

Genomic Alterations in Langerhans Cell Histiocytosis



Barrett J. Rollins, MD, PhD^{a,b,*}

KEYWORDS

• Langerhans cell histiocytosis • LCH • BRAF • MAP2K1 • MEK1

KEY POINTS

- Recurrent somatic genomic abnormalities occur in Langerhans cell histiocytosis (LCH), indicating that it is a neoplastic disease.
- Most mutations activate signaling enzymes that result in extracellular-signal-regulated kinase (ERK) activation.
- More than 50% of cases carry *BRAF* mutations and 10% to 28% carry *MAP2K1* mutations, but all cases show activation of ERK.
- Significant clinical responses to RAF family inhibitors have been reported in patients whose LCH cells carry *BRAF* mutations, indicating that these mutations are authentic drivers of disease in LCH.

INTRODUCTION

Long considered an enigmatic disease, LCH defies simple categorization. Its 4 clinically distinct syndromes (Hand-Schüller-Christian disease, Letterer-Siwe disease, eosinophilic granuloma, and Hashimoto-Pritzker disease) were unified by observations in the mid-twentieth century of a characteristically abnormal histiocyte with distinctive morphology, subcellular structures, and staining patterns that appears in all forms of the disease.^{1–3} Although this categorization has helped clarify thinking about LCH, it has not explained its pathogenesis or its impressively protean clinical manifestations.

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^a Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Boston, MA 02215, USA; ^b Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

* Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215.

E-mail address: barrett_rollins@dfci.harvard.edu

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Further progress in characterizing LCH has been impeded by a paucity of samples, a consequence both of the low prevalence of LCH^{4,5} and the small size of most tissue samples obtained for clinical purposes. However, technical advances in the genomic analysis of clinical material have been applied to LCH and have revolutionized the understanding of the fundamental nature of the disease. One of the most enabling innovations is the ability to perform robust multiplexed genetic testing on small amounts of archived clinical material, that is, formalin-fixed, paraffin-embedded samples acquired for diagnostic purposes.^{6–8} This ability has opened the archives of pathology departments around the world to genomic analyses, and the application of these technologies has revealed some of the first evidence for recurrent and pathogenetically relevant mutations in LCH. To date, these have been somatic mutations rather than germline alterations affecting risk. The result has been a clearer understanding of LCH as a neoplastic disease and the identification of therapeutically important molecular targets.

MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY ACTIVATION

BRAF

One of the first technologies capable of testing archived human samples for multiple alleles simultaneously was the Sequenom mass spectrometric genotyping platform.⁶ A modification specific for oncology applications known as OncoMap, which tests 983 specific mutations in 115 cancer-related genes, was applied to 61 LCH clinical samples.⁹ Overall, very few mutations were detected, a common finding in all subsequent studies (see later discussion) attesting to the stability of the LCH genome. However, a mutation in *BRAF* encoding the substitution of glutamate for valine at amino acid 600 (*BRAF* V600E) was observed in 57% of the samples.⁹ This mutation, which produces a constitutively active *BRAF* kinase, is the most commonly observed *BRAF* variant in cancer and is found in a variety of different cancer types in which it often plays a driver role in pathogenesis.^{10–14} The effect of mutationally activated *BRAF* is to stimulate signaling through the RAS/RAF/MEK/ERK pathway leading to constitutive transcription of genes involved in a variety of cellular responses including proliferation (**Fig. 1**).

The presence in LCH of recurrent activating mutations in *BRAF* has been confirmed in several studies using a variety of different detection techniques (**Table 1**). Among them are immunohistochemical studies with a V600E-specific antibody, which confirms that the variant is present specifically in LCH histiocytes,¹⁵ a fact that could previously be inferred only indirectly by molecular means.⁹ *BRAF* mutations occur in all clinical settings, including pediatric, adult, single system, and multisystem disease, and their overall prevalence in reported studies is 45% to 65%.^{9,15–24} Surprisingly, *BRAF* mutations appear at a nearly similar frequency in pulmonary LCH, a disease of adult smokers that has generally been thought to be polyclonal. However, about one-third of pulmonary LCH cases are clonal.²⁵ It is also possible that cases that are polyclonal in the aggregate actually comprise several clones of *BRAF* mutant disease that arose independently.

