may occur which also lead to constitutive activation of the signaling cascade. Some of these occur in LCH and are described below. These additional mutations along with the highly prevalent BRAF V600E mutation account for the fact that ERK phosphorylation is observed in nearly all cases of LCH regardless of *BRAF* mutational status [34, 46].

A large number of studies have confirmed the original observation [34] of the high prevalence of the T to A transversion at nucleotide position 1799 of *BRAF* which encodes the oncogenic substitution of glutamate for valine at amino acid position 600 (BRAF V600E) (Table 2.1). Although reported prevalences range from 16% to 64%, the studies which examine the largest cohorts suggest that BRAF V600E occurs in 45–65% of cases [34, 46–49]. Some of the variation in prevalence rates likely reflects the association between the presence of the mutation and younger age [34, 47, 50] although this association has not been seen in all studies [46]. A few studies suggest the possibility that mutation prevalence might be lower in East Asian populations [51, 52], but the number of samples tested is too small to make this a valid inference.

As noted above, pulmonary LCH is a syndrome seen almost exclusively in adult smokers. Because of this exposure history and the multifocal nature of the disease, pulmonary LCH was thought to be primarily an inflammatory



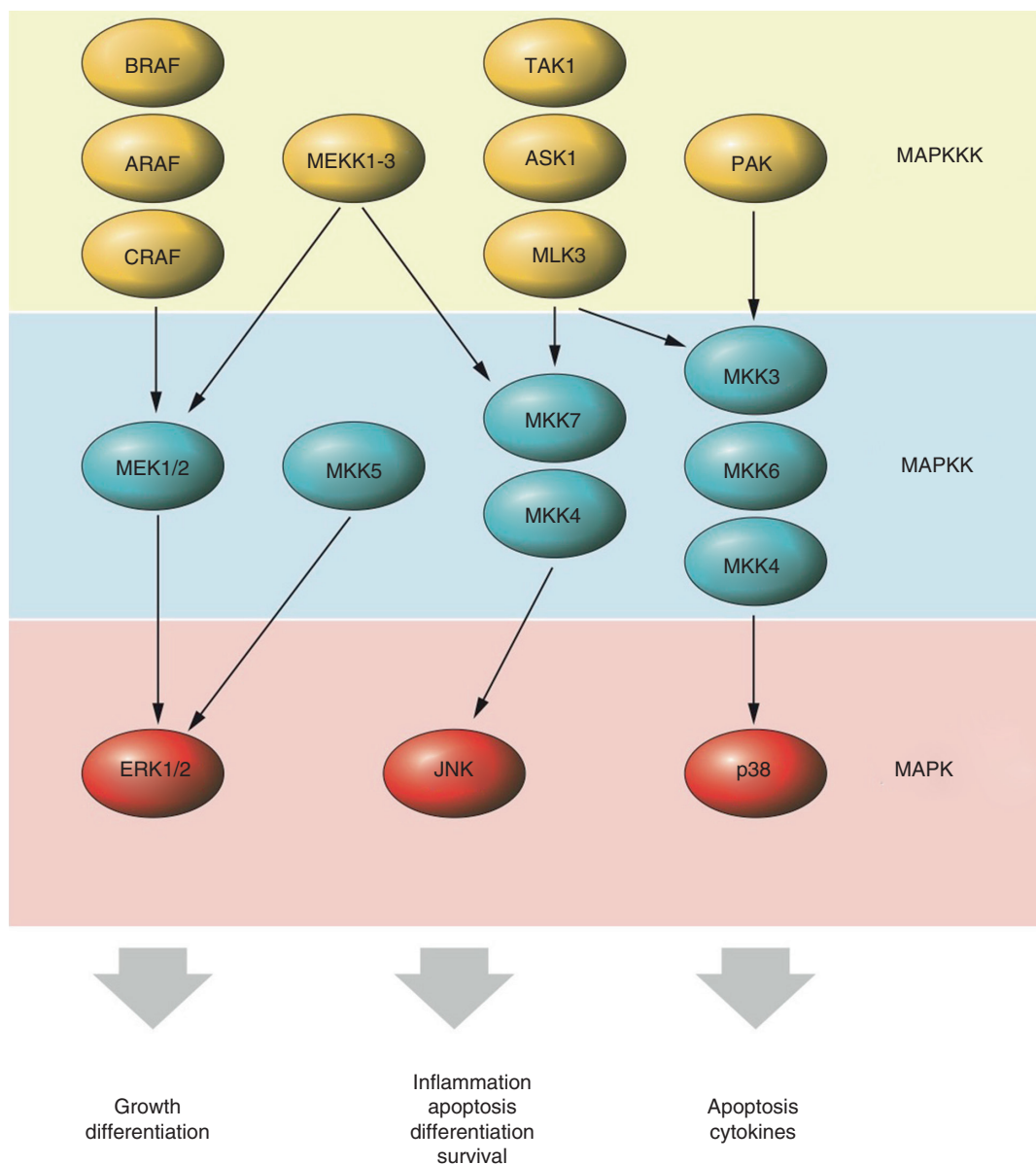
**Fig. 2.1** MAP kinase signal transduction pathway. Extracellular stimuli, such as those induced by growth factors (GFs), are transmitted to the nucleus by means of serial activation of kinases. GFs bind to their cognate receptors many of which, as shown here, have intrinsic tyrosine kinase activity which is stimulated by GF binding. Tyrosine kinase receptor activation leads, via GRB2 and SOS, to the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) bound to RAS. In this form, RAS phosphorylates and activates

