# Genomic Alterations in Langerhans Cell Histiocytosis

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**KEYWORDS**

• Langerhans cell histiocytosis • Histiocytic disorders • MAPK pathway • *BRAF*$^{V600E}$

**KEY POINTS**

- Langerhans cell histiocytosis (LCH) is a myeloid neoplastic disorder driven by activating somatic mutations in mitogen-activated protein kinase pathway gene that arise in myeloid precursor cells.
- Extent and severity of LCH is associated with differentiation potential of the cell of origin.
- *BRAF*$^{V600E}$ is the most common somatic mutation in LCH, and is associated with more severe systemic disease and risks of developing LCH-associated neurodegeneration.

## INTRODUCTION

Langerhans cell histiocytosis (LCH) is a myeloid neoplastic disorder characterized by inflammatory lesions and associated tissue destruction that can arise in almost any organ. Clinical presentations range from single lesions to disseminated disease that can be life-threatening (**Fig. 1**). The etiology of LCH was a topic of frequent debate with features of neoplasia/cancer (eg, clonality)[1] and features of immune dysregulation (eg, inflammatory infiltrate, Langerhans cell-like phenotype).[2] In 2010, somatic *BRAF*$^{V600E}$ point mutations were identified in the majority of a cohort of LCH lesion biopsies.[3] Subsequently, mutually exclusive activating mutations in mitogen-activated protein kinase (MAPK) pathway genes (primarily *BRAF* and *MAP2K1*) were discovered in almost all cases of LCH.[4–7] Additionally, *BRAF*$^{V600E}$ localized to myeloid precursors

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| Abbreviations | |
|---|---|
| CD34+ | cluster of differentiation 34+ |
| CI | confidence interval |
| COSMIC | Catalog of Somatic Mutations in Cancer |
| ECD | Erdheim-Chester disease |
| ERK | extracellular signal-regulated kinase |
| HS | histiocytic sarcoma |
| JXG | juvenile xanthogranuloma |
| KRAS | Kirsten Rat Sarcoma Viral Oncogene Homolog |
| LCH | Langerhans cell histiocytosis |
| LCH-ND | LCH-associated neurodegeneration |
| MAPK | mitogen-activated protein kinase |
| MH | malignant histiocytosis |
| NRAS | neuroblastoma RAS Viral Oncogene |
| ORR | overall response rate |
| PCR | polymerase chain reaction |
| *PIK3CA* | phosphatidylinositol-4,5-bisphosphate 3 kinase, catalytic subunit alpha |
| RDD | Rosai-Dorfman disease |
| RTKs | receptor tyrosine kinases |
| SEER | surveillance, epidemiology and end results |
| SNVs | single-nucleotide variants |

in blood and, in some cases, to cluster of differentiation 34 (CD34)+ hematopoietic stem cells in bone marrow. Ability to detect *BRAF*600 E in bone marrow and peripheral blood mononuclear cells from patients was associated with more extensive disease at presentation. The importance of cell of origin and MAPK pathway activation were further supported by the ability of enforced expression of $BRAF^{V600E}$ in myeloid precursors to drive an aggressive LCH-like phenotype in mice.[8] Based on these observations, we proposed a model in which MAPK activation in myeloid precursors drives disseminated LCH, whereas less extensive LCH is associated with somatic MAPK gene mutations in more differentiated myeloid cells[9] (**Fig. 2**).

## EPIDEMIOLOGY OF LANGERHANS CELL HISTIOCYTOSIS

LCH is the most common of all the histiocytic disorders and has an incidence similar to that of Hodgkin lymphoma and acute myelogenous leukemia.[10] While LCH may occur at any age, the median age at diagnosis is 30 months with an estimated incidence of 5 cases per 1,000,000 children aged 0 to 14 per year.[11] Incidence is highest in infants under 1 year of age and decreases steadily with increasing age,[12] as reflected by an incidence among adults of approximately 1 to 2 cases per million adults per year.[13] The male-to-female ratio is estimated to be 1.2:1.[14] Despite a relatively high 5-year survival rate of ~85% for patients with hematopoietic organ involvement and ~99% for multisystem patients without risk organ involvement,[15,16] up-front chemotherapy still fails in over 50% of cases, and approximately 10% of patients with high-risk LCH die from their disease.

Treatment failure results in recurrence rates nearing 50%,[17] and 40% of patients who relapse experience a second reactivation event within 2 years. Further, carriers of $BRAF^{V600E}$ experience a 2-fold increase in LCH recurrence risk compared to non-carriers (hazard ratio 2.17, 95% confidence interval [CI]: 1.06–4.46).[8] Treatment failure is also associated with an increased risk of morbidity in patients with both low-risk and high-risk LCH,[18] including liver fibrosis, diabetes insipidus, hearing loss, and LCH-associated neurodegeneration (LCH-ND).[16,18] Approximately 10% of LCH patients develop LCH-ND that may manifest decades after a presumed cure from LCH.[8]

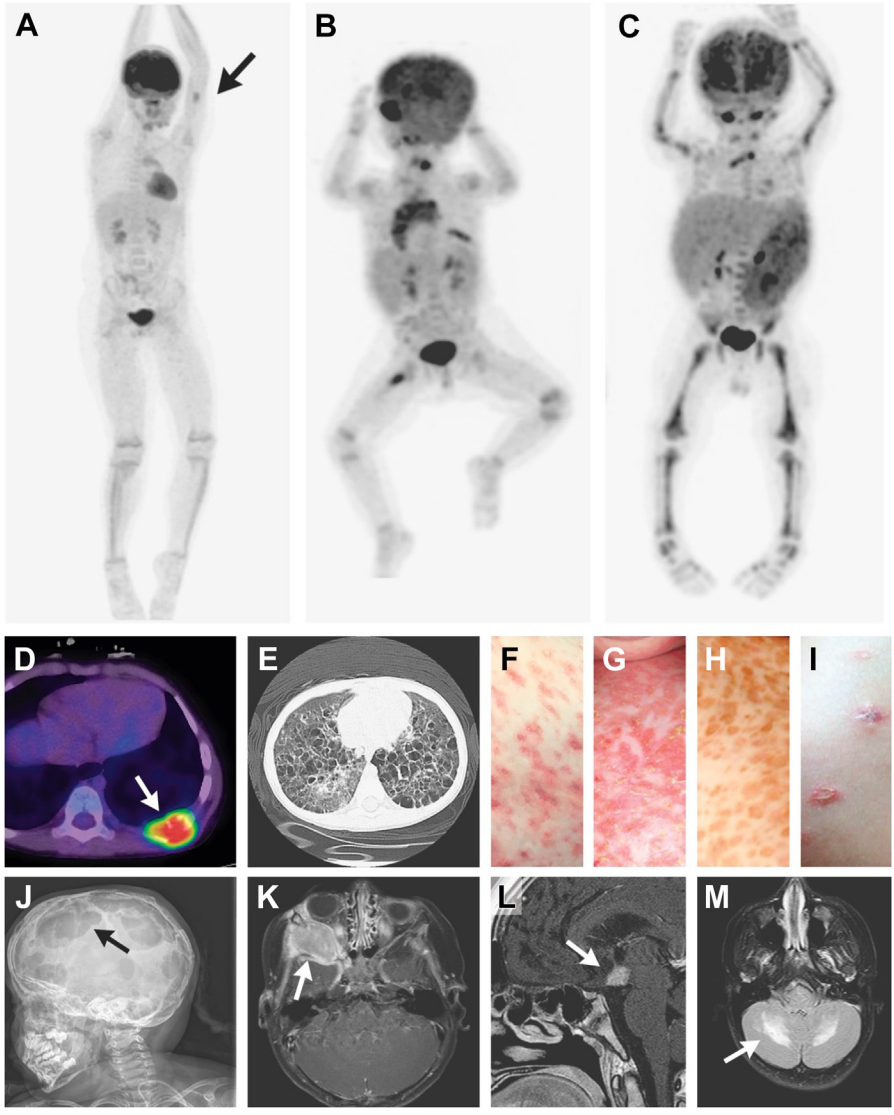


**Fig. 1.** Clinical spectrum of Langerhans cell histiocytosis (LCH). PET images show a single bone lesion involving the humerus (*A*, *arrow*); low-risk lesions involving the orbit, lymph nodes, bone (multifocal lesion), and thymus (*B*); and high-risk lesions involving the liver, spleen, and bone marrow (*C*). Other classic presentations include a lytic bone lesion (*D*, *arrow*), cystic lung lesions (*E*), and various skin lesions (*F* through *I*). Examples of LCH lesions involving the skull and brain include multifocal skull lesions (*J*, *arrow*), an orbital lesion (*K*, *arrow*), a pituitary lesion (*L*, *arrow*), and LCH-associated neurodegeneration (*M*, *arrow*). (*From New England Journal of Medicine*, Carl E. Allen and Miriam Merad and Kenneth L. McClain, Langerhans-Cell Histiocytosis, 379 (9), 856-868. Copyright © 2018. Massachusetts Medical Society. Reprinted with permission.)

