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EC collected and analyzed data and authored manuscript, JF collected and analyzed data and authored manuscript, SS and ACM reviewed radiologic findings, edited manuscript and provided radiologic figures, JP analyzed pathology specimens and edited manuscript. RL analyzed pathology specimens, MJ, MG, AB, and AN provided clinical data, SR performed ddPCR (HistioTrak) analysis, AK provided clinical data, supervised the entire study, and edited the manuscript.

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ABSTRACT

Standard treatment for Langerhans Cell Histiocytosis (LCH) is chemotherapy, with high failure rates. Since MAP-kinase activating mutations are found in most cases, BRAF- and MEK-inhibitors have been used successfully to treat patients with refractory or relapsed disease. However, data on long-term responses in children are limited and no data exist on their use as first-line therapy. We treated thirty-four patients (26 LCH, 2 Juvenile Xanthogranuloma, 2 Rosai-Dorfman Disease, 4 presumed single site-CNS histiocytosis) with either dabrafenib and/or trametinib, either as first line or after relapse or failure of chemotherapy. Sixteen patients aged 1.3-21 years, had disease that was recurrent or refractory to chemotherapy, 9 of whom had multisystem LCH with risk-organ involvement. With a median treatment duration of 4.3 years, 15 (94%) patients have sustained favorable responses. Eighteen patients aged 0.2-45 years received the inhibitor as first-line treatment. All of these have had sustained favorable responses, with a median treatment duration of 2.5 years. Three patients with presumed isolated CNS/pituitary-stalk histiocytosis demonstrated stabilization or improvement of disease. Overall, inhibitors were well tolerated. Five patients with single system LCH discontinued therapy and remain off therapy without recurrence. In contrast, all 4 patients with multisystem disease that discontinued therapy were restarted. Our data suggest that children suffering from histiocytoses can be treated safely, and effectively with dabrafenib or trametinib. Additional studies are needed however to determine the long term safety and optimal duration of therapy.
INTRODUCTION

Histiocytic diseases are rare neoplastic disorders resulting from the aberrant accumulation of cells of the monocyte lineage, namely macrophages, or dendritic cells (1). The most common and well-studied histiocytic disease is Langerhans Cell Histiocytosis (LCH), a condition characterized by accumulation of clonal CD1a+ and CD207+ cells (2). LCH affects approximately one in 200,000 children and is most common in children one to three years old, but can present at any age (3). Clinical manifestations of LCH are highly variable, ranging from isolated self-limiting lesions to multi-organ disease that is associated with significant morbidity and mortality. Traditionally, the first-line treatment for LCH has been vinblastine and systemic steroids, based on the last international randomized control study nearly a decade ago (called LCH-III) (4). Patients with multisystem-LCH with risk organ (liver, spleen, bone marrow) involvement (MS RO+) have a variable course, with only about 60% achieving no active disease (NAD) after one year on standard LCH-III therapy. In MS-LCH without risk organ involvement (MS RO-), there is still about 37% relapse/reactivation rate after 1 year of standard therapy with vinblastine and prednisone (4). Second-line agents have included cytarabine and/or cladribine, or clofarabine for recurrent disease or for disease that failed to respond to standard of care (5). These chemotherapy treatments carry a high risk of morbidity, require central access for the duration of treatment, and are not always effective in MS RO+ and CNS disease (6). These regimens are associated with severe hematologic toxicities, often requiring transfusion support, as well as delayed immune reconstitution and grade IV neutropenia, with associated bloodstream infections. These toxicities are a high price to pay given the persistent risk of disease reactivation both during and after therapy (5).
The ontology of LCH lesions has been linked to bone marrow derived myeloid dendritic precursor cells. Activating mutations in the mitogen-activated protein kinase (MAPK) pathway play a central role in the pathogenesis of LCH (7-13). Approximately 50-60% of LCH lesions harbor a recurrent activating mutation in the BRAF gene, specifically BRAF-V600E (14, 15). Furthermore, even in cases without the BRAF-V600E mutation, there is ubiquitous activation of downstream phosphorylated kinases, phospho-MEK and phospho-ERK. This suggested that activation of the BRAF-MEK-ERK axis is universal in this disease, regardless of BRAF-V600E mutational status. (16) Given these findings, there has been growing interest in BRAF-MEK-ERK pathway inhibitors for treatment of histiocytic diseases. Several studies have shown promise for BRAF and MEK inhibitors as salvage therapy for the treatment of high risk LCH, particularly in multisystem disease or those with risk organ involvement (17-24). The utility and feasibility of these treatments as first-line therapies has yet to be elucidated.