RAF family members which are MAP kinase kinase kinases (MAPKKK). Activated RAF kinases phosphorylate and activate MEK1 or MEK2 which are MAP kinase kinases (MAPKK). Activated MEK1 or MEK2 kinases phosphorylate and activate ERK1 or ERK2 which are MAP kinases (MAPK). Phosphorylated ERKs translocate to the nucleus where they stimulate transcription of genes that alter the state of the cell (Reprinted from Rollins [72], with permission from Elsevier)

response to environmental insults. In support of this mechanism, only one third of pulmonary cases have been found to be clonal [53]. Perhaps

surprisingly, then, the prevalence of BRAF V600E in pulmonary LCH approaches that seen in non-pulmonary LCH (Table 2.1). Much, but





**Fig. 2.2** The MAP kinase family. Arrows indicate substrates for the indicated kinase. Shown in yellow are members of the MAP kinase kinase kinase family. These include RAF family members (BRAF, ARAF, and CRAF) as well as MEKK1–3 and several other structurally related kinases. Shown in blue are members of the MAP kinase

kinase family including MEK1 and MEK2 as well as several MKK family members. Shown in red are the MAP kinase targets of these pathways including ERK1/2, JNK, and p38. Some of the physiological effects of activation of these MAP kinases are shown below each one (Reprinted from Rollins [72], with permission from Elsevier)

not all, of the prevalence could be accounted for by clonal cases, but the additional mutated cases could be the result of independent clones of LCH in a single patient, all of which carry the *BRAF* mutation.

Early studies examined relatively small patient cohorts, usually fewer than 100, and inferring correlations between the presence of *BRAF* V600E and clinical characteristics was difficult. For example, the original description of *BRAF*

**Table 2.1** Prevalence of BRAF V600E in LCH<sup>1</sup>

Report (Ref.)	Prevalence <sup>2</sup>
Badalian-Very et al. [34]	57% (35/61) 42% (5/12) <i>pulmonary only</i> 61% (30/49) <i>extrapulmonary</i>
Haroche et al. [40]	38% (11/29)
Sahm et al. [106]	38% (34/89) <sup>3,4</sup>
Satoh et al. [57]	56% (9/16) <sup>4</sup>
Wei et al. [49]	56% (28/50) 100% (1/1) <i>pulmonary only</i> 55% (27/49) <i>extrapulmonary</i>
Roden et al. [107]	33% (26/79) 28% (7/25) <i>pulmonary only</i> 35% (19/54) <i>extrapulmonary</i>
Berres et al. [46]	64% (64/100) <sup>4</sup>
Chilosi et al. [108]	46% (18/38) 63% (12/19) <i>pulmonary only</i> 32% (6/19) <i>extrapulmonary</i>
Méhes et al. [109]	53% (8/15)
Varga et al. [110]	54% (6/11) <i>adult cutaneous</i>
Bubolz et al. [111]	48% (23/48) 25% (1/4) <i>pulmonary only</i> 50% (22/44) <i>extrapulmonary</i>
Brown et al. [64]	45% (18/40)
Go et al. [51]	25% (7/28) <sup>5</sup>
Héritier et al. [47]	54.6% (173/315) <sup>4</sup>
Mourah et al. [48]	43% (27/63) 50% (13/26) <i>pulmonary only</i> 38% (14/37) <i>extrapulmonary</i>
Kamionek et al. [65]	34.6% <i>pulmonary only</i>
Sasaki et al. [52]	21% (4/19) <sup>6</sup>
Alayed et al. [50]	16% (8/50) <sup>7</sup>
Diamond et al. [63]	60% (6/10)

<sup>1</sup>Updated version of Table 2.1 in (72). The prevalence of any mutation in *BRAF* was taken from the indicated reference. When disease involving only the lungs (“pulmonary only”) was described, the prevalence of *BRAF* mutations in that disease subtype is indicated

<sup>2</sup>Prevalence rate is indicated with actual numbers shown in parentheses (number of cases with mutated *BRAF*/total number of cases)

<sup>3</sup>Detected by immunohistochemistry using VE-1 antibody

<sup>4</sup>No pulmonary-only cases

<sup>5</sup>Chinese population

<sup>6</sup>Japanese population

<sup>7</sup>Median age 36.5 years; presence of mutation correlated with young age