We recently demonstrated that LCH-ND is associated with migration of *BRAF*$^{V600E}$ + hematopoietic precursors to brain parenchyma (Wilk Immunity 2023).[19] The clinical course of LCH-ND is typically unrelenting and potentially fatal. Improved therapeutic strategies are thus clearly needed for the initial treatment of patients with LCH.

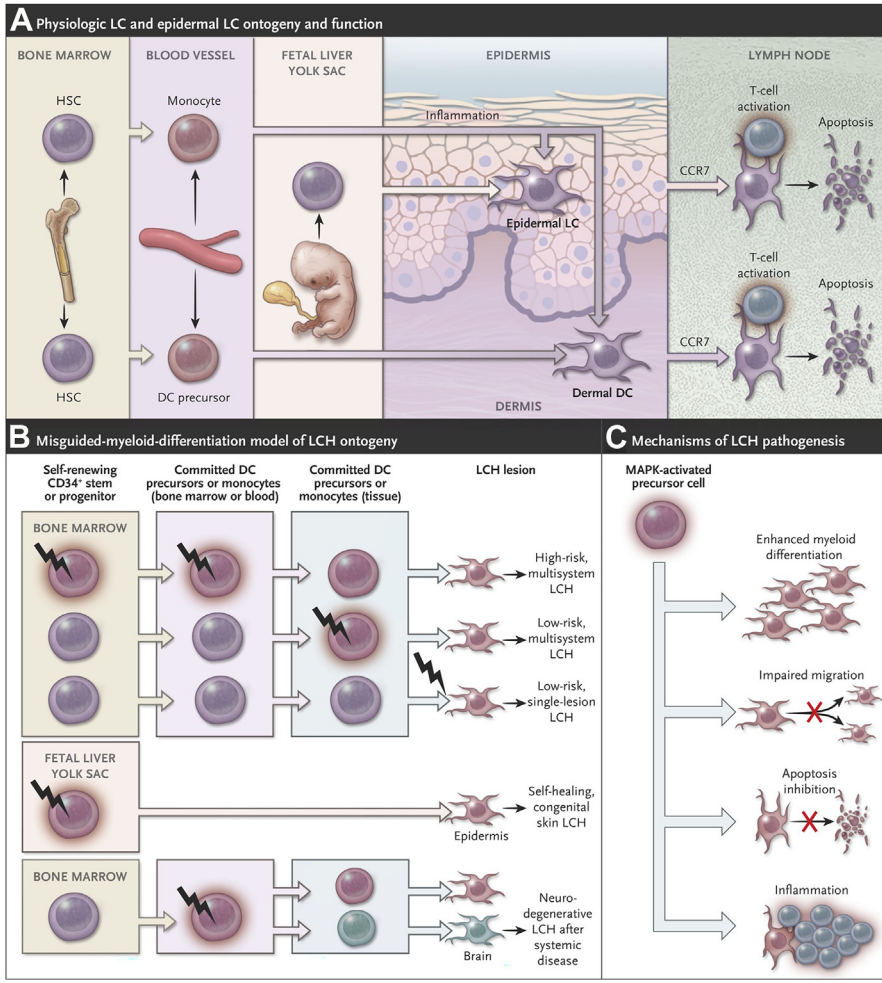


**Fig. 2.** (*A-C*) Models of LCH ontogeny and pathogenesis. Panel A shows physiologic Langerhans-cell (LC) and dermal dendritic-cell (DC) ontogeny and function. Under normal conditions, LC precursors arise from yolk-sac progenitors or fetal liver monocytes that seed the epidermis and are maintained locally by radio-resistant epidermal LC precursors in the steady state. Circulating DC-restricted precursors are constantly recruited to the skin to replenish dermal DCs. During injury or inflammation, bone marrow–derived monocytes can differentiate into epidermal CD207+ LC-like cells or dermal DC-like cells that replenish the damaged LC and dermal DC pool. CCR7 is required for activated epidermal LCs and dermal DCs to migrate through the lymphatics to the lymph node, where they recruit and activate T cells and are ultimately cleared through various mechanisms, including apoptosis. Panel B shows the misguided-myeloid-differentiation model of LCH ontogeny. According to this model, the stage of differentiation in which the myeloid cell acquires activating mitogen-activated protein kinase (MAPK) mutations determines the extent of LCH. High-risk, multisystem LCH arises from self-renewing stem or progenitor cells from bone marrow; low-risk, multisystem LCH arises from MAPK activation of committed DC precursors or monocytes; and a low-risk, single lesion arises from a regional DC precursor. Clinical data support a fetal-liver origin for self-healing, congenital skin LCH, and a hematopoietic origin for clonal cells that infiltrate the brain after systemic disease; a mouse model also suggests

Recent epidemiologic studies indicate there is significant variability in incidence of LCH across racial/ethnic groups in the United States.[20–23] In one study that utilized surveillance, epidemiology and end results (SEER) data, Hispanic children (0–19 years old) experienced higher age-standardized LCH incidence rates compared to non-Hispanics (RR = 1.63, 95% CI: 1.15–2.29), an effect that was strongest among Hispanic LCH cases diagnosed under 1 year of age (relative risk [RR] = 1.83, 95% CI: 1.03–3.24). Additionally, non-Hispanic Blacks experienced lower age-standardized LCH incidence rates compared to non-Hispanic Whites (RR = 0.41, 95% CI: 0.18–0.81), which was also more pronounced among those diagnosed less than 1 year of age (RR = 0.24, 95% CI: 0.03–0.91).[20] In a second SEER study that assessed all pediatric histiocytic disorders together, White children had a higher incidence compared with Blacks ($P < .05$) and those of Asian/Pacific Islander or Native American ancestry had the highest incidence rates of histiocytic disorders.[22] In a study that leveraged the Texas Cancer Registry, children born to 2 Hispanic parents were at a substantially increased risk of developing LCH compared to those children born to 2 non-Hispanic White parents (overall risk [OR]: 1.80; 95% CI: 1.13–2.87; *P*-for-trend = 0.01).[24] This finding is consistent with previous studies of LCH[20,23] and with the observation that children of mixed ancestry tend to exhibit cancer risks that more closely align with those of their racial/ethnic minority background.[25]

## SOMATIC MUTATIONS AND LANGERHANS CELL HISTIOCYTOSIS

Before the discovery of *BRAF*$^{V600E}$ as a recurrent genetic abnormality in LCH, several studies did not identify any recurrent genetic or epigenetic abnormalities. Those studies that reported cytogenetic abnormalities, loss of heterozygosity, or fractional allelic loss in patients with advanced disease, either yielded nonrecurrent findings or were nonreproducible in subsequent studies.[26–29] In 2010, mutations in *BRAF*$^{V600E}$ were identified in over 50% of LCH lesions, occurring specifically in CD1a-positive LCH histiocytes. This finding provided the first evidence of recurrent and reproducible driver mutations in LCH, confirming it as a clonal neoplastic disease.[3] Since then, various research groups have validated these findings and have further investigated the LCH genome, identifying recurrent mutually exclusive somatic activating mutations in MAPK pathway genes in ~85% of LCH lesions (most frequently *BRAF*$^{V600E}$) in an otherwise "quiet" genome.[4,5,7,9,30] Whole-exome sequencing reveals that pediatric LCH has a remarkably stable genome, exhibiting a small number of single-nucleotide variants (SNVs) compared to most other cancers: Chakraborty and colleagues, reported an average of 1 SNV per patient (0.03/Mb).[5] In cases where LCH arises along with or after another hematologic malignancy, leukemia/lymphoma-associated mutations such as *NOTCH1* can be shared by both phenoptyes.[31] Adult histiocytosis lesions typically have more somatic mutations than pediatric, with some mutations likely arising from clonal hematopoiesis.[32]

that it is possible for cells derived from the fetal yolk sac to drive neurodegeneration. Panel C shows mechanisms of LCH pathogenesis. MAPK activation in precursor cells contributes to the formation of LCH lesions through the following mechanisms: differentiation toward the LC phenotype, impaired migration through abrogation of CCR7 expression, and resistance to apoptosis, resulting in the accumulation of pathologic DCs and the development of an immune infiltrate that contributes to local and systemic inflammation. (*From New England Journal of Medicine*, Carl E. Allen and Miriam Merad and Kenneth L. McClain, Langerhans-Cell Histiocytosis, 379 (9), 856-868. Copyright © 2018. Massachusetts Medical Society. Reprinted with permission.)

## THE MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY AND LANGERHANS CELL HISTIOCYTOSIS

The RAS-RAF-MEK1/2-ERK1/2 pathway, also known as the MAPK, is a ubiquitous signaling platform that controls diverse fundamental physiologic processes such as cell differentiation, proliferation, and survival in response to extracellular environmental cues.[33] Constitutive activation of the MAPK pathway is one of the most frequently dysregulated signaling pathways in human cancers. The canonical MAPK signaling pathway transmits extracellular signals through receptor tyrosine kinases (RTKs), which activate RAS, followed by RAF, MEK, and finally, extracellular signal-regulated kinase (ERK) proteins (**Fig. 3**A). These proteins regulate specific nuclear targets and gene transcription programs. In LCH, activating mutations lead to constitutive ERK activation, affecting downstream transcriptional targets, including upregulation of *BCL2L1/bcl-xl* (rendering cells resistant to cell death) and downregulation of *CCR7* (causing pathologic LCH cells to accumulate).[3,8,9,34] MAPK activation in myeloid cells drives an oncogene-induced senescence phenotype characterized by local and systemic inflammation that mediate tissue destruction.[35] The mutually exclusive MAPK pathway somatic activating mutations in LCH are discussed in detail later and summarized in **Fig. 3**B.

