High-risk LCH in infants is serially transplantable in a xenograft model but responds durably to targeted therapy

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Key Points
- Infants with BRAF V600E–mutant LCH and secondary hemophagocytosis respond promptly and durably to monotherapy with dabrafenib.
- Murine xenografts of bone marrow from high-risk LCH patients with hemophagocytosis exhibit phenotypic features similar to human disease.

Langerhans cell histiocytosis (LCH) is a rare hematologic neoplasm characterized by a clonal proliferation of Langerhans-like cells. Genomic profiling has identified recurrent somatic activating mutations in the mitogen-activated protein kinase pathway, which are targetable by small-molecule inhibitors. However, key questions such as the curative potential of targeted therapy and the cell of origin remain unanswered. In this study, we describe clinical outcomes of a series of pediatric patients with multisystem BRAF V600E–mutant LCH, as well as the results of accompanying murine xenograft experiments. Four infants with LCH (range, 7-11 months at diagnosis) and secondary hemophagocytic lymphohistiocytosis were referred to our institution and subsequently treated with the BRAF V600E–specific inhibitor dabrafenib. All patients achieved complete clinical responses by 8 weeks of therapy, with remissions lasting a median of 36 months (range, 27-42 months). One infant successfully discontinued therapy long-term upon achieving a molecular response by real-time quantitative polymerase chain reaction (RT-qPCR). We further characterized the disease-propagating cell population in a subset of these patients by transplanting whole bone marrow into immunodeficient mice. Xenografted animals exhibited decreased survival with hematologic abnormalities, splenomegaly, and histiocytic infiltrates in the bone marrow resembling human disease. This process could also be secondarily transplanted, resulting in a comparable disease latency with similar histologic findings. These data further support the presence of a disease-initiating cell in the bone marrow compartment. We demonstrate that despite aggressive disease behavior in a xenograft model, these patients can achieve sustained clinical remissions with targeted monotherapy, with a select subset achieving molecular responses by RT-qPCR.

Introduction

Langerhans cell histiocytosis (LCH) is a rare hematologic neoplasm, characterized by the clonal proliferation and subsequent tissue accumulation of CD207+ Langerhans-like histiocytes.1 Though affecting all ages, LCH occurs in children with a frequency ranging from 4.1 to 8.9 per million per year2-4 with an increased incidence in children <1 year of age. While LCH has a highly variable clinical presentation, many children are cured with combination chemotherapy, yet the risk of relapse remains high despite contemporary treatment protocols.5 Furthermore, some children may...
experience significant morbidity such as diabetes insipidus (DI), anterior pituitary dysfunction, and end-stage liver disease. A rare but potentially fatal complication of systemic LCH is secondary hemophagocytic lymphohistiocytosis (HLH), which has been shown to occur in children with multisystem LCH with a cumulative incidence of 9.3%. Primary HLH arises from cytotoxic T-cell and macrophage hyperactivation because of deficiencies in normal negative regulatory signaling, whereas secondary HLH reflects massive immune system activation in response to an insult such as infection or malignancy. The development of HLH in the setting of multisystem LCH is associated with a poorer prognosis and ultimately decreased overall survival.

Genomic alterations resulting in activation of the mitogen-activated protein kinase pathway are a key molecular pathogenic feature of the histiocytic neoplasms and in LCH usually involve activating mutations in BRAF [12-14] and MAP2K1 [15-18]. While targeted therapy with BRAF V600E-specific inhibitors has demonstrated significant responses in adults [19,20] and children with LCH [21,22], longitudinal experience with targeted therapy in infants with risk-organ (RO) positive LCH remains limited. Furthermore, relapses have rarely occurred in similar histiocytic disorders despite the use of BRAF inhibitors, and the curative potential as well as the necessary duration of therapy remain unknown. We present our experiences with a series of pediatric patients with refractory RO-positive LCH and secondary HLH and their responses to targeted therapy, as well as the results of our functional experiments to characterize the biologic behavior of disease-initiating cells in this population of patients.

Methods

Patients

Patients with LCH were referred to Cincinnati Children’s Hospital Medical Center, and diagnostic biopsy samples underwent either comprehensive genomic profiling (Foundation Medicine, Cambridge, MA) or BRAF sequencing as part of routine clinical care. Dabrafenib was prescribed off-label at the discretion of the treating physician upon an informed consent discussion with the family. All patient data and samples were collected under an institutional review board–approved protocol (#2008-0483).