In the original OncoMap analysis, the median age of patients whose histiocytes contain *BRAF* V600E was less than the median age of patients whose histiocytes did not, and younger age was associated with the presence of the mutation in an unadjusted exact logistic model but not in the adjusted model.⁹ The presence of mutated *BRAF* did not correlate with any other clinical features, although the clinical annotation of that sample set was limited. In contrast, in the largest sample set analyzed to date, clinical annotation was much more complete and the presence of *BRAF* V600E

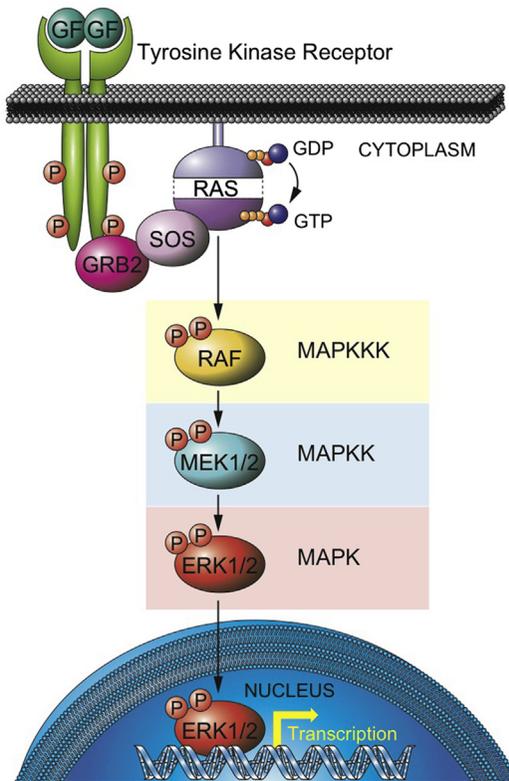


Fig. 1. The ERK signal transduction cascade. The ERK signaling cascade transmits stimuli for a variety of cellular responses, such as proliferation, from the cell surface to the nucleus. In this example, engagement of a tyrosine kinase growth factor receptor by its cognate ligand leads to phosphorylation of amino acids in the cytoplasmic domain of the receptor, which, in turn, activates RAS through GRB2 and SOS. Activated RAS activates RAF through phosphorylation; activated RAF phosphorylates and activates MEK1/2; activated MEK1/2 phosphorylates ERK1/2, which then translocates to the nucleus to stimulate transcription of genes involved in cell proliferation. RAF, MEK1/2, and ERK1/2 are members of the mitogen-activated protein (MAP) kinase kinase kinase (MAP4KKK), MAP kinase kinase (MAPKK), and MAP kinase (MAPK) families, respectively (see Fig. 2). Constitutive activation of components of this cascade would produce constitutive transcription of target genes and unrestrained cell proliferation. This class of activating mutations in genes encoding RAF proteins (BRAF and ARAF) and MEK1 has been described in LCH (see text).

correlated with disease relapse.¹⁶ That study also identified BRAF V600E in bone marrow–derived hematopoietic precursors from patients with high-risk LCH. In patients with low-risk disease, the mutation was detected only in lesional cells. This finding suggests that the appearance of a driver mutation earlier in ontogeny might lead to a more widely disseminated and aggressive form of LCH.

Like other BRAF-driven diseases, LCH is also associated with activating mutations of *BRAF* other than V600E. For example, another acidic amino acid substitution for V600, BRAF V600D (a substitution of aspartate for valine), is an activating mutation that occurs rarely in melanoma¹⁰ and has been reported in a single case of LCH.²⁶ A more commonly observed *BRAF* variant in melanoma, BRAF V600K (a substitution

Report	Prevalence ^a (%)
Badalian-Very et al, ⁹ 2010	57 (35/61)
	42 (5/12) pulmonary only
	61 (30/49) extrapulmonary
Haroche et al, ¹⁹ 2012	38 (11/29)
Sahm et al, ¹⁵ 2012	38 (34/89) ^{b,c}
Satoh et al, ²² 2012	56 (9/16) ^c
Wei et al, ²⁴ 2013	56 (28/50)
	100 (1/1) pulmonary only
	55 (27/49) extrapulmonary
Rodén et al, ²¹ 2014	33 (26/79)
	28 (7/25) pulmonary only
	35 (19/54) extrapulmonary
Berres et al, ¹⁶ 2014	64 (64/100) ^c
Chilosi et al, ¹⁸ 2014	46 (18/38)
	63 (12/19) pulmonary only
	32 (6/19) extrapulmonary
Méhes et al, ²⁰ 2014	53 (8/15)
Varga et al, ²³ 2014	54 (6/11) adult cutaneous
Bubolz et al, ¹⁷ 2014	48 (23/48)
	25 (1/4) pulmonary only
	50 (22/44) extrapulmonary

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.