### *BRAF$^{V600E}$*

The most common mutation found in LCH is *BRAF*$^{V600E}$, which is identified in 45% to 65% of cases according to studies that have examined the largest and most contemporary cohorts of patients.[3,6,8,36–42] In the Catalog of Somatic Mutations in Cancer (COSMIC) database (www.cancer.sanger.ac.uk), the most commonly mutated gene reported in samples from patients with LCH was *BRAF*, with 46% of samples from patients with LCH reported to have a mutation within *BRAF* out of those samples tested

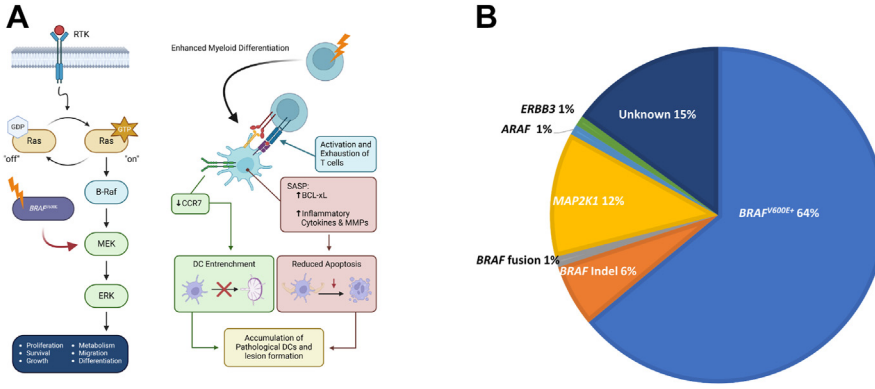


**Fig. 3.** Activating MAPK pathway mutations in LCH. As shown in Panel A, canonical MAPK signaling transduces extracellular signal through receptor tyrosine kinase, which activates RAS, then RAF, then MEK, and then extracellular signal-regulated kinase (ERK) proteins, which in turn regulate cell-specific nuclear targets and gene transcription programs. Activating mutations such as *BRAF* V600E drive constitutive ERK activation and downstream transcriptional targets, including *BCL2L1* (up-regulated) and *CCR7* (down-regulated). The pie chart in Panel B shows the proportions of cases with specific activating MAPK mutations in a pediatric series from one institution. (*From New England Journal of Medicine*, Carl E. Allen and Miriam Merad and Kenneth L. McClain, Langerhans-Cell Histiocytosis, 379 (9), 856-868. Copyright © 2018. Massachusetts Medical Society. Reprinted with permission.)

for a mutation within *BRAF*. In fact, over 98% of the mutations reported within *BRAF* in COSMIC for LCH lesions occurred at $BRAF^{V600E}$ (Table 1).

### *BRAF Indels*

Pathogenic insertions or deletions (indel) mutations in the *BRAF* gene, such as *BRAFN486_P490del, BRAFL485_P490delinsF, BRAFN486_T491delinsK, BRAFV487_T491del, and BRAF487_492delinsA,* have been reported in both pediatric and adult *cases.*[4,39–43]

### *BRAF Fusions*

*BRAF* fusions have also been documented, including a single case of a translocation that creates a FAM73A-BRAF fusion protein.[4] Additional *BRAF* fusions include *BICD2–BRAF*, *CSF2RA-BRAF*, *PACSIN2-BRAF*, *SPPL2A-BRAF*, *TMEM106B-BRAF, DOCK8-BRAF,* and *LMTK2-BRAF* fusions.[39,40,42,44,45]

Other *BRAF* single-nucleotide variants (SNVs) that produce a constitutively active BRAF kinase, apart from the well-known substitution of glutamate for valine at amino

**Table 1**
**Catalog of somatic mutations in cancer database**

| ***BRAF*** **Mutation, n (%)** | | ***MAP2K1*** **Mutation, n (%)** | |
|---|---|---|---|
| p.V600E | 597 (98.03) | p.Q58_E62del | 10 (20.41) |
| c.1511_1517 + 2dup | 2 (0.33) | p.E102_I103del | 8 (16.33) |
| c.1631_1637 + 2dup | 2 (0.33) | p.C121S | 3 (6.12) |
| p.V600D | 2 (0.33) | p.F53_Q58delinsL | 2 (4.08) |
| p.V640D | 1 (0.16) | p.K57_G61del | 2 (4.08) |
| p.V600K | 1 (0.16) | p.L101_E102del | 2 (4.08) |
| p.W604C | 1 (0.16) | p.V82M | 2 (4.08) |
| p.V600>DLAT | 1 (0.16) | p.A106T | 1 (2.04) |
| p.T599_V600insEAT | 1 (0.16) | p.A76V | 1 (2.04) |
| p.T639_V640insEAT | 1 (0.16) | p.E203K | 1 (2.04) |
| p.N526_P530del | 1 (0.16) | p.F53L | 1 (2.04) |
| p.N486_P490del | 1 (0.16) | p.G128D | 1 (2.04) |
| | | p.G128V | 1 (2.04) |
| | | p.G61_D65del | 1 (2.04) |
| | | p.H100_I103delinsPL | 1 (2.04) |
| | | p.I103_K104del | 1 (2.04) |
| | | p.I99_K104del | 1 (2.04) |
| | | p.L115P | 1 (2.04) |
| | | p.L63_D67del | 1 (2.04) |
| | | p.L98_K104delinsQ | 1 (2.04) |
| | | p.M94I | 1 (2.04) |
| | | p.Q56P | 1 (2.04) |
| | | p.Q56_G61delinsR | 1 (2.04) |
| | | p.Q58* | 1 (2.04) |
| | | p.R47Q | 1 (2.04) |
| | | p.R49C | 1 (2.04) |
| | | p.Y130H | 1 (2.04) |
| Total Mutations Reported | 611 (100.00) | Total Mutations Reported | 49 (100.00) |

* indicates predicted truncation.
Reported *BRAF* and *MAP2K1* mutations in Langerhans cell histiocytosis (LCH) lesions.

acid position 600, include *BRAF*$^{V600D}$, *BRAF*$^{V600}$insDLAT, BRAFG466E, BRAFE501K, BRAFT599I, *and BRAFV487Tfs*10*, which have been identified in a limited number of cases.[42,46,47]

### *MAP2K1*

Approximately 15% of LCH cases are associated with mutations in *MAP2K1*, which encodes MEK1. In COSMIC, *MAP2K1* was the second most mutated gene in samples from patients with LCH, with 17% of samples harboring a mutation in *MAP2K1*. Among the alterations in *MAP2K1*, deletions in exon 2 (N-terminal negative regulatory domain) and exon 3 (N-terminal catalytic core) are the most frequent (E102_I103del, p.Q58_E62del, p.F53_Q58delinsL), though point mutations in both exons have also been reported[5,6,30,34,37,39–42,48,49] (see **Table 1**).

The RAF kinase family comprises 3 structurally related members: ARAF, BRAF, and CRAF (or RAF1), all of which phosphorylate downstream members of the MEK family. Rare activating mutations of *ARAF* have been observed in patients who carry wild-type alleles of *BRAF*.[4,7,39,40]

### *RAS Isoforms*

Activating mutations in RAS family members may lead to constitutive phosphorylation of MEK and ERK. Kirsten Rat Sarcoma Viral Oncogene Homolog (*KRAS*) and Neuroblastoma RAS Viral Oncogene Homolog (*NRAS*) mutations have previously been reported in cases of pulmonary LCH,[37,49] and in LCH in conjunction with juvenile myelomonocytic leukemia[50]; these mutations have now also been documented in a few cases of LCH at other disease.[39,40,42]

### *PI3K/PTEN/AKT/mTOR Pathway Gene Mutations*

The PI3K/PTEN/AKT/mTOR pathway intersects with several downstream targets of the RAS/RAF/MEK/ERK pathways. Somatic mutations in the phosphatidylinositol-4,5-bisphosphate 3 kinase, catalytic subunit alpha (*PIK3CA*) gene have been identified in approximately 1% of LCH cases,[42,51] while no mutations in PTEN, AKT, or mTOR have been reported to date. PIK3CA mutations are not mutually exclusive with other MAPK pathway gene mutations.