Mice

Nonobese diabetic/LtSz-severe combined immunodeficiency interleukin-2 (IL-2) RG−/− SGM3 (NSGS) and nonobese diabetic/LtSz-severe combined immunodeficiency IL-2 RG−/− (NSG) mice were maintained under specific-pathogen–free conditions and in accordance with an Institutional Animal Care and Use Committee–approved protocol. Mice were kindly provided by the laboratory of James Mully (Cincinnati Children’s Hospital Medical Center) or purchased from The Jackson Laboratory. The strains are available as stock 013062 and stock 005557, respectively.

Patient-derived xenografts (PDXs)

Patient-derived samples underwent either Ficoll separation prior to cryopreservation, or if used fresh underwent RBC lysis using BD Pharm-Lyse (BD Biosciences). Mice were conditioned the day prior to transplant with a single intraperitoneal dose of busulfan (Sigma) at 30 mg/kg. The subsequent day, patient samples were treated with OKT3 (BioXCell, West Lebanon, NH) at a concentration of 1 µg per 1 × 10⁶ cells and injected IV via tail vein or intrafemorally as indicated. Blood counts were determined using a HemaVet 950FS analyzer (Drew Scientific, Miami Lakes, FL). Histological samples were fixed in 10% neutral buffered formalin and processed by the Research Pathology Core with clinical-grade antibodies as indicated. Micrographs were acquired on a Motic BA310 compound microscope equipped with an Olympus LC30 camera and captured with Olympus Lmicro software.

Flow cytometry, real-time quantitative polymerase chain reaction (RT-qPCR), and digital droplet PCR (ddPCR)

Bone marrow was collected from PDXs via aspiration or post-mortem as indicated. Single-cell suspensions were generated by crushing bones in media or mashing spleens through 40-µm cell strainers. Samples were stained in PBS/3% fetal bovine serum (flow buffer) and incubated for 1 hour at 4°C, followed by wash steps to remove unbound antibodies (described in supplemental Methods). Samples were acquired on a FACSCanto cytometer (BD Biosciences) by gating on live cells, singlets, and then cell populations of interest. Plots were analyzed with FlowJo V10 (BD Biosciences). Sorting was performed on a Sony SH800S (Sony Biotechnology). BRAF mutation status on xenograft samples was determined by extracting DNA from samples using the DNeasy Blood and Tissue Kit (Qiagen,) and analyzing 20 ng genomic DNA with the TaqMan Mutation Detection Assay (Thermo Fisher) for BRAF V600E (BRAF_476_mu) and the iTaq Universal Probes Supermix (Bio-Rad) according to the manufacturer’s instructions. Determination of mutation status was performed with the TaqMan Mutation Detection software (Thermo Fisher). Alternately, BRAF was amplified from genomic DNA using forward (5′-TACCATAAAC TTCCTATAATGCTG-3′) and reverse (5′-GTAATTCAGCAG CATCTCAGG-3′) primers (Integrated DNA Technologies) specific to BRAF exon 15 and MyTaq HS Red PCR Mastermix (Bioline) according to the manufacturer’s instructions, purified with the QiaQuick PCR Purification kit (Qiagen), and Sanger sequenced by the CCHMC DNA Sequencing and Genotyping Core. Chromatograms were visualized with FinchTV (Geospiza) ddPCR was performed on 200 ng genomic DNA as described in Heritier et al, with details (eg, probe sequences) provided in supplemental Methods.

Statistical analysis

Survival was calculated using the Kaplan-Meier method, and statistical significance determined using the log-rank test. Pairwise comparisons were performed using Student t test. All plots and statistical analyses were performed with GraphPad Prism 7 (GraphPad Software).