^a Prevalence is indicated with actual numbers shown in parentheses (number of cases with mutated *BRAF*/total number of cases).

^b Detected by immunohistochemistry using VE-1 antibody.

^c No pulmonary only cases.

Data from Refs. ^{9,15–24}

of lysine for valine),²⁷ has not yet been reported in LCH. In addition, an insertional mutation substituting an additional 4 amino acids, DLAT (aspartate-leucine-alanine-threonine), for valine at amino acid position 600 has been found in a single LCH case.²² Although this variant has not been tested directly for constitutive kinase activity, structural inferences suggest that its effects on constitutive activation of BRAF kinase should be similar to that of V600E.

ARAF

Although activating BRAF mutations are present in 45% to 65% of LCH cases, the histiocytes in all LCH cases examined to date show high levels of phosphorylated ERK, implying ERK pathway activation^{9,16} (see Fig. 1). This finding has led several groups to perform even broader analyses such as whole-exome sequencing to find mechanisms for ERK activation other than activating *BRAF* mutations.^{16,28} A surprising outcome of one whole-exome analysis was the discovery of an activating mutation of *ARAF*.²⁸ ARAF is another mitogen-activated protein (MAP) kinase kinase in the same protein family as BRAF (Fig. 2). The *ARAF* abnormality discovered in this study was actually a compound mutation comprising a single base substitution encoding leucine in place of phenylalanine at position 351 (F351L) and a 6-nucleotide deletion leading to

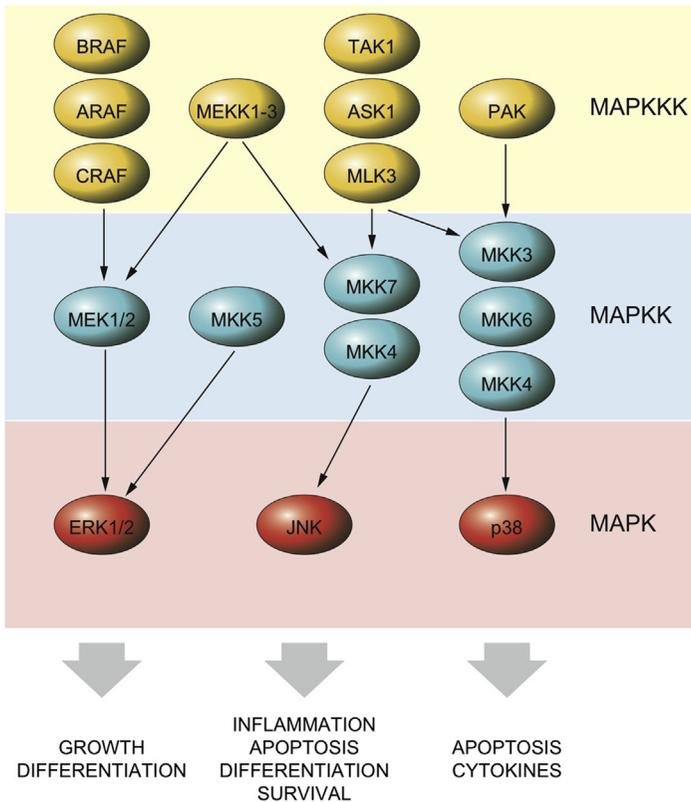


Fig. 2. MAP kinase families. Signaling from the cell surface to the nucleus occurs through serial activation of members of the MAP kinase family of proteins. Generically, signaling proceeds from MAP kinase kinase kinase (MAPKKK) enzymes to MAP kinase kinase (MAPKK) enzymes and then to MAP kinase (MAPK) enzymes. **Fig. 1** described the specific enzymes involved in growth and differentiation, portrayed here as the leftmost cascade. Other cascades result in activation of the MAP kinase JNK, which influences inflammation, apoptosis, differentiation, and survival, and the MAP kinase p38, which influences apoptosis and the release of cytokines. Note that there is cross talk between these cascades. In particular, the MAPKKKs MEKK1–3 stimulate the JNK pathway but can also stimulate the ERK1/2 pathway.