### *Receptor Tyrosine Kinase Gene Mutations*

Recurrent activating mutations RTKs such as CSF-1R (also known as macrophage colony-stimulating factor), ALK (eg, *KIF5B–ALK), and ERBB3 have* increasingly been recognized in a variety of histiocytic disorders. These mutations have been reported in a few cases of LCH.[5,9,39,42]

### *TP53*

Overexpression of p53, the protein product of the *TP53* gene, has been observed through immunohistochemistry. However, due to the low frequency of mutations in *TP53* or genes such as *MDM2*, the significance and basis for this overexpression are uncertain.[3,27]

Aside from the described *BRAF* fusions, no recurrent clonal translocations or copy number variations have been identified or confirmed in large-scale studies.[27]

## GERMLINE GENOMIC LANDSCAPE OF LANGERHANS CELL HISTIOCYTOSIS

While the somatic mutational landscape of LCH has been largely defined, less is understood of how the germline genome impacts LCH risk. Aricò and colleagues

reported studies of twins that suggest concordance rates for LCH of approximately 90% and 10% in monozygotic and dizygotic twins, respectively.[52,53] Monozygotic twins can develop LCH from shared mutated embryonic/fetal precursors; mechanisms for dizygotic twin co-occurrence are less clear. Rare instances of familial clustering have been reported. Overall, 1% of cases with LCH have an affected family member, and the familial occurrence of LCH may be in those of the same generation or across generations.[53–55] In a study of 41 cases with LCH and 140 controls, De Filippi and colleagues assessed the role of 20 candidate germline polymorphisms within cytokine genes and LCH risk.[56] Two LCH susceptibility loci were identified with genotype distributions that differed between cases and controls and also correlated with the degree of disease dissemination.

The current model of LCH pathogenesis based on random acquisition of mutations in specific myeloid cell lineages cannot alone explain the significant differences in LCH incidence across racial/ethnic groups or among twins and families. Therefore, to test the hypothesis that inherited genetic variation influenced the risk of LCH, we conducted genome wide association study (GWAS) of LCH, leveraging 118 LCH families from Texas Children's Cancer and Hematology Center. A germline mutation within *SMAD6* located on chromosome 15 significantly increased the risk of LCH (rs12438941; $P=7.99 \times 10^{-7}$). This effect was replicated in an independent validation cohort of 132 cases and 1645 controls and surpassed a level of genome-wide statistical significance ($P=1.29 \times 10^{-11}$; $OR_{summary} = 3.72$; 95% CI: 2.54–5.44) in the joint analysis.[57] SMAD6 has inhibitory roles in bone morphogenetic protein, transforming growth factor-β, and MAPK signaling pathways, which are determinants of Langerhans cell differentiation.[58,59] Further, this intronic locus is in a region with high DNA recombination rates adjacent to *MAP2K1* (~500 kb; **Fig. 4**). The identified *SMAD6* rs12438941 risk allele is enriched in Hispanics who are at the highest risk of developing LCH,[20,21,24] and absent in those of African ancestry who experience the lowest LCH incidence. Specifically, in 1000 Genomes Phase 3 data,[60] the *SMAD6* rs12438941 (A) risk allele is more common in Mexican ancestral (0.25) and Peruvian (0.36) populations compared to those of African ancestry [Americans of African ancestry in the Southwest United States (0.04); Gambian (0.00); and Nigerian (0.00)]. These findings provide evidence that the germline genome influences LCH risk and may vary across racial/ethnic or genomic ancestral groups to impact LCH pathogenesis.

## OTHER HISTIOCYTIC DISORDERS

The genetic mutations associated with various subtypes of histiocytosis, including Erdheim-Chester disease (ECD), Rosai-Dorfman disease (RDD), Juvenile xanthogranuloma (JXG), and malignant histiocytosis (MH)/histiocytic sarcoma (HS), exhibit distinct genetic alterations with varying prevalence (**Fig. 5**). However, in all these subtypes, the MAPK/ERK signaling pathway is the central mechanism driving these histiocytic disorders. ECD presents *BRAF* mutations in 50% to 60% of patients, *MAP2K1* mutations in about 25%, and rarer alterations in *NRAS*, *KRAS*, and *PIK3CA*. In RDD, *MAP2K1* mutations are found in approximately 15% of cases, with KRAS and NRAS mutations occurring in 10% to 20% of instances. JXG often involves mutations in the MAPK pathway, including *MAP2K1*, *NRAS*, *KRAS*, and *CSF1R*, with occasional *ALK* translocations and *NTRK1* gene fusions (such as *TMP3-NTRK1* and *PRDX1-NTRK1*). Rare *BRAF* mutations are also noted, particularly in cases associated with cranial or intracranial lesions. MH is characterized by epigenetic mutations in about 50% of cases, along with sporadic *KRAS* and *NRAS* mutations (reviewed in[61,62]).

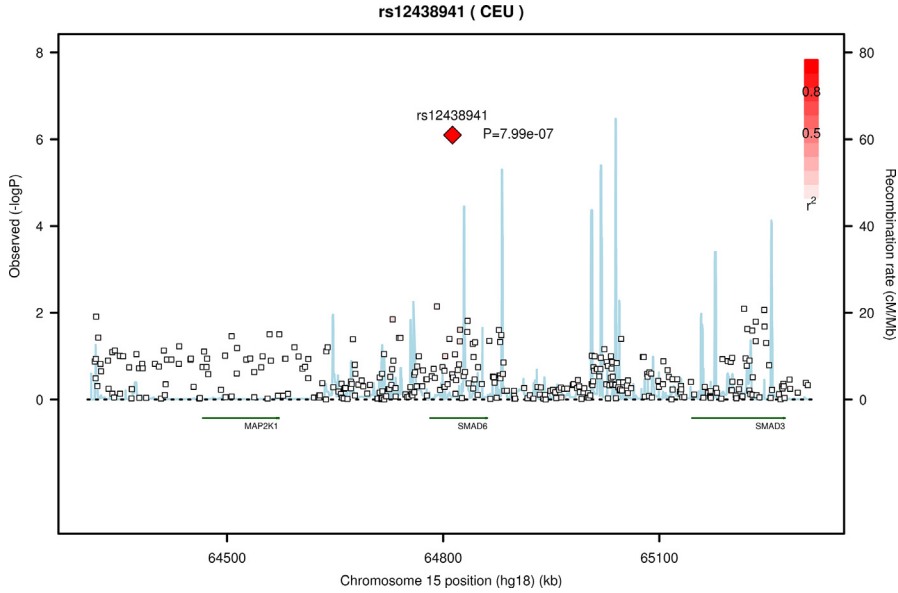


**Fig. 4.** Childhood LCH susceptibility GWAS results. Regional association plot highlighting the genomic region of the locus selected from discovery GWAS for replication. Recombination rate and linkage disequilibrium with SMAD6 rs12438941 (red diamond) shown for chromosome 15 using SNP Annotation and Proxy Search. Case-parent trio GWAS results were used to derive the *P* values used to create the figure. Each square represents a specific SNP in the genomic region, and the color of the filled squares depicts the r2 between that single nucleotide polymorphisms (SNP) and the most strongly associated SNP in our study population; the more highly correlated an SNP is with our locus of interest, the redder the box coloration. The blue lines indicate the recombination rate of this genomic region in the 1000 Genomes CEU (Northern Europeans from Utah) population.

## *BRAF*$^{V600E}$ IN OTHER CANCERS

*BRAF*$^{V600E}$ mutations that activate downstream MEK/ERK signal transduction have been identified in approximately 8% of all human tumors (Reviewed in[63,64]). Among pediatric cancers, activating MAPK mutations are found at high frequency, along with many other mutations in hematologic and lymphoid malignancies, rhabdomyosarcoma, glioblastoma multiforme, and neuroblastoma.[65,66] In general, these are rapidly growing tumors that require multiagent chemotherapeutics. In contrast, malignant nerve sheath tumors, pediatric low-grade gliomas, and LCH are relatively indolent tumors driven by mutually exclusive somatic mutations that cause pathologic MAPK activation in an otherwise stable genome,[5,7,67] and which may be responsive to MAPK inhibition (**Table 2**).

Mutations in MAPK pathway genes drive additional cancers that occur mainly among adults. Nearly 100% of hairy cell leukemia cases harbor *BRAF*$^{V600E}$,[68] and half of the related "hairy cell leukemia variant" are estimated to harbor other activating mutations in MAPK pathway genes, including *MAP2K1*.[69] Hyperactivation of the MAPK pathway is evident in ~90% of melanomas; ~50% of melanoma cases have *BRAF*$^{V600E}$, while 30% have mutations in *NRAS*.[70] In addition to LCH, Hispanics also experience higher rates of other MAPK pathway-driven malignancies, including papillary thyroid carcinoma[71] and melanoma,[72] compared to other minority groups.