Results

Dabrafenib achieves rapid clinical responses in refractory disease

Four pediatric patients with LCH and secondary HLH were successfully treated with BRAF inhibition (Table 1). Patients ranged in age from 7 to 11 months at the time of initial diagnosis. While the initial responses of patients 1 and 2 were previously described in an earlier study, we provide additional longitudinal follow-up data
in this study. One patient (patient 4) received initial evaluation and treatment at our institution, and the remaining 3 patients were transferred to our center with refractory and/or progressive LCH as well as secondary HLH. Three patients exhibited multisystem disease at initial diagnosis, though 2 were RO negative, and 1 patient had only single-system disease at initial diagnosis. Patients were initially treated with a variety of LCH therapeutic regimens; however, all experienced disease progression despite treatment. The patient with single-system disease (skin) at diagnosis was initially treated with topical and systemic steroids; however, she experienced worsening multisystem disease 5 months later. The patients with multisystem disease at diagnosis were treated with chemotherapy per the referring institution, including standard regimens such as vinblastine/stereoids, clofarabine, and cytarabine (as both single-agent and combination therapy). Two patients received 2 treatment regimens, and 2 patients received 3 treatment regimens for refractory LCH. All patients continued to experience progressive LCH with multisystem, high-risk disease, as well as subsequent secondary HLH. The 3 patients not initially treated at our institution for LCH were transferred to our center at the time of HLH onset.

Features of HLH present in the 4 patients included high and persistent fevers, refractory cytopenias, splenomegaly, hemophagocytosis in bone marrow, elevated inflammatory markers (ferritin, soluble IL-2 receptor), and elevated transaminases. Bone marrow, lymph node, and/or skin biopsies were positive for the characteristic \( \text{BRAF}^{V600E} \) mutation in all patients by molecular testing and immunohistochemistry. Due to the progressive and refractory nature of their LCH and secondary HLH symptoms in the setting of a targetable mutation, all patients were treated with the \( \text{BRAF}^{V600E} \)-specific inhibitor dabrafenib (range, 3-5 mg/kg per dose, by mouth, twice daily). The median age at the start of dabrafenib was 16 months (range, 12-23 months). After dabrafenib initiation, all patients experienced rapid resolution of clinical HLH signs and symptoms. Additionally, within 2 months of initiation of therapy, all patients showed a significant reduction in LCH activity, with improvement in lesions on imaging (Figure 1A) and/or biopsy. By 8 weeks of treatment with dabrafenib, bone marrow biopsy specimens obtained in 3 patients showed dramatic reductions of morphologic evidence of LCH and HLH, namely \( \text{BRAF}^{V600E} \)-positive histiocytes with active hemophagocytosis (Figure 1B-G). In all patients, clinical remissions were sustained on dabrafenib. The median duration of dabrafenib treatment within this cohort is 31 months (range, 12-42 months). While receiving treatment with dabrafenib, patients 1 and 2 continued to exhibit the presence of \( \text{BRAF}^{V600E} \)-positive histiocytes on multiple follow-up bone marrow biopsies, but both remained clinically asymptomatic (Figure 1H-L). Patients 3 and 4 achieved molecular responses, which we defined as undetectable \( \text{BRAF}^{V600E} \) by RT-qPCR on the bone marrow at 12 months after dabrafenib initiation. We performed ddPCR on these same negative marrow samples on a research basis in order to determine whether there may be molecular evidence of disease below the threshold of detection of RT-qPCR. Indeed, we were still able to detect extremely low-level mutant \( \text{BRAF}^{V600E} \) in both samples (0.058% mutant alleles in patient 3 and 0.021% in patient 4; Figure 2A). An additional surveillance marrow sample was obtained in patient 4 14 months after dabrafenib discontinuation; this marrow similarly had a minute number of detectable mutant \( \text{BRAF}^{V600E} \) alleles (Figure 2B) with a frequency comparable to her prior bone marrow (0.019%). Patient 4 remains in clinical remission at the time of this writing, 18 months off of dabrafenib.

Unfortunately, after 10 months off of dabrafenib, patient 3 presented to her local provider with signs and symptoms consistent with DI and

### Table 1. Clinical characteristics and responses of dabrafenib-treated patients

| Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|-----------|-----------|-----------|-----------|
| Age at LCH diagnosis, mo | 10 | 7 | 11 | 7 |
| Sex | Female | Male | Female | Female |
| Race | White | Hispanic | White | African American |