loss of amino acids 347 and 348 (Q347_A348del). Biochemical analysis of this variant demonstrated that it is a constitutively active kinase capable of transforming mouse embryo fibroblasts, suggesting that it is likely to be a driver mutation in this clinical case. This *ARAF* variant was sensitive to inhibition by vemurafenib *in vitro*.

A distinct *ARAF* mutation, T70M (methionine substituted for threonine at position 70), has also been described in a single mixed case of LCH and Erdheim-Chester disease (ECD).²⁹ Although this variant, which appears in the COSMIC (Catalog of Somatic Mutations in Cancer) database, has not been tested for RAF kinase activity, it is unlikely to be activated because it appeared in a case that also carried a *BRAF* V600E mutation. To date, no mutations in *CRAF* (also known as *RAF1*) have been described in LCH.

MAP2K1

Ongoing sequence analysis of LCH samples has uncovered additional somatic mutations that activate the ERK pathway. Most prominent are activating mutations in

MAP2K1, which encodes the MAP kinase kinase MEK1 (see Fig. 2). These mutations are found only in cases carrying wild-type *BRAF* alleles, supporting the notion that they are acting in the same signaling pathway as *BRAF*.^{29–31} The true prevalence of *MAP2K1* mutations is uncertain at present because their frequency differs substantially among the samples tested in various reports: 28% in a 40-sample cohort,³⁰ 19% in a different 36-sample cohort (of a 41-sample collection that included some mixed LCH/ECD and LCH/juvenile xanthogranuloma cases),²⁹ and 10% in a 30 sample cohort.³¹ It is possible that this disparity arises from differences in the clinical characteristics of the patients who comprised these cohorts, but the presence of *MAP2K1* mutations does not correlate with age, sex, sites of disease, or stage in these studies.

MAP2K1 mutations in LCH cluster vary specifically in 2 domains: an N-terminal negative regulatory domain and the N-terminal portion of the core kinase domain (Table 2). Some *MAP2K1* mutations found in solid tumors, leukemias, or lymphomas overlap these regions, but many more occur throughout the protein.^{32–36} Among the mutations in LCH are several that result in the same alterations of the *MAP2K1* protein but involve different nucleotide substitutions (see Table 2). For example, the substitution of serine for cysteine at position 121 (C121S) is created both by a substitution of G for C at nucleotide position 362, an alteration also observed in melanoma,³⁵ and by a substitution of A for T at position 361, creating a differently mutated codon that nonetheless still encodes serine at amino acid 121. Similarly, the deletion of amino acids

Nucleotide Position (Number of Samples)	Amino Acid Position
Negative Regulatory Domain	
140G>A ³⁰ (1)	R47Q
159_173del ^{29,30} (2)	F53_Q58delinsL
166_181del>C ³¹ (1)	F56_G61delinsR
167A>C ²⁹ (1)	Q56P
168_182del ³⁰ (1)	K57_G61del
170_184del ²⁹ (1)	Q58_E62del
172_186del ²⁹ (2)	Q58_E62del
Kinase Domain	
361T>A/383G>T ³⁰ (1)	C121S/G128V
361T>A ³¹ (1)	C121S
362G>C/383G>A ³¹ (1)	C121S/G128D
302_307del ²⁹ (1)	E102_I103del
303_308del ³⁰ (2)	E102_I103del
304_309del ³⁰ (2)	E102_I103del
299_307delinsCTC ³⁰ (1)	H100_I103delinsPL
295_312del ³⁰ (1)	I99_K104del
Both	
145C>T/316G>A ³⁰ (1)	R49C/A106T

Mutations in *MAP2K1* were taken from the published literature. The table sorts them into mutations that occur in the negative regulatory domain and the kinase domain. One case had mutations in both domains. The number of reported cases with the indicated mutation is shown in parentheses.