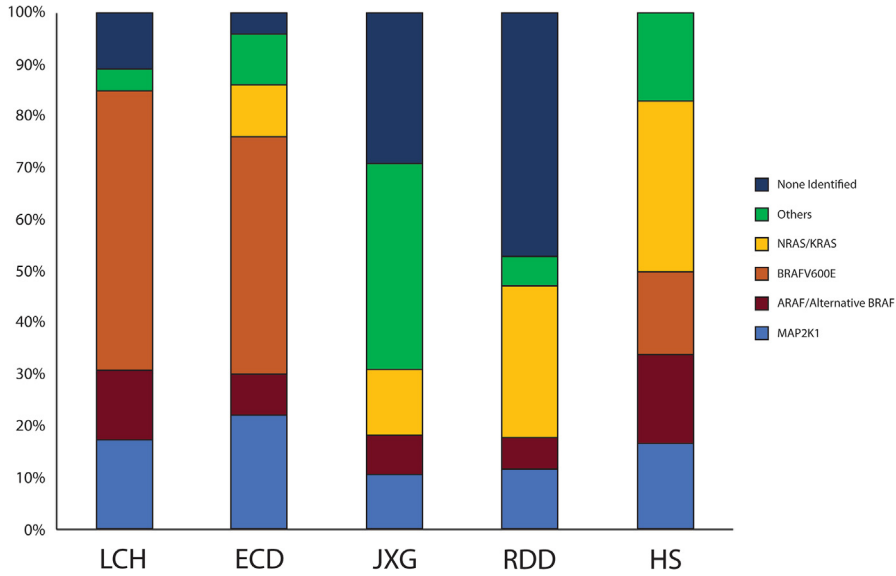


**Fig. 5.** Frequency of kinase mutations. Stacked bar graphs representing percentages of MAPK pathway mutations in each histologic subtype of histiocytosis. ECD, Erdheim Chester disease; HS, Histiocytic Sarcoma; JXG, Juvenile Xanthogranuloma; LCH, Langerhans cell histiocytosis; RDD, Rosai Dorfman Disease. (*Adapted from* Durham and colleagues, Nature Medicine 2019.)

Notably, the germline genome is also suggested to impact the incidence of *BRAF*$^{V600E}$ as racial/ethnic differences in mutational frequency exist in other MAPK-driven cancers[73] for which susceptibility loci have been identified,[74,75] and overall, Blacks and Asians have markedly lower frequencies of *BRAF*$^{V600E}$ compared to Whites.[76,77]

## MUTATIONS: DIAGNOSIS AND RISK IN LANGERHANS CELL HISTIOCYTOSIS

*BRAF*$^{V600E}$ has been linked to an earlier age at diagnosis, more extensive disease at presentation, risk of front-line treatment failure, risk organ involvement, and risk of LCH-ND in retrospective series.[8,36,41,78,79] These associations have not been replicated in adult patients with *BRAF*$^{V600E}$-driven LCH. A previous study involving 156 adults with LCH found that the *BRAF*$^{V600E}$ mutation was associated with a higher incidence of second malignancies, particularly hematological cancers, suggesting a potential clonal relationship in adult patients.[80]

Reports indicate a higher prevalence of BRAF indels in adult patients with multisystem LCH compared to those with single-system disease, and these mutations have been associated with poorer outcomes, including lower overall survival and progression-free survival.[42,81] In a recent study by Kemps and colleagues,[40] *BRAF* exon 12 deletions affecting the β3-αC loop were associated with a high prevalence of lung involvement in children. This aligns with molecular studies of adult LCH, which *BRAF* indels in patients with pulmonary involvement, often within the context of multisystem disease.[81–83]

In pediatric studies, no significant differences in outcomes have been observed among the less common mutations, like *MAP2K1*, small deletions in *BRAF* exon 12, or small duplications in BRAF exon 12. However, there are reports of increased bone involvement, particularly single-system bone involvement, in pediatric patients

**Table 2**
**Frequency of *BRAF*$^{V600E}$ in select cancers**

| Cancers with Mutually Exclusive Mitogen-Activated Protein Kinase Mutations and Few Additional Somatic Mutations | | Cancers Where Activating Mitogen-Activated Protein Kinase Mutations Among Many Other Somatic Mutations | |
|---|---|---|---|
| *Cancer type* | *Frequency BRAF*$^{V600E}$ *Mutated* | *Cancer type* | *Frequency BRAF*$^{V600E}$ *Mutated* |
| LCH | ~65% | Glioblastoma multiforme | 1%–2% of cases, particularly in epithelioid subtype |
| Pediatric low-grade glioma (pLGG) | 15% of all pLGGs and up to 75% and 50% of pleomorphic xanthoastrocytomas (PXA; grade II) and ganglioglioma (GGs; grade1), respectively | Rhabdomyosarcoma | Case reports |
| Malignant peripheral nerve sheath tumors | 6% overall, around 11.9% in sporadic cases, and 3% in cases associated with neurofibromatosis type 1 (*NF1*) | Neuroblastoma | Case reports |
| Classical hairy cell leukemia | 95%–100% | Non-small cell lung cancer | 2%–8% |
| Papillary thyroid carcinoma | 44% among adult-onset cases, while pediatric cases more often harbor *RET/PTC* rearrangements | Melanoma | 40%–60% |
| | | Colorectal | 8%–12% |

with *MAP2K1* mutations.[40,41] The lack of reported clinical associations is also biased by the smaller sample size.

## MUTATIONS: IMPLICATIONS FOR THERAPY IN LANGERHANS CELL HISTIOCYTOSIS

Over the past 14 years, discoveries have shown that hyperactivation of the MAPK pathway in myeloid precursors plays a central role in the formation and pathogenesis of LCH lesions. This insight provides a strong biological basis for using MAPK inhibitors to manage these patients.

Early trials conducted with adults suffering from LCH or the related ECD have indicated promising responses to MAPK-pathway inhibition. Adults with LCH and ECD participated in a phase 2 basket trial at Memorial Sloan Kettering featuring the MAPK inhibitor vemurafenib. The response rates were significant, with an overall response rate (ORR) of 61% according to the Response Evaluation Criteria in Solid Tumors and 100% ORR when evaluated using PET-computed tomography scans.[84,85] Similar results were documented in a French series that included adults with ECD and mixed ECD/LCH.[86]

It is important to note that vemurafenib and dabrafenib specifically target BRAFV600E, but they may cause paradoxic activation of MAPK signaling in normal cells. This limitation not only restricts the potential patient population but also contributes to toxicities. For instance, these inhibitors are ineffective against other BRAF mutations, including BRAF fusions with partner genes, insertions or deletions within exon 12,[5,43–45,87–90] and activating mutations in genes that act upstream of RAF, such as RAS. They are also ineffective against mutations in genes acting downstream of RAF, such as *MAP2K1*, which encodes MEK1. In such cases, selective MEK inhibitors could represent a valuable therapeutic option.[43,88,91,92]

To address some of the limitations of BRAF inhibitors, Diamond and colleagues initiated a trial using MEK inhibitors for all subjects with adult histiocytosis regardless of somatic mutation. This approach was grounded in the understanding that almost all activating mutations associated with histiocytic disorders occur in genes coding for MEK or proximal MAPK pathway genes. The trial reported metabolic ORR of 91%, irrespective of mutational status in adults, and led to the United States Food and Drug Administration (FDA's) approval of cobimetinib for treating histiocytic disorders in adults. However, one exception to this generally favorable outcome involves MAP2K1 exon 3 deletions (also termed class 3 mutants) and small duplications in BRAF exon 12.[43] The distinction between mutations in exon 2 and exon 3 of MAP2K1 is significant because exon 2 mutations are regulated by RAF, while exon 3 deletions are not. Furthermore, class 3 MAP2K1 mutants tend to exhibit higher activation of downstream extracellular signal-regulated kinase (ERK) *in vitro* compared to exon 2 mutations.[93,94]

The first child to be treated with a MAPK inhibitor was reported by Heritier and colleagues in 2015, resulting in a positive therapeutic response.[95] Since then, several case reports and 2 large case series involving children with systemic LCH and LCH-ND treated with BRAF or MEK inhibitors have reported remarkable responses, even in cases of aggressive disease.[96–98] Patients with LCH-ND have also shown some responses. However, the potential for recovery in patients with long-standing disease may be limited by the drug's ability to penetrate the blood-brain barrier and by preexisting central nervous system injury.[79] Where combining MAPK inhibitors (eg, $BRAF^{V600E}$ and MEK inhibitors) has demonstrated superior outcomes in more genomically complex disorders, such as melanoma, two-phase 1/2 trials utilizing dabrafenib, alone or in combination with trametinib to treat recurrent/refractory $BRAF^{V600E+}$ LCH had similar outcomes.[99]

Although the initial experiences with MAPK inhibitors have been transformative for patients with LCH, monotherapy with MAPK inhibitors has not proven curative. The LOVE study, which tracked adults with ECD after they achieved complete responses with MAPK inhibitors, found that over 75% of patients experienced a relapse within 6 months of stopping therapy.[100] Similarly, the European vemurafenib study reported rapid relapses in most patients after discontinuation of treatment.[96] Unlike other conditions with more complex mutational landscapes, resistance to MAPK inhibition does not typically develop in LCH; patients often respond to retreatment with the same MAPK inhibitor after relapse or progression off therapy.