**Disease risk**

| At diagnosis | Multisystem, low | Multisystem, high | Multisystem, low | Single system, low |
|-------------|-----------------|------------------|-----------------|-------------------|
| At initiation of dabrafenib | Multisystem, high | Multisystem, high | Multisystem, high | Multisystem, high |
| Location of disease at LCH diagnosis | Bone, skin | Skin, soft palate, spleen | Bone, skin | Skin |
| Treatments prior to dabrafenib | Methylpred/VBL, Dex/etop/CSA, clofarabine | Pred/VBL, clofarabine, AraC/clofarabine | Pred/MTX/6MP | Topical steroids, Pred |
| Disease location at dabrafenib initiation | Bone, bone marrow, skin | Bone, bone marrow, liver | Bone, bone marrow, lymph nodes, skin, thymus | Bone marrow, lymph nodes, skin, spleen |
| Age at dabrafenib initiation, mo | 23 | 17 | 15 | 12 |

**LCH response to dabrafenib**

| At 8 wk | CCR | CCR | CCR | CCR |
|---------|-----|-----|-----|-----|
| At last follow-up | CCR | CCR | Disease reactivation | CMR |
| Duration of dabrafenib use at last follow-up, mo | 42 | 38 | 24* | 12† |

**Dabrafenib toxicity**

| None | None | Transient skin rash | None |

*Discontinued 12 months after molecular response; targeted therapy resumed at 10 months after discontinuation due to relapse.
†Discontinued at molecular response.

CCR, complete clinical response; CMR, clinical and molecular response (by RT-qPCR); CSA, cyclosporin A; Dex, dexamethasone; Etop, etoposide; Methylpred, methylprednisolone; MTX, methotrexate; Pred, prednisolone; VBL, vinblastine.
pituitary stalk thickening on brain magnetic resonance imaging. While repeat PET/computed tomography was negative for other sites of disease, this was assumed to represent central nervous system LCH reactivation, and she was empirically started on trametinib as an alternative means of BRAF/MEK inhibition. At this point, her peripheral blood was still negative for BRAF V600E by RT-qPCR, but ddPCR detected an extremely small number of mutant BRAF alleles (Figure 2C). Based on this progression in patient 3 and the persistence of detectable BRAF-V600E by ddPCR, we recommended resumption of therapy for patient 4. However, the family declined therapy since the child remained in clinical remission.

Toxicities were minimal with dabrafenib. One patient (patient 2) experienced a patchy, erythematous rash after 2 months of therapy. He was treated with topical hydrocortisone and a 5-day course of oral prednisolone, after which his symptoms resolved. The rash has not recurred despite continuation of dabrafenib. No patients developed cutaneous squamous cell carcinoma.
Murine PDXs exhibit phenotypic similarities to human disease