Data from Refs. ^{29–31}

58 through 62 arises through an in-frame deletion of nucleotides 303 through 308 or 304 through 309. Finally, the frequently observed deletion of glutamate at position 102 (E102) and isoleucine at position 103 (I103) is produced through in-frame deletions starting at nucleotides 302, 303, or 304. This finding almost certainly reflects substantial selective pressure for these particular variant proteins, which can arise through a variety of alterations at the DNA level, which may be context- or patient specific.

These 2 regions of frequent mutation in LCH cases overlap those reported in variant hairy-cell leukemia or IGHV-34-positive classic hairy-cell leukemia, which, unlike nearly all true classic hairy-cell leukemia, do not carry *BRAF* mutations.³⁴ The frequency of *MAP2K1* mutations in these wild-type *BRAF* variants is 48%, close to the frequency in wild-type *BRAF* cases reported in one of the LCH cohorts.³⁰

Several of these variants have been tested biochemically and found to have constitutive MEK kinase activity.^{29,31} Some of the deletion variants have not been tested directly but are presumed also to be constitutive MEK kinases because the deleted regions overlap those that occur in variants known to be constitutively active. In at least 1 case, C121S/G128D, the effect of 2 simultaneous mutations in a single allele has been examined.³¹ The single C121S variant has considerable MEK kinase activity in vitro. The G128D substitution (aspartate for glycine at position 121), which has not been described as occurring by itself in LCH or any other disease, leads to some constitutive MEK kinase activity but far less than that of C121S. However, the variant carrying both substitutions is a more active kinase than C121S. This synergistic effect of 2 mutations is reminiscent of the *ARAF* mutations described earlier in which the presence of F351L, which is a weak kinase by itself, greatly enhances the ERK kinase activity of the Q347_A348del deletion.²⁸

MAP3K1

In the course of performing whole-exome sequencing on LCH samples, 2 mutations have been discovered in *MAP3K1*, which encodes MEKK1.²⁸ Both are frameshift deletions leading to truncated proteins: T799fs (a frameshift truncation at threonine at position 799) and L1481fs (a frameshift truncation at leucine at position 1481). (A third variant identified in the same study, E1286V [valine substituted for glutamate at position 1286], was presumed to be a germline single nucleotide polymorphism.) The effects of these mutations on ERK activation are unclear. Although *MAP3K1* encodes the MAP kinase kinase kinase MEKK1 (see Fig. 2), which is capable of phosphorylating MEK1,³⁷ the variants found in LCH were unable to be expressed, including the L1481fs variant in which the truncation is near the protein's C-terminus, suggesting that the variant is unstable. Thus, these are likely to be null alleles as are a large number of *MAP3K1* variants in solid tumors such as breast cancers.³⁸ The mechanisms by which these variants promote neoplastic growth are still unknown but may involve enhanced cell survival through activation of JNK (c-Jun N-terminal kinase) (see Fig. 2). In the absence of enhanced MEK1 kinase activity, these variants are unlikely to be contributing directly to ERK activation. This idea is supported by the observation that the T799fs variant occurs in a case that also carries *BRAF* V600E.

NRAS

A somatic *NRAS* mutation has been described in a case of mixed juvenile myelomonocytic leukemia (JMML) and LCH, the latter based on a CD1a-positive cutaneous infiltrate of characteristic cells.³⁹ Although this is a genomic abnormality characteristic of JMML, upregulation of the ERK signaling pathway through *NRAS* activation could also drive LCH. *NRAS* mutations, including Q61R (a substitution of arginine for glutamine at position 61), a known pathogenetic variant, have been described in *BRAF* wild

type ECD.^{40,41} This finding may be relevant to LCH considering the frequent appearance of LCH/ECD overlap syndromes.

PIK3CA

Because a patient with multisystem LCH experienced a prolonged clinical response to a pan-AKT inhibitor,⁴² mutationally activated *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) was anticipated to be a major driver in this disease. Four hotspot mutational sites in *PIK3CA* (encoding E542K, E545K, A1046T, and H1047R) have been assayed in LCH samples from 86 patients.⁴³ However, only 1 sample was identified in which a mutation encoding the E542K variant (lysine substituted for glutamate at position 542) was found; this sample did not contain *BRAF* V600E. This study is limited by its inability to detect any *PIK3CA* mutations other than these 4 hotspots. However, the low frequency of *PIK3CA* mutations in LCH inferred from this work is likely to be accurate because none of the whole-exome sequencing analyses reported to date describe mutations in *PIK3CA*.^{28,29}