Herein, we describe our experience using dabrafenib, a BRAF inhibitor, and trametinib, a MEK inhibitor to treat LCH and other histiocytic disorders. We initially treated several children whose disease was refractory to conventional treatments or had relapsed after therapy. Based on the observed responses and safety profile in these patients, we offered targeted therapies as first line therapy to newly diagnosed patients. All patients were treated off-label as described in detail under the methods section.

METHODS

Background rationale for patients treated with inhibitors

Several children were referred to us for refractory or relapsed LCH. With consistent positive results of dabrafenib and trametinib in these patients, we proposed a clinical trial to several
companies (17, 25). However, none were able to support a trial. We thus offered newly diagnosed patients the choice of treatment with conventional chemotherapy or an inhibitor. The justifications of this approach were the demonstrable efficacy of inhibitors in patients with refractory disease, and our goals of treatment, namely restoration of health and prevention of disease recurrence. The benefits and risks of conventional and targeted therapies were discussed in detail with the patient/parent, including the unknown potential for cure and unknown duration of optimal therapy for the latter. Patients and families were informed in detail about the off-label use of the agents and all were given the option of treatment with conventional chemotherapies. Patients receiving inhibitors were monitored for adverse effects with regular CBC, blood chemistries, and echocardiograms. CCHMC Institutional Review Board certified this retrospective study as exempt from oversight and from requiring informed consent.

Diagnosis was confirmed via histopathology when tissue samples were attainable (n=28) by expert pediatric pathologists (RL/JP). For LCH, the disease was classified as single system (SS) or multisystem (MS), with or without involvement of risk organs (liver, bone marrow, spleen). Mutations were identified by VE1-immunohistochemistry (BRAF-V600E) or NGS sequencing. Patients with diabetes insipidus and accompanying imaging findings of isolated pituitary stalk infiltration/thickening and/or loss of posterior pituitary bright spot were classified as isolated pituitary lesions. Other CNS manifestations (abnormal T2 signal in cerebellar nuclei or white matter, pontine lesions, cerebellar atrophy) were classified as isolated CNS LCH or neurodegenerative (ND) LCH. In cases of isolated pituitary or CNS disease, the presumed diagnosis was based on location and radiologic characteristics.

*Outcome Measures*
Clinical status and radiologic changes were used to assess response to therapy following the Histiocyte Society guidelines. Overall responses were classified as no active disease (NAD), active disease that was better since starting therapy (ADB), active disease that was progressive (ADP), or stable disease (ADS). For patients with irreversible diabetes insipidus (DI) the response was recorded separately from overall disease response. For example, in patients with MS-LCH and DI, complete resolution of non-pituitary disease was recorded as NAD with DI. Similarly, those with irreversible sclerosing cholangitis present at the time of initiating inhibitor, their overall disease response was separated from their liver disease (e.g., NAD with sclerosing cholangitis).

The imaging modalities used to assess response to treatment included positron emission tomography - computerized tomography scan (PET/CT), magnetic resonance imaging, computerized tomography scan, and ultrasound. For PET scans, the initial SUVmax was compared to the SUVmax of the same lesion on their first follow-up PET/CT for patients that had a PET/CT scan on record at the time of starting the inhibitor and at follow-up within one year of starting the medication. PET responses were considered as progressive disease, stable disease, improved disease, or complete resolution based on interval changes in SUVmax values, as well as number of lesions. There was not an absolute SUV value used as a negative cutoff. When looking at the response of LCH lesions to treatment, lesions were compared to the local background. For example, we would determine if a skeletal lesion continued to have uptake greater than the adjacent uninvolved bone or if a liver lesion still had uptake above uninvolved liver. This classification was then used in conjunction with clinical status to determine the overall response.