To test our hypothesis that LCH with HLH originates from a hematopoietic stem/progenitor cell (HSPC) within the bone marrow compartment, diagnostic bone marrow from patients 3 and 4 or from another LCH patient without marrow involvement were xenografted into both NSGS and NSG mice. All recipient mice achieved engraftment of human cells based on human CD45 expression (Figure 3A), with decreased survival in transplant recipients (Figure 3B) and development of a disease phenotype resembling features seen in patients. Both strains of mice developed splenomegaly, which trended larger in xenografts from patients with marrow involvement but did not reach statistical significance (Figure 3C). Mice similarly generally exhibited mild anemia and thrombocytopenia (Figure 3D). Total white blood cell, monocyte, and neutrophil counts were not significantly different between groups (supplemental Figure 1A). Two of the NSG mice did not display signs of distress but upon sacrifice at the end of the observation period exhibited similar hematologic abnormalities as well as detectable BRAF V600E by RT-qPCR. The median survival of NSGS mice was 60 days (Figure 3B). On histologic examination of necropsy specimens, bone marrow demonstrated an expansion of CD163+ macrophages and frequent histiocytes with increased S100 staining and scattered CD1a positivity. Spleens showed varying degrees of effacement of the splenic architecture, with recapitulation of follicular structures.
Figure 3. Patient-derived bone marrow successfully engrafts primarily and secondarily in immunodeficient mice. (A) Representative flow cytometry scatterplots of human (h; x-axis) vs mouse (m; y-axis) engraftment of xenograft recipients following transplant of bone marrow from either RO-positive patients with hematopoietic involvement (BM+, right) or from an LCH patient without bone marrow involvement (BM−, left). (B) Survival curves of xenografted experimental animals. Survival between control and NSGS mice was statistically significant (*p < .01) by log-rank test. (C) Comparison between spleen sizes in control and experimental groups, with representative spleen images. (D) Hematologic abnormalities observed in BM+ and BM− transplanted animals. Error bars represent mean ± standard deviation. Statistical significance determined by Student t test. (E) Bone marrow histology from representative animals (original magnification ×400). (F) Representative flow scatterplots of bone marrow and spleens from a secondary transplant recipient animal. (G) Bone marrow from secondary transplant NSGS mouse shows a similar infiltration of histiocytes with abundant eosinophilic cytoplasm (left, original magnification ×400) and cytospins of splenocytes demonstrate frequent hemophagocytosis (white arrowheads) with numerous large, activated and vacuolated macrophages (right, original magnification ×1000). H&E, hematoxylin and eosin stain; WBC, white blood cell.
consisting primarily of human-derived B cells, as evidenced by immunohistochemistry and flow cytometry (supplemental Figure 1B), with variable numbers of CD163+ cells present. The degree of B-cell proliferation noted in spleens led us to question whether these could have originated from a transformed myeloid progenitor with lymphoid potential. To test this, we selected 2 representative animals with abundant CD20+ positivity in the spleen and purified human CD20+ cells by flow sorting followed by DNA extraction and PCR amplification of BRAF exon 15 for Sanger sequencing. We reasoned that if these did originate from a BRAF-mutant multipotent progenitor, the mutation would be readily detectable; however, both samples were wild-type for BRAF (supplemental Figure 1C). The liver parenchyma of animals transplanted with bone marrow from patients with RO-positive LCH and bone marrow involvement also exhibited nodules of CD163+ and S100-positive cells (supplemental Figure 1D). Additionally, bone marrow, liver, and spleen samples exhibited varying degrees of regional CD207 (Langerin) staining, typically within collections of histiocytes (supplemental Figure 1E). All bone marrow and spleen samples assayed from bone marrow–positive xenograft recipients were positive for the BRAF V600E mutation as determined by RT-qPCR (supplemental Table 1). As a comparison, bone marrow collected from an LCH patient without hematopoietic involvement was transplanted into mice as well. None of these mice became moribund during the observation period.

To test the self-renewal capacity of disease-initiating cells from our xenograft model, we performed secondary transplants of explanted cells. Cell doses ranged from 2.5 × 105 to 1.04 × 107 per animal. Samples obtained from primary NSGS animals failed to achieve human engraftment, consistent with previously published experience.29 However, in 4 recipient NSGS mice transplanted with either bone marrow or splenocytes from primary NSG xenografts, 1 died before it could be sampled, and 2 out of 3 subsequent mice examined demonstrated robust human engraftment as determined by flow cytometry for human CD45 (Figure 3F). These mice had hemoglobin counts of 12 and 11.9 g/dL and platelet counts of 240 and 1159 × 109/L, respectively. Both mice became moribund by days 31 and 44 and exhibited similar hematologic abnormalities and histologic findings on necropsy (Figure 3G) as well as molecular evidence of the BRAF V600E mutation (supplemental Table 1).