Tumor Protein 53

Most LCH cases show increased expression of tumor protein p53 (TP53) as determined by immunohistochemistry.⁴⁴ Although this suggests the possibility of an abnormality in the TP53 pathway, mutations in TP53 are rare. A single report describes an LCH case containing the R175H variant (histidine substituted for arginine at position 175), which has been characterized as a cancer-associated abnormality.^{9,45–47} To date, no mutations in the TP53 regulator MDM2 (mouse double minute 2 homolog) have been described.^{28,29,44,48} It is still possible that TP53 overexpression is a driver abnormality in LCH and that it occurs as the result of mechanisms such as epigenetic dysregulation that have not yet been tested in LCH samples. However, its near universality in LCH parallels that of ERK activation and suggests that TP53 overexpression may occur in response to constitutive activation of RAS-RAF-MEK-ERK pathway.

Others

Few additional DNA variants have been described in LCH. In one survey of a small number of LCH cases by whole-exome sequencing, no other potentially relevant substitutions or deletions were described.²⁸ In another whole-exome sequencing study of 41 cases, 29 mutations targeted the RAS-RAF-MEK-ERK pathway, whereas 23 were nonrecurrent in a variety of genes, including *PICK1* and *PIK3R2*, which, although not directly in that pathway, might theoretically affect ERK activation.²⁹ Among these was a substitution encoding ERBB3 P921Q (glutamine substituted for proline at position 921), which occurred in a *BRAF* wild type case.

Translocations and Copy Number Changes

Before the era of next-generation sequencing, some analyses suggested the presence of chromosome-level alterations in LCH. One report described a clonal t(7;12)(q11.2;p13) translocation in 1 case as well as nonclonal translocations in this case and 3 more.⁴⁹ Since then, however, there have been no additional reports of this or any other translocation in LCH. A careful study of 31 samples found no translocations and documented diploid genomes in all cases.⁴⁸

Copy number changes have been investigated using array comparative genomic hybridization (CGH) and quantitative polymerase chain reaction (PCR). One study applied array CGH to 7 bone lesions and found several copy number alterations scattered through the genome and recurrent loss of heterozygosity at some loci.⁵⁰ PCR analysis of a 24-sample set documented fractional allelic loss in LCH samples,

which was more frequent in patients with multisystem disease than in those with single-system or low-risk disease.⁵¹ However, these findings were not confirmed in a later study that used high-density single nucleotide polymorphism arrays.⁴⁸

Based on the absence of recurrent chromosomal abnormalities in these reports, it may not be surprising that whole-exome sequencing analyses have also failed to identify recurrent copy number changes or translocations in LCH samples.

CLINICAL IMPLICATIONS

The discovery of activating *BRAF* and *MAP2K1* mutations in LCH suggests the possibility that RAF and MEK1 inhibitors might be clinically useful. To date, however, only scattered case reports support this notion. For example, among 3 patients with *BRAF* V600E-positive ECD who were treated with the RAF inhibitor vemurafenib, 2 also had LCH involving skin or lymph nodes.⁵² Vemurafenib led to striking clinical responses in both the ECD and LCH components in the 2 patients with mixed disease. The response lasted throughout the 4-month period of ongoing treatment and observation. A follow-up study reported on vemurafenib treatment of 8 patients with ECD *BRAF* V600E-positive ECD, 4 of whom had concurrent LCH.⁵³ Responses were again seen in all patients, and no resistance was observed during the follow-up period of 6 to 16 months.

In another report, a 45-year-old woman with pure, *BRAF* V600E-positive LCH who had become refractory to standard treatment (steroids and vinblastine and then cladribine) responded dramatically to vemurafenib.¹⁷ Within 6 weeks of treatment, metabolic activity of her LCH lesions as measured by PET (positron emission tomography) with fludeoxyglucose had returned to background and her anatomic lesions had stabilized. Unfortunately, local regulations required discontinuation of this off-label use of vemurafenib after 12 weeks of treatment. Her disease progressed 6 months later.