*BRAFV600E mutation detection by droplet digital PCR (ddPCR)*
When feasible, patients with a known BRAFV600E mutation had their peripheral blood analyzed for the presence of circulating mutant cells by real-time PCR or by droplet digital PCR (using DNA derived from peripheral blood mononuclear cells, available at our institution as HistioTrak). The platform for HistioTrak is a standard digital droplet PCR (BioRad, Inc) system that is highly optimized to maximize signal to noise ratio at mutation levels that are below 0.01% VAF. Fractional abundance or VAF < 0.001% is considered a negative result. The term “significant or not significant” is not used for clinical reporting of HistioTrak results.

RESULTS

This study is a single-center retrospective chart review of 34 patients with histiocytic disease who were treated with either dabrafenib and/or trametinib. Patient characteristics and outcomes are tabulated in Table 1 and Table 2. Thirty-four patients (12 female, 22 males) aged 0.2 to 45 years old were treated with targeted inhibitor therapy. Sixteen patients had received systemic treatment for their histiocytosis prior to initiation of the inhibitor and 18 received the inhibitor without prior treatment (see details under methods). The median age of diagnosis was 2.3 years and the median age at the start of inhibitor therapy was 1.9 years. Patient characteristics, average length of time of inhibitor treatment, risk organ involvement, mutation, site(s) of disease, inhibitor used, histiocytic disease classification response at last follow-up, and adverse effects are detailed in Table 1 for those with relapsed/refractory disease and in Table 2 for those treated with a targeted inhibitor as first-line.

Patients receiving inhibitors who were refractory to prior treatment
Details of the 16 patients who received the targeted inhibitor after undergoing earlier histiocytic disease specific therapy can be viewed in Table 1. Thirteen patients had biopsy confirmed LCH; 9 patients had multisystem (MS) risk organ positive (RO+) disease, 4 patients had MS RO- (negative) disease. Of these, 1 had MS RO+ LCH with CNS involvement and 1 had MS RO- LCH with pituitary stalk involvement. One patient had isolated CNS involvement (central DI, progressive ataxia and cognitive dysfunction) that could not be biopsied due to location of disease, however their imaging findings were highly characteristic of LCH (loss of posterior pituitary bright spot, abnormal T2 signal in white matter and cerebellum, and progressive cerebellar volume loss). Two patients had systemic Rosai-Dorfman Disease (RDD) (patient 15 lymph node and CNS disease; patient 16 skin, bone and lymph node). Of the 14 patients with tissue samples available for analysis, 13 had mutations in the BRAF-MEK-ERK pathway (BRAFV600E n=12, MAP2K1 n=1). In this cohort, the median age of initiation of inhibitor treatment was 2.4 years (1.3-31). Seven patients received dabrafenib, 7 received trametinib, and 2 patients received both drugs simultaneously. The rationale for the combination therapy for patient #14 was the unknown mutation and the lack of data on impact of inhibitors in CNS LCH at the time of initiation of treatment, while for patient #15 dabrafenib was added to help reduce the acneiform rash with trametinib (26). The median length of time this group has been treated with the inhibitor is 4.3 years (range 0.3 – 7.3 years). As shown in Figure 1, in this group, 6 patients with LCH now have NAD, 6 have NAD with residual organ damage (4 with DI, 1 with sclerosing cholangitis, and 1 with both DI and sclerosing cholangitis). Of the two patients with RDD, one has stable disease, while the other suffered progressive disease. The patient with isolated CNS disease (patient #14) had improvement of neurological symptoms and function.
Patients receiving inhibitor as first-line therapy