Discussion

In this case series, we describe our institutional experience with pediatric RO-positive LCH and secondary hemophagocytosis and the rapid clinical responses seen with targeted therapy. Using PDXs and primary/secondary transplant experiments with samples obtained from these patients, we further provide evidence that in pediatric patients with LCH and secondary HLH, the disease-propagating cells reside in the bone marrow, which can be serially transplanted in mice. Despite this behavior in our xenograft model, these patients are still able to attain durable complete clinical remissions and in certain cases molecular responses with targeted monotherapy. In select high-risk patients, the mutant BRAF V600E allele can be detected in the CD34+ fraction of the HSPC compartment.3 27,28 This has previously been experimentally demonstrated through the use of murine xenografts in the related adult histiocytic neoplasm ECD.27 Our findings provide further supporting evidence that in patients with high-risk LCH with HLH, the disease-initiating cell also originates in the bone marrow, as evidenced by the development of systemic disease in our PDX animals. The development of HLH in our system with both cytokine-expressing (NSGS) and non–cytokine-expressing (NSG) mice suggests that the mechanism underlying hemophagocytosis in our system may be distinct from the model presented by Wunderlich et al.29 Whereas the development of HLH in their system is most likely a consequence of cell-extrinsic factors, namely granulocyte macrophage colony-stimulating factor (and other cytokine)–mediated stimulation of myeloid cells in the marrow, we hypothesize that cell-intrinsic mitogen activating protein kinase activation may be a major driver of hemophagocytosis in our system. In patients, the rapid resolution of hyperinflammatory symptoms upon initiating a BRAF inhibitor, as well as the abundance of BRAF V600E–positive histiocytes, favors this hypothesis as well. A limitation of this system though is that we cannot rule out whether the xenograft phenotype and large burden of BRAF V600E–mutant cells may be in part due to absence of functional cell-mediated immunity in NSG/S mice. Indeed, preliminary evidence suggests that there may be some degree of effector T-cell exhaustion in the inflammatory component of some LCH lesions.30 Future experiments may determine whether humanization of mice with donor-derived immune systems will impact the progression of LCH in xenografts. Notably, attempts to graft on-therapy bone marrow samples in our study were unsuccessful despite ongoing evidence of persistent BRAF V600E–positive cells in the patient bone marrow biopsy specimens. We hypothesize that this may be due to impaired fitness of a mutated HSPC following dabrafenib treatment or other yet-uncharacterized host factors such as posttherapy disease burden.

We specifically elected to use dabrafenib rather than vemurafenib based on a more favorable side effect profile. While no head-to-head comparison has been performed between the 2 drugs, it appears that dabrafenib likely results in a decreased incidence of toxicities and adverse events (cutaneous and otherwise) compared with vemurafenib.31–33 The rapid improvements seen with HLH symptoms despite refractoriness to all previous therapies is consistent with previous individual reports in the literature.21,22,34 Remission of clinical LCH activity was sustained for over 24 months in all patients (>36 months in patients 1 and 2), suggesting that responses to dabrafenib may be durable. Despite dramatic clinical responses, 2 patients demonstrated the frank persistence of BRAF V600E in the bone marrow by standard molecular testing (RT-qPCR) or immunohistochemistry. This suggests that in these patients, a transformed neoplastic founder HSPC is not eradicated by targeted therapy alone. Patients 3 and 4 both achieved what we deemed a molecular response, namely no detectable BRAF V600E in bone marrow samples by RT-qPCR at 12 months after starting dabrafenib. However, a minute fraction of mutant BRAF alleles was still detectable by ddPCR when we performed a post hoc longitudinal analysis of these marrow samples. The clinical significance of this finding is unclear; while this represents the detection of cells bearing mutant BRAF V600E, we are not able to determine whether these cells retain disease-initiating potential or if they instead represent residual differentiated myelomonocytic cells that may no longer retain disease-initiating potential due to maturation. Further supporting the ambiguity of this finding is the fact that patient 3 developed DI while off of dabrafenib, which is a phenomenon well described even in patients who are treated with conventional systemic chemotherapy.35 However, patient 4 remains in clinical remission even after a prolonged period of time off of targeted therapy. We identified a single case report describing a molecular response in an adult patient treated with combined BRAF and MEK inhibition.36
in which circulating \textit{BRAF} V600E DNA became undetectable in the peripheral blood on therapy, although this was also determined using qPCR. In a recent study that used comparatively more sensitive ddPCR, a majority of patients still exhibited detectable \textit{BRAF} V600E alleles despite being on targeted therapy with vemurafenib.\textsuperscript{34} Despite this, these combined clinical and molecular responses are intriguing and suggest that it is possible to achieve molecular responses with monotherapy alone even in patients with hematopoietic involvement and secondary HLH, of whom a select few may even be candidates for a trial of therapy discontinuation.