Definitive assessment of the efficacy of RAF inhibition in LCH requires trials that attempt to measure this directly. Clinical trials are open at the time of this writing that may fulfill this need, such as a trial of dabrafenib in children and adolescents with *BRAF* V600 mutation-positive diseases, including LCH (NCT01677741). The presence of *MAP2K1* mutations in *BRAF* wild-type LCH suggests that MEK1 inhibitors such as trametinib need to be tested. By analogy to other diseases such as melanoma, treatment with both a RAF inhibitor and a MEK1 inhibitor, such as trametinib, may prevent the appearance of resistance.⁵⁴ Such a trial of trametinib in combination with dabrafenib in children and adolescents with *BRAF* V600 mutation-positive diseases including LCH has been started (NCT02124772).

SUMMARY

The application of advanced genomic analytical technologies to LCH has led to a new and deeper understanding of this disease. These tools have revealed recurrent mutations in RAF and MEK family members, indicating that LCH is a neoplasm driven by ERK pathway activation. This inference is tentatively supported by the striking clinical responses to RAF inhibitors in patients whose LCH cells carry *BRAF* V600E mutations, including patients who have failed first- and second-line therapies.^{17,52,53} Rigorous demonstration of the pathogenetic role of ERK pathway mutations in LCH awaits completion of trials of RAF and MEK inhibitors.

Mutations in RAF and MEK family members are found in 70% to 75% of LCH cases.^{9,15–24} The fact that ERK activation has been documented in all cases examined to date^{9,16} means that the causes for this activation remain unknown in 25% to 30% of LCH cases. Although the total number of LCH samples analyzed by whole-exome sequencing is still small, they are consistently found to have a small number of single

nucleotide variants, insertions, or deletions compared with other solid tumors that are ERK-driven, such as melanoma.^{28,29} Translocations or copy number changes involving relevant loci could provide additional mechanisms for ERK pathway activation. Although recurrent abnormalities of these types have not yet been described in LCH, it is not clear if the technical quality of the assessments performed to date have been sufficiently high to rule them out. Epigenetic alterations could also lead to ERK pathway activation, and global, unbiased analyses of LCH samples for such changes are likely to be the next approach taken by many groups. The challenge for epigenetic analyses, as for RNA sequencing analyses, is determining the appropriate control tissue to make comparisons against. It is also possible that ERK pathway activation in some cases may be non-cell autonomous. For example, overexpression of the macrophage colony-stimulating factor (M-CSF) receptor by LCH cells could lead to heightened responses to ambient M-CSF, including constitutive activation of the ERK signaling pathway. Although this mechanism would not be detected by ordinary genomic analyses of LCH cells, overexpression of receptor tyrosine kinases such as the M-CSF receptor would be detected by careful RNAseq analysis.

Although the presence of mutations in RAF and MEK family members in all subtypes of LCH unifies the disease under a single nosologic category, the fact remains that their clinical manifestations vary widely. So far, no correlations have been found between the presence of specific mutations and a variety of clinical attributes or outcomes other than a higher prevalence of *BRAF* mutations in patients who relapse.¹⁶ Larger numbers of samples drawn from specific clinical subtypes of LCH will have to be analyzed for a larger variety of alterations, including those mentioned earlier, to uncover a possible genomic basis for their disparate clinical behaviors.

The hallmark of treating ERK pathway-driven solid tumors with single-agent RAF inhibitors has been the induction of a profound clinical response followed within a few months by regrowth of the tumor. In contrast, although the number of patients with LCH treated with vemurafenib and reported in the literature is small, they all experienced a clinical benefit from RAF inhibition for as long as they were being treated.^{17,52,53} It is striking that there are no reports yet of the development of resistance; this is also the experience reported from the treatment of a larger number of patients with ECD.⁵³

One of the main differences between LCH (or ECD) and cancers such as melanoma is the low frequency of genomic alterations in the former compared with the latter. This finding is likely a reflection of genomic stability in LCH, which reduces the probability that LCH cells will generate daughter cells with resistance to RAF inhibitors. This scenario is reminiscent of chronic myelogenous leukemia in which BCR-ABL is the sole driver in most cases and treatment with imatinib can be effective for many years before resistance appears.⁵⁵ It remains to be seen how long patients with LCH can be treated with RAF inhibitors without developing resistance, but, for now, it seems that closest therapeutic analog of LCH may be chronic myelogenous leukemia.

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