Eighteen patients were treated with targeted inhibitors as first-line therapy (Table 2). Thirteen patients had biopsy confirmed LCH; 7 patients had single system (SS) disease, 3 patients had MS RO- disease, (1 with CNS involvement), and 3 patients had MS RO + disease. Four patients with SS LCH had solitary bone lesions and received treatment due to location (CNS-risk) and/or due to rapid growth of the lesion or persistence of pain. There were 2 patients with isolated CNS or pituitary stalk lesion(s) that could not be biopsied due to location of disease, however their imaging findings were highly characteristic of LCH (abnormal T2 signal in white matter and deep cerebellar nuclei). One patient with isolated CNS disease (#32) was found to have circulating BRAFV600E+ cells via peripheral blood ddPCR, suggesting LCH as the likely diagnosis (Figure 2). Two patients had progressive systemic Juvenile Xanthogranuloma (JXG). In this cohort, 4 patients received dabrafenib, 13 received trametinib, and one patient (#23) was initially treated with trametinib but then switched to dabrafenib due to side effects. Of the 13 patients with tissues samples available for analysis, 12 had mutations in the BRAF-MEK-ERK pathway (BRAFV600E n=9, GAB2-BRAF fusion n=1, BRAF indel n=2). One patient with JXG had a TFG-RET fusion that was identified after the patient had experienced a dramatic response to treatment with trametinib. In this group, the median age of treatment initiation was 5.5 years (0.2-45) and the median treatment duration was 2.5 years (range 0.3-6.4 years). As depicted in Figure 3, twelve patients with LCH currently have NAD, one patient with MS RO- LCH which included CNS disease now has NAD with resolution of DI and improved clinical neurocognition. Of the two patients with systemic JXG (patient 33 with skin, liver, spleen, bone marrow disease and patient 24 with CNS and skin disease), one has
NAD while the other has NAD with DI. Three patients had isolated CNS or pituitary stalk disease, two of whom are currently improved, while one has stable disease.

Overall, no patients in either group experienced progression or worsening of disease on therapy, including notably patients with MS RO+ disease. When available, there was a universal decrease in PET scan activity upon treatment with trametinib and/or dabrafenib (Supplemental Figure 1). Eleven patients (4 MS RO+ LCH, 7 SS LCH) were trialed off inhibitor therapy with 4 experiencing relapses of disease ranging from 3 weeks to 1 year after discontinuation with median time of 5 months. Three of the 9 relapsed/refractory MS RO+ patients had their therapy paused. All 3 had disease recurrence at 3 weeks, 5 months and 10 months after cessation of inhibitor, all of whom achieved NAD after inhibitor was resumed. The remaining 6 patients with RO+ disease were not trialed off therapy and remain with NAD. One patient with SS multifocal bone disease (no prior chemotherapy) suffered recurrence upon stopping therapy after 1 year and similarly experienced resolution of disease when inhibitor was resumed. Only one patient receiving inhibitor as first line therapy with MS RO+ LCH (patient 17) was taken off therapy per parental preference and given that peripheral blood and bone marrow BRAFV600E PCR (real time) were both negative. This patient remains with NAD, however therapy was resumed 18 months later once it was discovered that peripheral blood HistioTrak (ddPCR for BRAF-V600E) was positive. Six patients with SS disease did not experience recurrence following cessation of therapy (4 patients with SS unifocal bone disease who were treated due to location of the lesion being CNS-risk or due to rapidly growing lesion or persistent symptoms and 2 patients with SS multifocal bone disease). These patients were treated for a median time of 1.8 years (range 0.3 months to 4 years) and the median time off therapy was 11 months (range 4 months to 3.2 years).
Dosing and Side Effects