V600E in LCH reported that the median age of patients carrying the mutation was younger than the age of those who did not; further, the presence of BRAF V600E was associated with

younger age in an unadjusted exact logistic model but not in an adjusted model [34]. Mutational status was also not associated with specific clinical presentations, e.g., single-system disease versus disseminated disease. In a larger study of 100 patients, BRAF V600E was not correlated with young age, but the mutation did predict for disease relapse despite not being correlated with disseminated disease or the clinical definition of high risk [46]. In contrast, a recent report on 315 pediatric patients showed that the presence of BRAF V600E was associated with involvement of so-called risk organs (bone marrow, spleen, or liver) and the skin (but not single-system skin disease with spontaneous regression) with odds ratios of 6.35 and 3.675 [47]. BRAF V600E did not correlate with bone involvement. The presence of the mutation also correlated with disease involvement of the CNS and pituitary. Patients whose histiocytes expressed BRAF V600E were resistant to standard vinblastine/prednisone therapy, had a higher rate of relapse (as in [46]), and had more debilitating long-term complications. Thus, in this large pediatric study, BRAF V600E was present in patients with more aggressive disease. This study also showed a correlation between the presence of the mutation and younger age.

Substitution of glutamate for valine at amino acid position 600 is not the only molecular abnormality that produces a constitutively active BRAF kinase. In melanoma, for example, substitution of another acidic amino acid, aspartate, for valine at this position (V600D) is also an activating mutation [54]. This alteration has been reported in one case of LCH [55]. BRAF V600K, a substitution of lysine for valine at this same position, is seen more commonly in melanoma than V600D [56] but has not been described in LCH to date. An unusual four amino acid substitution for V600, aspartate-leucine-alanine-threonine (DLAT), has been reported in a single LCH case [57]. Like the other substitutions seen in LCH, this is predicted on the basis of structural considerations to lead to a constitutively active kinase. In-frame deletions of *BRAF* were identified in 6 of 25 cases analyzed by whole-exome or targeted sequencing [58]. These deletions are

predicted to shorten the  $\beta 3/\alpha C$ -helix loop and lock it in the so-called “helix-in” conformation that favors dimer formation, a conformation predicted to be resistant to inhibition by vemurafenib, a first generation RAF kinase inhibitor [59, 60]. A single example of a translocation generating a *FAM73A-BRAF* fusion protein has been described [58]. This fusion is predicted to have constitutive BRAF kinase activity because the kinase domain of *BRAF* is intact, while the auto-inhibitory domain has been replaced by the fusion partner. *BRAF* duplications such as those seen in pediatric gliomas [61, 62] have not been described in LCH.

**ARAF** The RAF kinase family consists of three structurally related members, ARAF, BRAF, and CRAF (or RAF1), all of which phosphorylate members of the MEK family (Fig. 2.2). Because they are so closely related, these proteins, all MAP kinase kinase kinases, may substitute for one another in some circumstances. This may be the case in LCH in which activating mutations of *ARAF* have been found in patients who carry wild-type alleles of *BRAF*. The first such report described an unusual compound mutation in which a single-nucleotide variant results in a substitution of leucine for phenylalanine at amino acid 351 (F351L) accompanied by a six-nucleotide in-frame deletion resulting in loss of amino acids 347 and 348 (Q347\_A348del) [43]. Both alterations occur in the kinase domain close to the homolog of amino acid 600 in BRAF. Expressing the ARAF variant in vitro demonstrated that it has constitutive MEK kinase activity. It is also capable of transforming mouse embryo fibroblasts suggesting that it could be an oncogenic driver in this *BRAF* wild-type patient. Notably, the ARAF variant is inhibited by clinically relevant concentrations of vemurafenib, suggesting that mutational screening in the clinical management of LCH should extend beyond *BRAF*. Interestingly, ARAF F351L has been found in a single case of juvenile xanthogranuloma (JXG), a C Group histiocytosis [63].

A different mutation in *ARAF*, namely, methionine substituted for threonine at position 70 (T70M), was described in a case of combined

LCH and ECD [44]. Although this variant has not been examined for constitutive MEK kinase activity, it occurred in a case that carried BRAF V600E suggesting that T70M is likely not to be an activating mutation. No mutations in CRAF have been described to date in LCH.

**MAP2K1** *MAP2K1* encodes MEK1, a MAP kinase kinase (Fig. 2.2). Mutations in *MAP2K1* have been described in LCH and, as expected, are found only in cases in which *BRAF* is not mutated suggesting that *BRAF* and *MAP2K1* exert their effects within the same signaling pathway in LCH [44, 50, 64–66]. The prevalence of *MAP2K1* mutations in LCH varies between 10% and 30% (Table 2.2). Differences in prevalence may relate to differences in study cohort composition. Overall, however, *MAP2K1* mutations appear to comprise approximately 50% of the *BRAF* wild-type cases. So far, the presence of *MAP2K1* mutations does not correlate with age or extent of disease.