## LANGERHANS CELL HISTIOCYTOSIS BEYOND BRAF

In conclusion, ERK phosphorylation is found in nearly all LCH cases, regardless of *BRAF* mutation status or alternative mutations identified.[3,8,40] Approximately 85% of LCH cases exhibit mutually exclusive MAPK pathway mutations, including *BRAF* mutations in about 65% (which may include rare fusion events), activating mutations in *MAP2K1* in roughly 15%, and a few mutations in *ARAF*, *KRAS*, *NRAS*, *CSFR1*, and *PIK3CA* (as reviewed[9,38]). This indicates that around 15% of LCH cases lack a documented genetic cause for ERK pathway activation. Potential mechanisms for this gap could include epigenetic changes or the overexpression of various receptor tyrosine kinases or their ligands, resulting from mutations in promoter regions or epigenetic alterations affecting expression levels. Currently, quantitative polymerase chain reaction (PCR), next-generation sequencing platforms, and whole exome sequencing are primarily utilized to investigate the genetic drivers of histiocytic disorders. However, it is essential to adopt additional molecular techniques, such as whole genome sequencing or fusion gene analyses on biopsy samples, as well as next generation sequencing (NGS) analyses of peripheral blood mononuclear cells for cases with minimal infiltration of pathologic histiocytes, to clarify the genetic underpinnings of the disease and enhance genotype-phenotype correlations.

After years of using vinblastine and prednisone for pediatric LCH—initially, without a clear understanding of LCH biology—we now face challenges with the rapid development of new MAPK inhibitors outpacing our clinical trials. MAPK inhibitors are increasingly employed as first-line or second-line therapies or in various combinations. Most data on their use in pediatric LCH are limited to case series, with no extensive clinical trials in initial treatment settings. Prospective trials are crucial to enhance our understanding of how the pharmacodynamic properties of these agents, including central nervous system penetration, affect toxicity and treatment response persistence. While manageable acute toxicities like skin rash and gastrointestinal symptoms are common, severe side effects, including secondary malignancies, have been reported.[101] Long-term chronic inhibitor therapy side effects, including impacts on growth and development in children, are largely unrecognized in case studies and series.

Chemotherapy for LCH may not always be effective, but it offers a potential cure. While MAPK inhibition shows significant activity, it often does not result in a cure, leading to rapid relapse post therapy. Combining MAPK inhibition with chemotherapy may be synergystic.[102,103]

In addition to chemotherapy and MAPK inhibitors, recent mechanistic insights offer potential novel therapeutic targets. Preclinical data support inhibiting apoptosis with BH3 mimetics,[35] utilizing senolytic agents that target the senescence-associated secretory phenotype (eg, sirolimus),[35] blocking differentiation through histone deacetylase inhibitors,[8] focusing on antigen targets (eg, CSF1R, SIRP$\alpha$),[104,105] and/or

leveraging immune checkpoint inhibitors (eg, PD-1)[106] could improve durability of responses to MAPK inhibition.

Historically, patients at high mortality risk from LCH are identified by disease presentation site.[16,107] Recent molecular data may improve risk stratification. For example, somatic *BRAF* mutations in LCH lesions are associated with increased systemic disease and LCH-ND risk.[8,36,40,41,78,79] Detecting $BRAF^{V600E}$ in blood samples, via cell-free DNA or peripheral blood mononuclear cells, correlates with systemic disease.[8,108] $BRAF^{V600E}$ allele frequency can indicate disease burden in LCH patients treated with chemotherapy.[8,108,109] Custom droplet digital PCR primers can identify similar mutation patterns in those with other MAPK pathway mutations.[110] The lack of association between peripheral $BRAF^{V600E}$ allele frequency and clinical response in patients treated with MAPK inhibition suggests that this approach blocks pathologic differentiation of mutated cells, but does not kill them.[96–98] Incorporating minimal detectable and residual disease strategies in future trials may provide useful risk stratification tools to inform therapy.

As many new therapies effectively prevent death and achieve unmatched response rates, the insights from our patients and their families are becoming crucial in shaping the best experiences during and post treatment. Our goal now is to discern and apply the most effective therapy for each patient with LCH, focusing on molecular drivers, disease extent, and persistence of LCH clones, to ensure long-term survival, minimal morbidity, and optimal quality of life.

## CLINICS CARE POINTS

- LCH is a myeloid neoplastic disorder driven by MAPK activation in myeloid precursors.
- Activating mutations in MAPK pathway genes, most commonly $BRAF^{V600E}$, are detected in almost all cases LCH. $BRAF^{V600E}$ is associated with extensive disease at presentation and risk of developing LCH-associated neurodegeneration.
- The current standard of care, basically unchanged for >30 years, consists of vinblastine/prednisone for all patients requiring systemic therapy. High relapse rates and morbidity associated with active disease highlight the need for developing more effective curative strategies.
- The majority of patients with relapsed and refractory LCH are treated off study with an increasing range of strategies. Prospective trials incorporating standardized response criteria are essential to defining optimal therapies.
- Early studies and reports with MAPK inhibitors (eg, vemurafenib, dabrafenib) demonstrate high response rates, but do not cure the majority of patients once therapy is discontinued. The impact of long-term MAPK inhibition in children is not known.
- Long-term side effects of MAPK inhibitors, including impacts on growth and development in children, remain inadequately studied.
- Combined treatment approaches with MAPK inhibitors and chemotherapy are under exploration.
- Preclinical data support potential therapies targeting apoptosis, immune modulation, and senescence-associated pathways.

## DISCLOSURES

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## REFERENCES


1. Willman CL, Busque L, Griffith BB, et al. Langerhans'-cell histiocytosis (histiocytosis X)–a clonal proliferative disease. N Engl J Med 1994;331(3):154–60.
2. Nezelof C, Basset F, Rousseau MF. Histiocytosis X histogenetic arguments for a Langerhans cell origin. Biomedicine 1973;18(5):365–71.
3. Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood 2010;116(11):1919–23.
4. Chakraborty R, Burke TM, Hampton OA, et al. Alternative genetic mechanisms of BRAF activation in Langerhans cell histiocytosis. Blood 2016;128(21):2533–7.
5. Chakraborty R, Hampton OA, Shen X, et al. Mutually exclusive recurrent somatic mutations in MAP2K1 and BRAF support a central role for ERK activation in LCH pathogenesis. Blood 2014;124(19):3007–15.
6. Diamond EL, Durham BH, Haroche J, et al. Diverse and targetable kinase alterations drive histiocytic neoplasms. Cancer Discov 2016;6(2):154–65.
7. Nelson DS, Quispel W, Badalian-Very G, et al. Somatic activating ARAF mutations in Langerhans cell histiocytosis. Blood 2014;123(20):3152–5.
8. Berres ML, Lim KP, Peters T, et al. BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. J Exp Med 2014;211(4):669–83.
9. Allen CE, Merad M, McClain KL. Langerhans-cell histiocytosis. N Engl J Med 2018;379(9):856–68.
10. Berres ML, Merad M, Allen CE. Progress in understanding the pathogenesis of Langerhans cell histiocytosis: back to Histiocytosis X? Br J Haematol 2015;169(1):3–13.
11. Alston RD, Tatevossian RG, McNally RJ, et al. Incidence and survival of childhood Langerhans cell histiocytosis in Northwest England from 1954 to 1998. Pediatr Blood Cancer 2007;48(5):555–60.
12. Guyot-Goubin A, Donadieu J, Barkaoui M, et al. Descriptive epidemiology of childhood Langerhans cell histiocytosis in France, 2000-2004. Pediatr Blood Cancer 2008;51(1):71–5.
13. Baumgartner I, von Hochstetter A, Baumert B, et al. Langerhans'-cell histiocytosis in adults. Med Pediatr Oncol 1997;28(1):9–14.
14. Gulati N, Allen CE. Langerhans cell histiocytosis: version 2021. Hematol Oncol 2021;39 Suppl 1(Suppl 1):15–23.
15. Chow TW, Leung WK, Cheng FWT, et al. Late outcomes in children with Langerhans cell histiocytosis. Arch Dis Child 2017;102(9):830–5.
16. Gadner H, Minkov M, Grois N, et al. Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. Blood 2013;121(25):5006–14.
17. Minkov M, Steiner M, Pötschger U, et al. Reactivations in multisystem langerhans cell histiocytosis: data of the international LCH Registry. J Pediatr 2008;153(5):700–5.e2.
18. Haupt R, Nanduri V, Calevo MG, et al. Permanent consequences in langerhans cell histiocytosis patients: a pilot study from the histiocyte society-late effects study group. Pediatr Blood Cancer 2004;42(5):438–44.
19. Wilk CM, Cathomas F, Torok O, et al. Circulating senescent myeloid cells infiltrate the brain and cause neurodegeneration in histiocytic disorders. Immunity 2023;56(12):2790–802.e6.
20. Ribeiro KB, Degar B, Antoneli CB, et al. Ethnicity, race, and socioeconomic status influence incidence of Langerhans cell histiocytosis. Pediatr Blood Cancer 2015;62(6):982–7.