Patients treated with trametinib were prescribed an oral starting dose of 0.025 mg/kg daily. For young children, the 0.5 mg tablet was dissolved in 5 ml of clear liquid and the appropriate dose calculated for each patient. For patients treated with dabrafenib, the starting dosage was an oral formulation of 3-5 mg/kg daily in two divided doses. The contents of the 50 mg capsule were dissolved in 5 ml of clear liquid and the dose calculated for each child based on weight. Each dose was prepared fresh. Attributable side effects for each inhibitor are listed in Table 1 and Table 2. Most of the listed side effects were considered minor. The most common reported side effect was skin rash with trametinib in 6/20 patients. The only reported side effect of dabrafenib was nausea in 1/11 patients. Four out of twenty patients on trametinib required dose adjustment due to side effects of skin rash or abdominal pain, which resolved upon dose reduction. Only one patient stopped trametinib due to side effects. Patient #23 had hair-thinning and was transitioned from trametinib to dabrafenib per parental preference. No patients on dabrafenib needed dose adjustment.

Peripheral blood BRAF V600E analysis in select patients

For patients with BRAF-V600E associated disease, peripheral blood monitoring was performed either by real-time PCR or more recently by ddPCR. The results of the most recent testing are shown in Tables 1 and 2. In most patients with MS RO+ LCH, circulating BRAF-V600E+ cells were detectable even after their disease was inactive (NAD). Notably, in several patients we did not detect circulating BRAF-V600E by real-time PCR, but did see low level detection by ddPCR, highlighting the importance of using methodologies with higher sensitivity to detect the presence of residual disease cells.
DISCUSSION

In 2017, we reported dramatic sustained clinical responses in refractory multisystem LCH to BRAF-MEK pathway targeted inhibitors (25). Although limited by number of pediatric patients, several other reports have also shown sustained clinical responses with targeted BRAF inhibitors dabrafenib and/or vemurafenib in histiocytosis, which has been most notably in refractory disease (17-19). Moreover, a large pediatric international observational study recently demonstrated that vemurafenib was effective at controlling refractory LCH with the BRAFV600E mutation, but not curing the disease as shown by reactivation after stopping therapy (20). A recently published prospective phase 1/2 study evaluated single-agent dabrafenib or the combination of dabrafenib and trametinib in pediatric patients with recurrent/refractory LCH. Both groups demonstrated clinical efficacy and manageable toxicity, with most responses ongoing at the end of the study (24). Outside of pediatrics, these inhibitors have also shown efficacy in adults with histiocytic disorders (27-29). The efficacy of targeted inhibitors as a first-line monotherapy therapy is largely unknown, as most patients in these studies have been previously treated with conventional therapies and either did not respond or relapsed. Given the dramatic, consistent, and sustained responses seen in patients with refractory high-risk disease, we aimed to treat patients newly diagnosed with histiocytic disorders with inhibitors as first-line therapy to achieve rapid and durable disease control. Ideally, such novel treatments should be administered as part of a prospective clinical trial. Unfortunately, none of the manufacturers of the available BRAF or MEK inhibitors were able to support such a trial. With the overall treatment failure rates of ~40% with chemotherapy compared to the almost 100% response rate with the targeted inhibitors, we chose to treat patients with the latter FDA-approved drugs off-
label, with the goal of restoring the patient’s health and preventing disease recurrence. The secondary benefits were the ease of administration while also sparing patients the potential side effects, toxicity and morbidity of traditional chemotherapeutic agents.