In addition to the individual case reports we cite in this article, we attempted to summarize the published literature regarding the use of targeted therapy in LCH (Table 2). All studies demonstrated high overall response rates. In the 3 studies with pediatric patients,\textsuperscript{34,37,38} treatment-related toxicities were largely manageable. Two trials investigated the effect of therapy discontinuation; in the LOVE study, which consisted of adults with ECD treated with targeted therapy, 75% of patients who discontinued BRAF inhibitors (15/20) experienced disease recurrence, with a median time to relapse of 6 months.\textsuperscript{39} In the pediatric vemurafenib study by Donadieu et al, which was published while this manuscript was under review, a majority of RO-positive patients (20 out of 22) experienced disease reactivation upon targeted therapy discontinuation, with a 12-month reactivation rate in the RO-positive cohort of 95%.\textsuperscript{34} Despite this, all patients who resumed BRAF inhibitors after recurrence experienced clinical improvement, suggesting that these diseases retain susceptibility to targeted therapy even with treatment interruptions.\textsuperscript{34,39} Taken together, the results of the studies are insufficient to determine when, if at all, therapy may be discontinued, and in which patients. Given that patient 4 remains in a clinical remission despite her original multisystem involvement and persistent minimal residual disease, akin to leukemia risk stratification is applicable to treating LCH with targeted therapy. Specifically, even if ultrasensitive molecular techniques can detect molecular evidence of mutant \textit{BRAF}, there may be a threshold of disease below which sustained clinical remissions are feasible even without continued therapy. However, these hypotheses must be tested prospectively in a formal clinical trial.

Even though all patients achieved prompt clinical remissions with dabrafenib, it is unclear why patients 1 and 2 have not achieved molecular responses despite prolonged courses of treatment.

### Table 2. Published experience with targeted BRAF inhibitors

| Authors          | Year | Agents                   | Disease(s)               | Patients, n | Response                                                                 | Main toxicities (n)                                                                 |
|------------------|------|--------------------------|--------------------------|-------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Haroche et al\textsuperscript{20} | 2013 | Vemurafenib              | ECD, LCH/ECD             | 3           | Patient 1: improvement in all lesions, 4 mo (mean SUV change – 70%) Patient 2: resolution of skin lesions, PET improvement, 1 mo (mean SUV change – 57%) Patient 3: resolution of skin lesions, significant improvement on imaging, 2.5 mo | Keratosis pilaris (2), pruritus, skin erythema (3)                                      |
| Haroche et al\textsuperscript{40} | 2015 | Vemurafenib              | ECD, LCH/ECD             | 8 (including 3 from a 2013 report) | ORR, 100%; metabolic response: PR, 8 patients; cardiac infiltration: SD, 1 patient; PR, 7 patients | Keratosis pilaris (8), arthralgia (5), photosensitivity (5), QT prolongation (1), BCC (1), SCC (1) |
| Kieran et al\textsuperscript{38}   | 2015 | Dabrafenib              | HGG, LGG, LCH, OST       | 27          | HGG: PD, 2 patients; PR, 3 patients; CR, 3 patients LGG: PD, 1 patient; SD, 6 patients; PR, 8 patients LCH: CR, 2 patients OST: PD, 1 patient; SD, 1 patient | Mitrail regurgitation (1), arthralgia (1), HoTN, fever, DIC (1)                        |
| Cohen et al\textsuperscript{39}    | 2017 | Cobimetinib, dabrafenib, vemurafenib | ECD, LCH/ECD              | 54          | ORR: 88% (91% in V600E mutated) PMD, 2 patients; SMD, 4 patients; PMR, 35 patients; CMR, 7 patients | Cobimetinib: acne (8), nausea/vomiting (4), rhabdomyolysis (4), vemurafenib: photosensitivity, pil keratosis (16), arthralgia (7) |
| Diamond et al\textsuperscript{11}  | 2018 | Vemurafenib              | ECD, LCH                 | 26          | ORR: 61.5%; PD, 0 patients; SD, 9 patients; PR, 14 patients; CR, 2 patients; NE, 1 patient; PFS: 86% (2 y) OS: 96% (2 y) | Grade 3/4: cutaneous SCC (11), hypertension (7), maculopapular rash (6), increased Ipase (4), arthralgia (3), actinic keratos (2), hyperkeratosis (2), fatigue (1), prolonged QT interval (1), headache (1) |
| Bhatia et al\textsuperscript{12}   | 2018 | Dabrafenib (including 5 patients s/p vemurafenib) | ECD, ECD/LCH             | 11          | ORR: 100%; PMR, 8 patients (3 maintained PMR); CMR, 3 patients | Grade 3: fever (1) grade 2: fatigue (2), fever (1), keratosis pilaris (1), arthralgia (1) |
| Eckstein et al\textsuperscript{37} | 2019 | Dabrafenib, trametinib, vemurafenib | LCH                      | 21          | ORR: 86% PD, 1 patient; SD, 2 patients; PR, 14 patients; CR, 4 patients; PFS: 37% (median, 14 mo) OS: 90% (median, 14 mo) | Grade 3 dabrafenib: orbital swelling (1), hypokalemia (1), dabrafenib + trametinib: skin rash (1) Vemurafenib: uveitis (1), arthralgia (1), gastritis (1), neutropenia (1) |
| Donadieu et al\textsuperscript{34} | 2019 | Vemurafenib              | LCH                      | 54          | ORR: 100% CR, 38; PR, 16 | Grade 3: panniculitis (4), nosebleed (tumor necrosis) (1), clonus, neck and limbs (1) |