*MAP2K1* mutations in cancers, leukemias, and lymphomas tend to cluster in the N-terminal negative regulatory domain and in the catalytic domain. Mutations in the N-terminal regulatory domain include both single-nucleotide variants as well as in-frame deletions which presumably derepress the kinase. Mutations occurring in the catalytic domain are single-nucleotide variants which lead to amino acid substitutions that activate the kinase [67–71]. *MAP2K1* mutations in

**Table 2.2** Prevalence of *MAP2K1* mutations in LCH<sup>1</sup>

Report (Ref.)	Prevalence <sup>2</sup>
Brown et al. [64]	27.5% (11/40)
Chakraborty et al. [44]	33% (7/21)
Nelson et al. [66]	10% (3/30)
Alayed et al. [50]	12% (6/50)
Kamionek et al. [65]	18% (5/28) <sup>3</sup>
Mourah et al. [48]	11.5% (3/26) <sup>3</sup>
Diamond et al. [63]	40% (4/10)

<sup>1</sup>Prevalence of mutations in *MAP2K1* (encoding MEK1) in ECD. All mutations are included whether or not they have been tested for encoding constitutively active MEK1

<sup>2</sup>Prevalence rate is indicated with actual numbers shown in parentheses (number of cases with mutated *MAP2K1*/total number of cases)

<sup>3</sup>All pulmonary cases



LCH map to the same areas and often include previously reported alterations such as a C to G transversion at nucleotide position 362 which results in a substitution of serine for cysteine at amino acid 121 (C121S). This is a frequent alteration in melanoma [70]. However, some LCH mutations are novel. For example, the C121S substitution is created in at least one case by a different nucleotide variant: an A to T transversion at position 361 which creates a different codon but one that still encodes serine at amino acid position 121 [66]. This appears to be a unique mutation in LCH. Many of the deletions in the N-terminal negative regulatory domain in LCH are identical or overlap with deletions reported in other diseases [72].

Many of the MEK1 variants found in LCH samples have been expressed *in vitro* and have constitutive ERK activity [44, 66]. Not all of the deletion variants have been tested, but because they occur in the same region as other deletion variants known to have constitutive kinase activity, they are presumed to be activating mutations as well. One case reported by Nelson et al. [66] was found to have a compound mutation: C121S and G128D. Each variant was tested and found to have constitutive ERK activity *in vitro* (C121S > G128D), but the combination had much more activity than either variant alone.

**MAP3K1** Whole-exome sequencing identified two LCH samples with mutations in *MAP3K1*, a MAP kinase kinase kinase that encodes MEKK1 (Fig. 2.2) [66]. In both cases, deletions produced frameshifts that encode truncated proteins: T799fs and L1481fs. Because MEKK1 can phosphorylate MEK1 [73], an attempt was made to test whether the variants were able to do so. However, no stable expression could be achieved, and the mutations are presumed to be null alleles similar to many MEKK1 variants in other cancers, including breast cancer [74]. If these variants contribute to LCH pathogenesis at all, they are unlikely to do so through ERK activation. This inference is supported by the fact that the T799fs variant was found in a case carrying BRAF V600E [66].

**RAS** Activating mutations of RAS family members could result in constitutive phosphorylation of MEK and ERK and might explain some cases of LCH with wild-type *BRAF* and *MAP2K1*. Interestingly, however, RAS mutations are rare in LCH. One analysis of 30 pulmonary LCH cases found two instances of KRAS mutations (G12A and G12D) [65], and both occurred, as expected, in *BRAF* wild-type backgrounds. A second, independent report on 26 pulmonary LCH cases found one case with KRAS G12V again in a *BRAF* wild-type background [48]. This study may underestimate the true prevalence of *KRAS* mutations since the authors examined single-nucleotide variants only at amino acid position 12. There are no reports to date of *KRAS* mutations in non-pulmonary LCH. This may reflect the more specific mutational effects of smoking on the induction of *KRAS* mutations.

Pathogenetic variants of *NRAS* and, in particular, substitutions of lysine or arginine for glutamine at position 61 (Q61K or Q61R) have been reported in LCH. The same study of pulmonary LCH that identified a single case with KRAS G12V also found that 42% of the cases examined (11 of 26) contained *NRAS* Q61K or Q61R [48]. Notably, seven of these occurred in patients whose total biopsy material also contained single-nucleotide variants encoding BRAF V600E. However, by genotyping individual foci of CD1a-positive LCH histiocytes in several of these patients, the authors could demonstrate that each focus contained cells expressing either BRAF V600E or *NRAS* Q61K/R but not both. Thus, the mutations are mutually exclusive as would be expected based on their convergence on ERK. This observation also supports the notion, described above, that pulmonary LCH may be comprised of multiple independent clones that only appear in the aggregate to be non-clonal. A single case report described an *NRAS* G12D variant in mixed juvenile myelomonocytic leukemia (JMML) and LCH [75]. This mutation is characteristic of JMML, and its presence in this case likely reflects its driver status in that disease and not in LCH, since it was found in blood samples rather than tissue LCH samples, which were not tested.