In our study, most of the patients had LCH, and consistent with the existing literature, the majority harbored BRAFV600E or another BRAF-MEK-ERK pathway activating mutation. All the patients with LCH or JXG, regardless of the mutation showed a favorable response to dabrafenib or trametinib, ranging from stabilization of disease (CNS disease, sclerosing cholangitis of liver) to complete clinical and radiographic resolution. The responses were comparable in patients treated with either dabrafenib or trametinib. Trametinib and dabrafenib each led to sustained clinical responses in patients with all classifications of disease, either as first line therapy or in recurrent/refractory disease (Figures 1 and 3). Dabrafenib is effective only in the case of BRAF-V600E, while trametinib, being a MEK inhibitor has a wider application. Once we observed responses to trametinib in patients whose disease was driven by other mutations in BRAF or in MAP2K1, we treated all subsequent patients with trametinib, regardless of the driver mutation. One reasonable approach is to use a BRAF-inhibitor in patients with BRAF-V600E-associated disease, and a MEK-inhibitor in all the others. Our data suggest that a MEK-inhibitor may be useful in all patients, regardless of the driver mutation. None of the patients in our cohorts had disease associated with RAF-independent mutations in MAP2K1 (Class III), which are known to be resistant to allosteric MEK-inhibitors such as trametinib (30). Given the tolerability and efficacy in patients with either BRAF or MAPK21 mutations, MEK inhibitors such as trametinib may be utilized initially in all patients while awaiting identification of the mutation.
It is well known that patients with MS RO+ LCH are at considerable risk for disease progression despite conventional chemotherapy treatments (4). In concordance with previous studies, we saw sustained dramatic clinical and radiologic responses, even in MS RO+ patients that were refractory to chemotherapy (18, 19, 21). Moreover, three patients with MS RO+ LCH treated with the inhibitor as first line therapy had NAD at follow-up that has been sustained, making these inhibitors a potential novel therapeutic option for pediatric patients with high-risk disease. The range of disease response to therapy was largely based on classification of disease, with the known irreversible effects such as diabetes insipidus (DI) or sclerosing cholangitis/liver cirrhosis persisting despite therapy (e.g. Patients #4 and #8). The goals of treatment in these situations are to preserve organ function and prevent further progression (31). Unfortunately, by the time these two patients were referred to us for inhibitor therapy, they both had already developed extensive fibrosis in the liver, which was irreversible. In patients treated with inhibitors as first-line therapy, we hope to decrease or prevent the development of these permanent consequences. In fact, no patients in our study (in either cohort) developed DI, cirrhosis, or ND LCH while on targeted therapy. While CNS penetration of MEK inhibitors varies by drug, they remain a mainstay of brain tumor therapy for low grade gliomas, neurofibromatosis type I (NF1) and metastatic melanoma (32). We report significant improvement for the patients in our cohort with CNS disease treated with MEK inhibition, including those with isolated CNS disease, in accordance with what was described by McClain et al in their 2018 report on CNS LCH (33). The only patient whose disease did not respond to targeted inhibitor was Patient #16, who had recurrent/refractory Rosai-Dorfman Disease (RDD) for 12 years that did not respond to numerous treatments and did not have an identifiable mutation on multiple biopsies. Trametinib was tried due to persistent, severe knee pain in an area
of perceived active disease. However, symptoms persisted, and trametinib was stopped when the
disease progressed.

The adverse effects of trametinib and dabrafenib in our study were generally mild and
well tolerated with only one patient experiencing side effects significant enough to stop therapy
(patient #26). It is also notable that responses were sustained at smaller doses as seen in patients
#11, #30, and #28. The discontinuation rate of these inhibitors in our study is less than that
reported in melanoma treatment of 11.5-15.7% (34). Although an increase in risk skin-cancers
has been attributed to BRAF and MEK inhibitors in the adult population, none of our patients
developed skin cancer during their treatment (35). As the average length of treatment in our
study was just over three years and three months, the longer-term tolerability remains to be
elucidated.