21. Bhatia S, Nesbit ME Jr, Egeler RM, et al. Epidemiologic study of Langerhans cell histiocytosis in children. J Pediatr 1997;130(5):774–84.
22. Golpanian S, Tashiro J, Gerth DJ, et al. Pediatric histiocytoses in the United States: incidence and outcomes. J Surg Res 2014;190(1):221–9.
23. Venkatramani R, Rosenberg S, Indramohan G, et al. An exploratory epidemiological study of Langerhans cell histiocytosis. Pediatr Blood Cancer 2012; 59(7):1324–6.
24. Peckham-Gregory EC, McClain KL, Allen CE, et al. The role of parental and perinatal characteristics on Langerhans cell histiocytosis: characterizing increased risk among Hispanics. Ann Epidemiol 2018;28(8):521–8.
25. Chow EJ, Puumala SE, Mueller BA, et al. Childhood cancer in relation to parental race and ethnicity: a five-state pooled analysis. Cancer 2010;116(12):3045–53.
26. Betts DR, Leibundgut KE, Feldges A, et al. Cytogenetic abnormalities in Langerhans cell histiocytosis. Br J Cancer 1998;77(4):552–5.
27. da Costa CE, Szuhai K, van Eijk R, et al. No genomic aberrations in Langerhans cell histiocytosis as assessed by diverse molecular technologies. Genes Chromosomes Cancer 2009;48(3):239–49.
28. Murakami I, Gogusev J, Fournet JC, et al. Detection of molecular cytogenetic aberrations in langerhans cell histiocytosis of bone. Hum Pathol 2002;33(5): 555–60.
29. Chikwava KR, Hunt JL, Mantha GS, et al. Analysis of loss of heterozygosity in single-system and multisystem Langerhans' cell histiocytosis. Pediatr Dev Pathol 2007;10(1):18–24.
30. Nelson DS, van Halteren A, Quispel WT, et al. MAP2K1 and MAP3K1 mutations in Langerhans cell histiocytosis. Genes Chromosomes Cancer 2015;54(6): 361–8.
31. Rodig SJ, Payne EG, Degar BA, et al. Aggressive Langerhans cell histiocytosis following T-ALL: clonally related neoplasms with persistent expression of constitutively active NOTCH1. Am J Hematol 2008;83(2):116–21.
32. Papo M, Diamond EL, Cohen-Aubart F, et al. High prevalence of myeloid neoplasms in adults with non-Langerhans cell histiocytosis. Blood 2017;130(8): 1007–13.
33. Lavoie H, Gagnon J, Therrien M. ERK signalling: a master regulator of cell behaviour, life and fate. Nat Rev Mol Cell Biol 2020;21(10):607–32.
34. Brown NA, Furtado LV, Betz BL, et al. High prevalence of somatic MAP2K1 mutations in BRAF V600E-negative Langerhans cell histiocytosis. Blood 2014; 124(10):1655–8.
35. Bigenwald C, Le Berichel J, Wilk CM, et al. BRAF(V600E)-induced senescence drives Langerhans cell histiocytosis pathophysiology. Nat Med 2021;27(5): 851–61.
36. Heritier S, Emile JF, Barkaoui MA, et al. BRAF mutation correlates with high-risk langerhans cell histiocytosis and increased resistance to first-line therapy. J Clin Oncol 2016;34(25):3023–30.
37. Mourah S, How-Kit A, Meignin V, et al. Recurrent NRAS mutations in pulmonary Langerhans cell histiocytosis. Eur Respir J 2016;47(6):1785–96.
38. Rollins BJ. Genomic alterations in langerhans cell histiocytosis. Hematol Oncol Clin North Am 2015;29(5):839–51.
39. Durham BH, Lopez Rodrigo E, Picarsic J, et al. Activating mutations in CSF1R and additional receptor tyrosine kinases in histiocytic neoplasms. Nat Med 2019;25(12):1839–42.

40. Kemps PG, Zondag TCE, Arnardottir HB, et al. Clinicogenomic associations in childhood Langerhans cell histiocytosis: an international cohort study. Blood Adv 2023;7(4):664–79.
41. Hélias-Rodzewicz Z, Donadieu J, Terrones N, et al. Molecular and clinicopathologic characterization of pediatric histiocytoses. Am J Hematol 2023;98(7):1058–69.
42. Lang M, Chang L, Cai H, et al. BRAF deletion in adult patients with Langerhans cell histiocytosis correlates with multisystem disease and poor outcome. Clin Cancer Res 2024. https://doi.org/10.1158/1078-0432.Ccr-24-1802.
43. Héritier S, Hélias-Rodzewicz Z, Chakraborty R, et al. New somatic BRAF splicing mutation in Langerhans cell histiocytosis. Mol Cancer 2017;16(1):115.
44. Zarnegar S, Durham BH, Khattar P, et al. Novel activating BRAF fusion identifies a recurrent alternative mechanism for ERK activation in pediatric Langerhans cell histiocytosis. Pediatr Blood Cancer 2018;65(1). https://doi.org/10.1002/pbc.26699.
45. Zanwar S, Abeykoon JP, Dasari S, et al. Clinical and therapeutic implications of BRAF fusions in histiocytic disorders. Blood Cancer J 2022;12(6):97.
46. Kansal R, Quintanilla-Martinez L, Datta V, et al. Identification of the V600D mutation in Exon 15 of the BRAF oncogene in congenital, benign langerhans cell histiocytosis. Genes Chromosomes Cancer 2013;52(1):99–106.
47. Satoh T, Smith A, Sarde A, et al. B-RAF mutant alleles associated with Langerhans cell histiocytosis, a granulomatous pediatric disease. PLoS One 2012;7(4):e33891. https://doi.org/10.1371/journal.pone.0033891.
48. Alayed K, Medeiros LJ, Patel KP, et al. BRAF and MAP2K1 mutations in Langerhans cell histiocytosis: a study of 50 cases. Hum Pathol 2016;52:61–7.
49. Kamionek M, Ahmadi Moghaddam P, Sakhdari A, et al. Mutually exclusive extracellular signal-regulated kinase pathway mutations are present in different stages of multi-focal pulmonary Langerhans cell histiocytosis supporting clonal nature of the disease. Histopathology 2016;69(3):499–509.
50. Ozono S, Inada H, Nakagawa SI, et al. Juvenile myelomonocytic leukemia characterized by cutaneous lesion containing Langerhans cell histiocytosis-like cells. Int J Hematol 2011;93(3):389–93.
51. Héritier S, Saffroy R, Radosevic-Robin N, et al. Common cancer-associated PIK3CA activating mutations rarely occur in Langerhans cell histiocytosis. Blood 2015;125(15):2448–9.
52. Aricò M, Danesino C. Langerhans' cell histiocytosis: is there a role for genetics? Haematologica 2001;86(10):1009–14.
53. Arico M, Scappaticci S, Danesino C. *The genetics of Langerhans cell histiocytosis*. Histiocytic disorders of children and adults: basic science, clinical features and therapy. Cambridge University Press; 2005.
54. Aricò M, Haupt R, Russotto VS, et al. Langerhans cell histiocytosis in two generations: a new family and review of the literature. Med Pediatr Oncol 2001;36(2):314–6.
55. Beverley PC, Egeler RM, Arceci RJ, et al. The nikolas symposia and histiocytosis. Nat Rev Cancer 2005;5(6):488–94.
56. De Filippi P, Badulli C, Cuccia M, et al. Specific polymorphisms of cytokine genes are associated with different risks to develop single-system or multisystem childhood Langerhans cell histiocytosis. Br J Haematol 2006;132(6):784–7.

57. Peckham-Gregory EC, Chakraborty R, Scheurer ME, et al. A genome-wide association study of LCH identifies a variant in SMAD6 associated with susceptibility. Blood 2017;130(20):2229–32.
58. Yasmin N, Bauer T, Modak M, et al. Identification of bone morphogenetic protein 7 (BMP7) as an instructive factor for human epidermal Langerhans cell differentiation. J Exp Med 2013;210(12):2597–610.
59. Jung SM, Lee JH, Park J, et al. Smad6 inhibits non-canonical TGF-beta1 signalling by recruiting the deubiquitinase A20 to TRAF6. Article. Nat Commun 2013; 4:2562.
60. NCBI 1000 genomes browser, phase 3 may 2013 call set. Available at: http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/. Accessed March 20, 2018.
61. Pai P, Nirmal A, Mathias L, et al. Molecular mutations in histiocytosis: a comprehensive survey of genetic alterations. Mol Biotechnol 2024. https://doi.org/10.1007/s12033-024-01072-2.
62. Koh K-N, Yoon SH, Kang SH, et al. Advancements in the understanding and management of histiocytic neoplasms. Blood Res 2024/07/04 2024;59(1):22.
63. Niault TS, Baccarini M. Targets of raf in tumorigenesis. Carcinogenesis 2010; 31(7):1165–74.
64. Michaloglou C, Vredeveld LC, Mooi WJ, et al. BRAF(E600) in benign and malignant human tumours. Oncogene 2008;27(7):877–95.
65. Pui CH, Carroll WL, Meshinchi S, et al. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol 2011;29(5):551–65.
66. Shukla N, Ameur N, Yilmaz I, et al. Oncogene mutation profiling of pediatric solid tumors reveals significant subsets of embryonal rhabdomyosarcoma and neuroblastoma with mutated genes in growth signaling pathways. Clin Cancer Res 2012;18(3):748–57.
67. Zhang J, Wu G, Miller CP, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet 2013;45(6):602–12.
68. Tiacci E, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy cell leukemia. N Engl J Med 2011;364(24):2305–15.
69. Waterfall JJ, Arons E, Walker RL, et al. High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. Brief Communication. Nat Genet 2014;46(1):8–10. Available at: http://www.nature.com/ng/journal/v46/n1/abs/ng.2828.html#supplementary-information.
70. Wellbrock C, Arozarena I. The complexity of the ERK/MAP-Kinase pathway and the treatment of melanoma skin cancer. Front Cell Dev Biol 2016;4:33.
71. Weeks KS, Kahl AR, Lynch CF, et al. Racial/ethnic differences in thyroid cancer incidence in the United States, 2007-2014. Cancer 2018;124(7):1483–91.
72. Wu X-C, Eide MJ, King J, et al. Racial and ethnic variations in incidence and survival of cutaneous melanoma in the United States, 1999-2006. J Am Acad Dermatol 2011;65(5, Supplement 1):S26.e1–13.
73. Yoon HH, Shi Q, Alberts SR, et al. Racial differences in BRAF/KRAS mutation rates and survival in stage III colon cancer patients. J Natl Cancer Inst 2015; 107(10). https://doi.org/10.1093/jnci/djv186.
74. Law MH, Bishop DT, Lee JE, et al. Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. Article. Nat Genet 2015;47(9):987–95.
75. Whiffin N, Hosking FJ, Farrington SM, et al. Identification of susceptibility loci for colorectal cancer in a genome-wide meta-analysis. Hum Mol Genet 2014; 23(17):4729–37.