Of course, the mutation could theoretically promote ERK activation if it were also present in LCH histiocytes.

**PI3K/PTEN/AKT/mTOR** The PI3K/PTEN/AKT/mTOR pathway converges on many of the same downstream targets as the RAS/RAF/MEK/ERK pathway [76], and it is possible that activating mutations in LCH in the former may produce outcomes similar to mutations in the latter. This possibility was supported by a report that a patient with multisystem LCH enrolled on a clinical trial of an AKT inhibitor had a prolonged clinical response [77]. An LCH-specific trial demonstrated responses in 5 of 17 patients (29%) some of whom had relapsed or refractory disease [78]. However, to date, no mutations in *PTEN*, *AKT*, or *mTOR* have been reported in LCH. Targeted assessment of four hotspot mutations in *PIK3CA* (E542K, E545K, A1046T, and H1047R) was performed in 86 LCH patients and revealed only a single case with the E542K variant in a *BRAF* wild-type background [79]. The low frequency of *PIK3CA* mutations in this allele-specific assessment is likely to be generally true since no *PIK3CA* mutations were described in whole-exome sequencing analyses of LCH performed to date [44, 66].

**TP53** Although the histiocytes in most cases of LCH overexpress p53 as determined by immunohistochemistry [80], its mechanistic basis is unclear. Mutations in the *TP53* gene are rare in LCH with only one report of a case with TP53 R175H [34], a presumed oncogenic variant [81–83]. There are no reports of mutations in p53 regulators such as *MDM2*. The role of p53 overexpression in LCH pathogenesis is unknown. On one hand, it could be a driver abnormality that occurs via epigenetic alterations; on the other hand, p53 overexpression could be a response to constitutive ERK activation.

**Others** Based on the low overall frequency of single-nucleotide variants in clinical LCH samples described above, it is not surprising that few additional DNA variants have been described. In one whole-exome sequencing study of 41 LCH samples, 29 mutations that targeted the RAS/

RAF/MEK/ERK pathway were found [44]. An additional 23 mutations were found in a variety of genes, which might theoretically impact that pathway including *PICK1* and *PIK3R2*, and an ERBB3 P921Q variant in a *BRAF* wild-type background [44].

**Translocations and Copy Number Variations** An early survey of cytogenetic abnormalities in LCH described a clonal t(7;12)(q11.2;p13) translocation in one case and non-clonal translocations in the same case plus three more; none were recurrent [30]. A subsequent study of 31 cases showed that all were diploid and contained no translocations [31]. As described above, a single example of a translocation producing a *BRAF* fusion protein has been reported [58].

Array comparative genomic hybridization (array CGH), quantitative PCR, and next-generation sequencing have all been used to examine copy number changes in LCH. The array CGH study examined seven bone lesions and described several copy number changes throughout the genome and hints of recurrent loss of heterozygosity at some loci [32]. A separate PCR study found fractional allelic loss at a higher prevalence in multisystem disease than single-system or low-risk disease [33]. However, a later study which used high-density SNP (single-nucleotide polymorphism) arrays failed to confirm these findings [31]. None of the next-generation sequencing studies published to date describe recurrent copy number variations.

**Summary** Essentially all LCH histiocytes show constitutive activation of ERK. In a little over three quarters of these cases, activation has a genetic explanation: activating mutations of *BRAF* in about 50% (including rare fusion events); activating mutations of *MAP2K1*, in about 20–25%; and a smattering of mutations in *ARAF*, *KRAS*, *NRAS*, and *PIK3CA*. This leaves about 20–25% of LCH without an as yet documented genetic basis for ERK pathway activation. This is the “dark matter” of LCH pathogenesis. Epigenetic alterations may eventually account for much of the missing mechanisms underlying ERK activation. It is also

possible that overexpression of receptor tyrosine kinases or their ligands could provide autocrine or paracrine stimulation of ERK sufficient to cause LCH histiocyte accumulation. This could arise from epigenetic alterations that affect expression levels or from mutations in promoter regions which have not been thoroughly examined in the sequencing projects reported to date.

### Erdheim-Chester Disease

As in LCH, the genome of ECD histiocytes is very close to normal: an average of seven SNVs per adult patient and five SNVs per pediatric patient in a whole-exome analysis [63]. However, also like LCH, the discovery of recurrent mutations in the ERK activation pathway places ECD squarely in the neoplastic disease category.

**BRAF** The prevalence of mutations encoding BRAF V600E in ECD is 50–60% (Table 2.3) and is similar to the prevalence seen in LCH. One study showing 100% of ECD patients expressing this *BRAF* variant examined a very small sample (18 patients), and this prevalence rate has not been reproduced [84]. To date, none of the rarer activating mutations occasionally observed in LCH have been reported in ECD. However, at least two translocations involving *BRAF* have been described [63]. One results in a novel RNF11-BRAF fusion, which produces a constitutively active MEK kinase with about the same

activity as BRAF V600E. The second translocation is also novel and results in a CLIP2-BRAF fusion, which is expressed, but its transforming activity has not been demonstrated [60].