One of the great medical challenges in treating patients with BRAF and MEK inhibitors
has been the question of when to stop therapy. In our study, all patients with SS solitary bone
lesions and two of three patients with SS multifocal bone disease experienced sustained
remissions upon discontinuation of therapy. It is known that some patients with SS solitary bone
lesions may experience resolution of disease without treatment. As such, these lesions are not
always treated and are instead monitored over time. In cases where the lesion involves the cranial
bones outside the calvarium however, treatment is recommended due to the risk of progression to
the CNS. We thus cannot state with certainty if the responses seen in this cohort were due to
therapy or due to spontaneous remission. The rapid resolution of symptoms and the
accompanying radiographic improvement are consistent with treatment effect. One patient with
SS multifocal bone disease (#28) in whom therapy was stopped at one year suffered a recurrent
bone lesion shortly upon discontinuation of trametinib. Resumption of treatment resulted in a
rapid response that has been sustained. Additionally, all MS-LCH patients who stopped therapy experienced recurrence of disease, but regained NAD once inhibitor was reinitiated. These data support others’ conclusions that potentially outside of solitary bone lesions, one-year of inhibitor-treatment is insufficient. Further, in MS-LCH, inhibitors likely do not eradicate disease cells but rather induce clinical (silent) remission (19, 20). We detected very low levels of circulating mutant cells in the blood of many MS-LCH patients, even upon attaining complete clinical remission on inhibitor therapy. This phenomenon is thought to be due to the presence of presumed long-lived, but scantily represented, mutant bone marrow progenitor cells that serve as the reservoir of disease (10, 13, 17). However, given the clinical and radiologic remission, the inhibitor therapy does effectively inhibit (renders static) the mutant cell(s) from causing systemic and tissue-specific inflammatory damage to the end-organs, and thus likely prevents permanent, irreversible consequence in these children (ie. diabetes insipidus, neurodegeneration, liver cirrhosis secondary to sclerosing cholangitis) along with quickly ameliorating the hyper-inflammatory HLH-like cytokine storm in MS RO+ infants which carries a high risk of mortality (36).

A highly sensitive minimal residual disease marker is needed to guide physicians on the duration of therapy based on the molecular detection of occult disease. Unfortunately, testing the blood for the circulating mutant BRAFV600E cells by RT-PCR has not proven to accurately monitor molecular remission, as clinical relapses have been noted after cessation of therapy despite negative RT-PCR results (17). In fact, when these same samples were re-tested by ddPCR on circulating mononuclear cells (not cell-free DNA), low level mutational burden was found. We have now developed this assay as a clinical test, called HistioTrak to serve as a high sensitivity biomarker for minimal residual disease. As predicted, majority of patients with MS
LCH had detectable circulating BRAF-V600+ cells by HistioTrak, even years after inhibitor therapy. Remarkably, HistioTrak helped us diagnose the etiology of isolated DI in a patient whose MRI demonstrated pituitary-stalk infiltration (Figure 2). Although the DI persists, the pituitary stalk infiltration resolved rapidly with trametinib therapy and the child remains otherwise asymptomatic, with no clinical evidence of neurologic dysfunction. However, his peripheral blood HistioTrak remains positive in spite of treatment with trametinib for 2 years. Incorporation of molecular assays such as HistioTrak will help improve the diagnosis, treatment, and monitoring of patients with histiocytic diseases, reducing the risk of long-term complications such as neurodegenerative disease.

Despite the successful clinical remissions reached by targeted therapies in histiocytic disorders, our study is limited in being a retrospective review, not a prospective clinical trial. As such, it is difficult to directly deduce the efficacy of these therapies. We also recognize the inability of these agents to cure MS-RO+ LCH. Additionally, since these drugs are relatively new, the long-term safety of treatment is unknown, including impacts on fertility. The utility of inhibitors in patients with isolated DI is also unknown, and as such treating these patients with inhibitors is not universally accepted. However, despite these limitations, our experience suggests that targeted therapies are safe and appear more efficacious in controlling disease than conventional chemotherapy, as shown by the consistent response in both refractory and newly diagnosed disease. To demonstrate efficacy rigorously, prospective studies are needed. Given the rarity of this disease, it is our hope that these aggregate results will lead to prospective clinical trials that will help answer the question of efficacy. In addition, we believe that the inability to completely eradicate mutant cells and achieve cure (defined as absence of disease without therapy) should not be the reason to discard or not utilize these life-preserving targeted therapies.
There are tantalizing reports of disease eradication being attained by combining targeted therapy with chemotherapy (37). The utility and safety of such combination approaches will need to be evaluated in larger groups of patients, probably once a targeted therapy is approved and readily available for use in children.