76. Qiu T, Lu H, Guo L, et al. Detection of BRAF mutation in Chinese tumor patients using a highly sensitive antibody immunohistochemistry assay. Article. Sci Rep 2015;5:9211.
77. Abdulkareem FB, Sanni LA, Richman SD, et al. KRAS and BRAF mutations in Nigerian colorectal cancers. W Afr J Med 2012;31(3):198–203.
78. Heritier S, Barkaoui MA, Miron J, et al. Incidence and risk factors for clinical neurodegenerative Langerhans cell histiocytosis: a longitudinal cohort study. Br J Haematol 2018;183(4):608–17.
79. McClain KL, Picarsic J, Chakraborty R, et al. CNS Langerhans cell histiocytosis: common hematopoietic origin for LCH-associated neurodegeneration and mass lesions. Cancer 2018;124(12):2607–20.
80. Acosta-Medina AA, Kemps PG, Zondag TCE, et al. BRAF V600E is associated with higher incidence of second cancers in adults with Langerhans cell histiocytosis. Blood 2023;142(18):1570–5.
81. Chen J, Zhao AL, Duan MH, et al. Diverse kinase alterations and myeloid-associated mutations in adult histiocytosis. Leukemia 2022;36(2):573–6.
82. Jouenne F, Chevret S, Bugnet E, et al. Genetic landscape of adult Langerhans cell histiocytosis with lung involvement. Eur Respir J 2020;55(2). https://doi.org/10.1183/13993003.01190-2019.
83. Cao XX, Duan MH, Zhao AL, et al. Treatment outcomes and prognostic factors of patients with adult Langerhans cell histiocytosis. Am J Hematol 2022;97(2):203–8.
84. Diamond EL, Subbiah V, Lockhart AC, et al. Vemurafenib for BRAF V600-mutant erdheim-chester disease and langerhans cell histiocytosis: analysis of data from the histology-independent, phase 2, open-label VE-BASKET study. JAMA Oncol 2018;4(3):384–8.
85. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. N Engl J Med 2015;373(8):726–36.
86. Haroche J, Cohen-Aubart F, Emile JF, et al. Reproducible and sustained efficacy of targeted therapy with vemurafenib in patients with BRAF(V600E)-mutated Erdheim-Chester disease. J Clin Oncol 2015;33(5):411–8.
87. Chen SH, Zhang Y, Van Horn RD, et al. Oncogenic BRAF deletions that function as homodimers and are sensitive to inhibition by RAF dimer inhibitor LY3009120. Cancer Discov 2016;6(3):300–15.
88. Foster SA, Whalen DM, Özen A, et al. Activation mechanism of oncogenic deletion mutations in BRAF, EGFR, and HER2. Cancer Cell 2016;29(4):477–93.
89. Yap J, Deepak R, Tian Z, et al. The stability of R-spine defines RAF inhibitor resistance: a comprehensive analysis of oncogenic BRAF mutants with in-frame insertion of αC-β4 loop. Sci Adv 2021;7(24). https://doi.org/10.1126/sciadv.abg0390.
90. Jain P, Surrey LF, Straka J, et al. BRAF fusions in pediatric histiocytic neoplasms define distinct therapeutic responsiveness to RAF paradox breakers. Pediatr Blood Cancer 2021;68(6):e28933. https://doi.org/10.1002/pbc.28933.
91. Poulikakos PI, Zhang C, Bollag G, et al. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature 2010;464(7287):427–30.
92. Karoulia Z, Gavathiotis E, Poulikakos PI. New perspectives for targeting RAF kinase in human cancer. Nat Rev Cancer 2017;17(11):676–91.
93. Gao Y, Chang MT, McKay D, et al. Allele-specific mechanisms of activation of MEK1 mutants determine their properties. Cancer Discov 2018;8(5):648–61.

94. Hanrahan AJ, Sylvester BE, Chang MT, et al. Leveraging systematic functional analysis to benchmark an in silico framework distinguishes driver from passenger MEK mutants in cancer. Cancer Res 2020;80(19):4233–43.
95. Heritier S, Jehanne M, Leverger G, et al. Vemurafenib use in an infant for high-risk langerhans cell histiocytosis. JAMA Oncol 2015;1(6):836–8.
96. Donadieu J, Larabi IA, Tardieu M, et al. Vemurafenib for refractory multisystem langerhans cell histiocytosis in children: an international observational study. J Clin Oncol 2019;37(31):2857–65.
97. Eckstein OS, Visser J, Rodriguez-Galindo C, et al, NACHO-LIBRE Study Group. Clinical responses and persistent BRAF V600E(+) blood cells in children with LCH treated with MAPK pathway inhibition. Blood 2019;133(15):1691–4.
98. Cournoyer E, Ferrell J, Sharp S, et al. Dabrafenib and trametinib in Langerhans cell histiocytosis and other histiocytic disorders. Haematologica 2024;109(4):1137–48.
99. Whitlock JA, Geoerger B, Dunkel IJ, et al. Dabrafenib, alone or in combination with trametinib, in BRAF V600-mutated pediatric Langerhans cell histiocytosis. Blood Adv 2023;7(15):3806–15.
100. Cohen Aubart F, Emile JF, Carrat F, et al. Targeted therapies in 54 patients with Erdheim-Chester disease, including follow-up after interruption (the LOVE study). Blood 2017;130(11):1377–80.
101. Tardieu M, Neron A, Duvert-Lehembre S, et al. Cutaneous adverse events in children treated with vemurafenib for refractory BRAF(V600E) mutated Langerhans cell histiocytosis. Pediatr Blood Cancer 2021;68(9):e29140.
102. Evseev D, Osipova D, Kalinina I, et al. Vemurafenib combined with cladribine and cytarabine results in durable remission of pediatric BRAF V600E-positive LCH. Blood Adv 2023;7(18):5246–57.
103. Karri V, Lin H, Velazquez J, et al. Clinical, radiological and molecular responses to combination chemotherapy with MAPK pathway inhibition in relapsed and refractory Langerhans cell histiocytosis. Br J Haematol 2024;204(5):1882–7.
104. Allen CE, Li L, Peters TL, et al. Cell-specific gene expression in Langerhans cell histiocytosis lesions reveals a distinct profile compared with epidermal Langerhans cells. J Immunol 2010;184(8):4557–67.
105. Okamoto T, Murata Y, Hasegawa D, et al. Targeting of SIRPα as a potential therapy for Langerhans cell histiocytosis. Cancer Sci 2023;114(5):1871–81.
106. Sengal A, Velazquez J, Hahne M, et al. Overcoming T-cell exhaustion in LCH: PD-1 blockade and targeted MAPK inhibition are synergistic in a mouse model of LCH. Blood 2021;137(13):1777–91.
107. Gadner H, Grois N, Potschger U, et al. Improved outcome in multisystem Langerhans cell histiocytosis is associated with therapy intensification. Blood 2008;111(5):2556–62.
108. Hyman DM, Diamond EL, Vibat CR, et al. Prospective blinded study of BRAFV600E mutation detection in cell-free DNA of patients with systemic histiocytic disorders. Cancer Discov 2015;5(1):64–71.
109. Heritier S, Helias-Rodzewicz Z, Lapillonne H, et al. Circulating cell-free BRAF(V600E) as a biomarker in children with Langerhans cell histiocytosis. Br J Haematol 2017;178(3):457–67.
110. Milne P, Abhyankar H, Scull B, et al. Cellular distribution of mutations and association with disease risk in Langerhans cell histiocytosis without BRAFV600E. Blood Adv 2022;6(16):4901–4.