**ARAF** Whole-exome sequencing, targeted gene panel sequencing, and transcriptome sequencing of 44 *BRAF* wild-type ECD cases found ARAF mutations in ten for a prevalence of 23% [63]. Among unselected ECD cases, one could impute a prevalence of approximately 11% (10/88) which is much higher than the prevalence seen in LCH [43, 44]. The minority of ARAF mutations encoded amino acid substitutions in the kinase domain. Although the effects of these substitutions on ARAF kinase activity are largely unknown, one of the variants (S214A) was reported as an activating mutation in a non-small cell lung cancer case, which responded to treatment with sorafenib [85]. This variant was found in an ECD patient who had relapsed after multiple therapies and was similarly responsive to sorafenib [63].

**MAP2K1** Whole-exome and transcriptome sequencing of 14 ECD cases found two with *MAP2K1* mutations; targeted sequencing of 18 archived *BRAF* wild-type cases found nine more for an overall prevalence of about 22% (assuming a 50% prevalence of *BRAF* mutations) [63]. *MAP2K1* mutations are found in 50% of the *BRAF* wild-type ECD cases in this series. These included deletions and SNVs in the N-terminal regulatory domain and kinase domain which overlap those found in LCH. However, the C121S variant commonly observed in LCH was not seen in ECD. As expected, cases with mutations in *MAP2K1* did not contain mutations in *BRAF*, *ARAF*, *NRAS*, *KRAS*, or *PIK3CA*.

**RAS** Mutations in RAS family members have a significant prevalence in ECD. The first description of a *KRAS* mutant (G12S) came from an analysis of mutations in cell-free DNA from the plasma and urine of histiocytosis patients [86]. This same mutation was documented in tissue taken from a cardiac lesion in the same patient. No *KRAS* mutations were seen in a broader survey of tissues from 44 ECD patients [63].

**Table 2.3** Prevalence of *BRAF* mutations in ECD<sup>1</sup>

Report (Ref.)	Prevalence <sup>2</sup>
Haroche et al. [40]	57.5% (46/80) <sup>3</sup>
Emile et al. [112]	
Emile et al. [88]	
Cangi et al. [84]	100% (18/18)
Mazor et al. [113]	50% (3/6)
Cao et al. [114]	68.8% (11/16) <sup>4</sup>
Diamond et al. [63]	50% (7/14)

<sup>1</sup>Prevalence of *BRAF* mutations in ECD

<sup>2</sup>Prevalence rate is indicated with actual numbers shown in parentheses (number of cases with mutated *BRAF*/total number of cases)

<sup>3</sup>Cumulative prevalence from various components of the 80 patient cohorts reported in these three papers

<sup>4</sup>Chinese population

In contrast, *NRAS* mutations are recurrent in ECD although the prevalence is still low. After a case report from 2013 [87], a French study of 80 patients with ECD found *NRAS* mutations in three (3.7%) [88]. The amino acid substitutions were all known to be activating and included G12D, Q61K, and Q61R. As expected, these appeared in *BRAF* wild-type cases. Another analysis of archived material from 18 *BRAF* wild-type ECD cases found *NRAS* mutations in three, including G12D and Q61K/R variants [63]. This 16.6% prevalence among *BRAF* wild-type cases implies an overall prevalence among all ECD patients of about 8%, similar to the French study.

***PIK3CA*** Through a combination of allele-specific genotyping and exon sequencing, *PIK3CA* mutations were found in 7 of 58 ECD patients in the French cohort (12.1%) [88]. Among the 41 patients with *BRAF* mutations, there were 4 concurrent *PIK3CA* mutations (10.0%) while there were 3 *PIK3CA* mutations in the 17 remaining *BRAF* wild-type patients (17.6%), suggesting that *PIK3CA* mutations occur independently of *BRAF* mutational status. This may mean that the *PIK3CA* mutations in ECD exert their effects in a pathway that does not overlap ERK activation pathways. A second study found three *PIK3CA* mutations in 18 *BRAF* wild-type samples for a prevalence of 16.7% among the *BRAF* wild-type cohorts and an imputed overall prevalence of 3/36 or 8.3% among all ECD patients [63].

***Others*** Whole-exome sequencing of 14 ECD cases revealed nonrecurrent SNVs in a variety of genes that could have a plausible role in pathogenesis [63]. Several occurred in genes encoding members of the JNK/p38 pathway and in genes involved in epigenetic and transcriptional regulation. The contributions of these alterations, if any, to the development or behavior of ECD are unknown.