BCC, basal cell carcinoma; CMR, complete metabolic remission; CR, complete response; DIC, disseminated intravascular coagulation; ECD, Erdheim-Chester disease; HGG, high-grade gliomas; HoTN, hypotension; LGG, low-grade gliomas; NE, not evaluable; ORR, overall response rate; OS, overall survival; OST, other solid tumors; PD, progressive disease; PFS, progression-free survival; PMD, progressive metabolic disease; PMR, partial metabolic remission; PR, partial response; SCC, squamous cell carcinoma; SD, stable disease; SMD, stable metabolic disease; SUV, standardized uptake value.
Biopsy specimens from patients 1, 3, and 4 underwent targeted sequencing at the time of dabrafenib initiation, and no other known driver mutations were identified, including any previously associated with LCH or other neoplasms. The clinical responses we observed with single-agent dabrafenib further supports our conclusion that mutant BRAF was the sole driver of disease in these patients. Though we did not have sufficient material from patients 1 and 2 for xenograft experiments with pretherapy diagnostic bone marrow, we hypothesize based on the clinical bone marrow studies performed that the disease-propagating cells in these patients also originate in the bone marrow compartment. One notable difference that distinguishes patients 3 and 4 from the others is the lack of prior treatment with multiple rounds of intense, cytotoxic chemotherapy. Samples from patient 1 were sequenced at our institution after prior cytotoxic therapy and did not appear to acquire any additional potential driver mutations. The mechanisms underlying this observation are unclear. It is possible that heavy pretreatment with conventional chemotherapy may potentially render disease-propagating cells resistant to eradication by targeted therapy. Alternately, it is plausible that the disease in patients 1 and 2 was intrinsically more resistant to all therapies due to a yet unknown biological process. Further correlation and investigation of a larger cohort will be required to determine if this observation holds true and elucidate the reasons behind this phenomenon.

In summary, our clinical case series demonstrates that targeted therapy can quickly achieve disease control in infants with BRAF V600E–mutant high-risk LCH and secondary hemophagocytosis. We also provide an in vivo xenograft model of high-risk LCH that may allow further dissection of biological questions such as the cell of origin while potentially serving as a new platform for testing novel therapies. Overall, these results demonstrate that targeted therapies such as dabrafenib are a viable treatment option for BRAF V600E–mutant RO-positive LCH with hematopoietic involvement, with durable long-term clinical remissions, without the need for bone marrow transplantation. Given the high rates of treatment failure with conventional salvage therapy and the apparently manageable toxicities observed in our case series and other published studies with pediatric LCH patients, we believe a larger clinical trial is warranted to formally evaluate the efficacy of targeted therapy upfront in infants with high-risk LCH and HLH, in the interests of obviating the need for toxic salvage regimens and bone marrow transplant. Given the limited number of patients in our case series, larger and longer-term studies are necessary in order to determine (1) the appropriate duration of treatment, (2) the potential for long-term disease control and eventual cure, and eventually (3) the potential modifying effects of targeted therapy on the incidence of delayed sequelae of LCH such as DI and neurodegenerative disease.

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Authorship

Contribution: L.H.L., C.K., and A.R.K. designed the study, reviewed clinical data, conducted and oversaw experiments, and analyzed the data; L.H.L., J.C., M.W., and M.B. performed experiments and analyzed data; C.K., M.S.G., A.N., and R.B.L. conducted clinical management and data collection, and analyzed data; and all authors contributed to writing the manuscript.

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