***Translocations and Copy Number Variations*** Several translocations resulting in potentially actionable protein fusions were discovered in a transcriptome and targeted RNA sequencing analysis of ECD cases [63]. They appeared in *BRAF* wild-type cases and included an

*RNF11-BRAF* fusion, a *CLIP2-BRAF* fusion, two *KIF5B-ALK* fusions, and an *LMNA-NTRK1* fusion. In all cases, the kinase domain of the downstream partner was intact. The *RNF11-BRAF* fusion imparted factor-independent growth to Ba/F3 cells and made them sensitive to MEK inhibition similar to the effects of *BRAF* fusions in other diseases [89, 90]. Similarly, the *KIF5B-ALK* fusion made Ba/F3 cells factor independent, but, in this case, their growth was sensitive to an ALK inhibitor. There are no published reports of copy number changes in ECD. There is a single report of a balanced translocation t(12;15;20)(q11;q24,p13.3) in an ECD case [39] which has not been reported again.

**Summary** Like LCH, about half of ECD cases are driven by activating mutations of *BRAF* and another 25% by activating mutations in *MAP2K1*. Unlike LCH, mutations in *ARAF* are somewhat more common as are mutations in *NRAS* and *PIK3CA*. Also more common in ECD are translocations leading to fusions that activate oncogenic driver kinases including, so far, *BRAF*, *ALK*, and *NTRK1*. The result is that there is much less “dark matter,” i.e., cases without identified driver genomic alterations, in ECD. Nonetheless, 8–10% of ECD cases have an unexplained pathogenesis, and it will be important to test some of the rare, one-off mutations for their potential function. Epigenetic mechanisms may also contribute to transformation in ECD, and these have yet to be rigorously investigated.

### Indeterminate Cell Histiocytosis

The rarity of ICH and the ongoing disputes about its diagnostic criteria have made molecular analysis of this disease challenging. One report describes a case of mixed angioimmunoblastic T cell lymphoma and ICH in which the ICH cells stained for *BRAF* V600E [41]. If more cases were to be described with *BRAF* mutations, this might lead to a reconsideration of ICH as a variant of LCH, given its CD1a positivity. However, a recent collection of four ICH cases showed same clonal translocation in three which results in a *ETV3-NCOA2* gene fusion [42]. *ETV3* encodes the transcriptional repressor, Ets variant

3 (also known as METS and PE-1 and an ERK2 substrate [91]); *NCOA2* (also known as GRIP1 and TIF2) encodes nuclear receptor coactivator 2 which is a transcriptional coregulator [92]. The pathophysiological role played by this fusion, if any, in ICH is unclear although translocations involving *NCOA2* have been observed in a variety of sarcomas, solid tumors, and hematologic malignancies [93–97]. This recurrent translocation provides substantial support for the idea that ICH is a nosologically distinct histiocytosis.

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### Implications of Genomic Alterations for Identifying the Cell of Origin in Histiocytic Diseases

Inferences about histiocytoses' cells of origin have been based on the phenotype of the abnormal histiocyte. In the case of LCH, the expression of CD1a and CD207 and the presence of Birbeck granules are features shared by mature LCs, and LCH was presumed to arise as a result of oncogenic activation or inflammatory stimulation of LCs [3, 4]. However, several lines of evidence suggest that this model is incorrect. For example, the pattern of global gene expression by LCH cells is much closer to that of immature myeloid dendritic cells than LCs [98]. In addition, the mutation encoding BRAF V600E was identified in circulating CD14+ monocytes and CD11c+ myeloid DCs in patients with high-risk disease and was also present in CD34+ bone marrow cells in some of the high-risk patients [46]. Interestingly, circulating cells carrying mutated *BRAF* were not detectable in patients with single-system disease and were present only in a few patients with multifocal low-risk disease. This has led to the proposal that the acquisition of the T1799A transversion is a transforming event and can occur in any of the several precursor cells in the myeloid dendritic cell lineage. Transformation in an early precursor (e.g., CD34+ stem cells) leads to multisystem high-risk disease, while transformation in a later, more differentiated cell leads to localized or lower-risk disease. Some support for this hypothesis comes from genetically engineered mouse models in which the

gene encoding BRAF V600E is conditionally expressed [46]. Directed expression of mutated *BRAF* to CD207-expressing cells produces a mild, limited histiocytic disease while directing expression to CD11c-expressing cells results in a systemic histiocytosis.

Similarly, the phenotype of ECD histiocytes has led to the suggestion that they are derived from macrophages. As in LCH, however, mutated *BRAF* [84] and *NRAS* [88] alleles have been found in circulating CD14+ cells of some ECD patients suggesting the possibility that a less mature precursor cell may have undergone transformation. The existence of driver mutations in these disorders will eventually enable a detailed analysis of the transformation state of well-defined stem and precursor cells. This will provide a clearer picture of the ontogeny of histiocytosis cells.

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### Implications of Genomic Alterations for the Treatment of L Group Histiocytoses

The presence in LCH and ECD of mutations known to be oncogenic drivers in cancer strongly suggests, but does not prove, that they are also drivers in these diseases. Real proof of their driver status comes from the remarkable clinical responses to inhibitors of the activated proteins encoded by these mutations. Unfortunately, no clinical trial outcome data are available yet for the histiocytoses, but a significant number of case reports and descriptions of small cohorts support the efficacy of RAF or MEK inhibition in these diseases [96–102].

The first published report of the effect of treating ECD and LCH with a RAF inhibitor described three patients with refractory BRAF V600E-expressing ECD, two of whom also had LCH involvement of skin or lymph nodes. Treatment with vemurafenib led to major clinical responses in all three patients, and the response persisted for the duration of reported follow-up (4 months) [99]. The same investigators later described a larger cohort of eight BRAF V600E-positive ECD patients, four of whom also had LCH. Again,



all had responses to vemurafenib that lasted for the duration of follow-up (6–16 months) [100]. In both reports, disease activity was easily monitored by PET scanning. Single case reports also describe responses to vemurafenib in specific clinical settings including brainstem involvement by ECD/LCH [101] and spinal cord involvement by ECD [102]. A so-called basket study designed to treat patients having a wide variety of diseases with BRAF variants at position 600 included several with ECD and LCH which were lumped together in the analysis [103]. The overall response rate of the combined diagnostic group was 43% (6 of 14) although some disease regression was observed in 12 of 14 patients and symptomatic improvement occurred in all. Median treatment duration in the study period was 5.9 months, and no patient progressed while on vemurafenib. Four patients discontinued the drug because of adverse events, and one of these patients progressed while off drug. A similar example of treatment-dependent persistence of response was reported in an 8-month-old patient with multisystem LCH [104]. She had a dramatic response to vemurafenib, but when the drug was discontinued after 90 days of treatment, she relapsed in the skin. Re-treatment with vemurafenib was effective. Finally, the only published report of vemurafenib resistance in LCH described an adult patient who had a very good response to vemurafenib for 20 months at which time she progressed on therapy [105].

Similar early signals of efficacy for MEK inhibition have been published. Two ECD patients have been described who had failed multiple lines of conventional therapy and whose histiocytes had *MAP2K1* mutations: K57N and Q56P [63]. Both patients have experienced major and prolonged responses to the MEK inhibitors trametinib, in the first case, and cobimetinib in the second. One note of caution, however, is that some of the *MAP2K1* mutations that occur in LCH, e.g., C121S, have been described as resistant to MEK inhibitors [70] suggesting that not all *MAP2K1* mutations may be biomarkers for sensitivity to MEK inhibitor treatments.

The common threads that run through these scattered reports are as follows: (1) patients

whose histiocytoses carry targetable mutations respond dramatically to cognate inhibitors; (2) patients do not generally develop resistance to the inhibitors, at least during the periods of follow-up described in the reports; and (3) disease reappears when targeted therapies are withdrawn. These observations suggest that LCH and ECD are “single-pathway” diseases, i.e., the proliferative and antiapoptotic thrust depends almost entirely on ERK activation through RAF and MEK family members. Further, the non-emergence of resistance is consistent with the very low frequency of mutations in these diseases (see above). A stable genome is much less likely to generate mutations that permit bypass pathways to appear. In many ways, this scenario is reminiscent of chronic myeloid leukemia and its response to ABL inhibitors. It remains to be determined whether LCH and ECD can be cured by prolonged treatment with targeted agents and whether resistance will eventually emerge through mutations in the target proteins.

## Conclusions

The recent discoveries of recurrent genomic abnormalities in the L Group histiocytoses have had several important implications. First, they provide insight into the fundamental nature of these diseases. The fact that so many of the alterations result in activation of authentic oncogenic drivers indicates that these diseases are neoplastic in nature. Second, these discoveries provide new information about pathogenesis and the development of the histiocytoses. The fact that clonal genetic abnormalities can be found in precursor cells, e.g., CD34+ bone marrow cells, indicates that the transforming event can occur early in the ontogeny of the abnormal histiocytes and suggests the possibility that clinical behavior may be determined by the specific precursor population or stem cell that first suffers the mutagenic hit. Finally, these discoveries provide a road map for therapeutics. The histiocytosis community can apply the lessons learned in other ERK-driven diseases such as melanoma to treatment of LCH and ECD. We have already seen early evidence that RAF and

MEK inhibitors have substantial activity in patients whose abnormal histiocytes carry mutations in the genes encoding the targets of those inhibitors. It is now essential to design clinical trials to determine which patient populations might benefit from these targeted therapies. In particular, the reappearance of disease after withdrawing these drugs indicates that, despite their efficacy, single targeted therapies are not curing patients. In contrast, vinblastine and prednisone can cure LCH in the appropriate population. Future goals will be to determine which patients should receive cytotoxic chemotherapy, who should receive single targeted agents, and who should receive combination therapy.

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