In summary, patients with histiocytic disorders can be treated safely, and effectively with the targeted BRAF inhibitors such as dabrafenib (for those with BRAF-V600E mutant disease) or a MEK inhibitor such as trametinib (in disease caused by any BRAF and most MAP2K1 mutations). Although our data suggest that it may be possible to discontinue inhibitor therapy in single system disease, future prospective studies are needed to determine when and if patients with multisystem disease can safely discontinue therapy. The development of highly sensitive molecular testing for MRD may help clinicians make this decision and should be incorporated into these future studies. The availability of an efficacious, well-tolerated treatment for patients with high-risk disease offers a breakthrough therapeutic choice in a potentially fatal condition. Likewise, this also presents a new therapeutic possibility for patients with neurodegenerative disease for whom currently no effective therapy is available. Prospective studies are warranted to further determine the long-term efficacy and tolerability of inhibitors as first line therapy as well as the length of treatment.
References:


TABLES

TABLE 1 Trametinib and/or Dabrafenib in Relapsed or Refractory Disease. Disease classifications, mutations, and response to trametinib and or dabrafenib in patients who received prior therapy for their histiocytic disorder.
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<th>Age (yrs)/Sex</th>
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<td>3</td>
<td>0.8/M</td>
<td>LCH</td>
<td>MS, RO + skin, bone, LN, liver, spleen</td>
<td>BRAF V600E</td>
<td>1) VBL, CS 2) ARA-C 3) CAFdA</td>
<td>1.9</td>
<td>DAB</td>
<td>7.3</td>
<td>NAD</td>
<td>Y</td>
<td>5 mo</td>
<td>none</td>
<td>-</td>
<td></td>
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<tr>
<td>4</td>
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<td>LCH</td>
<td>MS, RO + skin, LN, bone, spleen, liver</td>
<td>BRAF V600E</td>
<td>1) VBL, CS 2) CAFdA</td>
<td>1.3</td>
<td>DAB</td>
<td>5.0</td>
<td>NAD + sclerosing cholangitis</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>Low +</td>
<td></td>
</tr>
<tr>
<td>5</td>
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<td>LCH</td>
<td>MS, RO + skin, bone, spleen, BM</td>
<td>BRAF V600E</td>
<td>1)VBL, CS 2) CAFdA 3) ARA-C, CAFdA</td>
<td>1.4</td>
<td>DAB</td>
<td>6.3</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
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<td>LCH</td>
<td>MS, RO + skin, bone, liver, spleen, BM, LN</td>
<td>BRAF V600E</td>
<td>1) VBL, CS 2) CAFdA 3) CS, vemurafenib, VBL</td>
<td>2.9</td>
<td>DAB</td>
<td>3.6</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td></td>
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<tr>
<td>7</td>
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<td>LCH</td>
<td>MS, RO +, CNS skin, bone, CNS, spleen</td>
<td>BRAF V600E</td>
<td>VBL, CS</td>
<td>1.7</td>
<td>TRA</td>
<td>4.4</td>
<td>NAD + DI</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td></td>
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<tr>
<td>8</td>
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<td>LCH</td>
<td>MS, RO + skin, liver, PS</td>
<td>BRAF V600E</td>
<td>1) VBL, CS 2) ARA-C, CS</td>
<td>3.8</td>
<td>TRA</td>
<td>2.1</td>
<td>NAD+ DI + cholangitis</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>0.5/M</td>
<td>LCH</td>
<td>MS, RO + skin, BM, liver, spleen</td>
<td>BRAF V600E</td>
<td>1) VBL, CS 2) CAFdA</td>
<td>1.3</td>
<td>DAB</td>
<td>4.2</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>0.7/M</td>
<td>LCH</td>
<td>MS, RO- PS, skin bone</td>
<td>BRAF V600E</td>
<td>1) VBL, CS 2) ARA-C 3) CAFdA</td>
<td>7.3</td>
<td>TRA</td>
<td>4.4</td>
<td>NAD + DI</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>-</td>
<td>Low +</td>
</tr>
<tr>
<td>11</td>
<td>1.2/M</td>
<td>LCH</td>
<td>MS, RO- skin, bone, inner ear mass</td>
<td>BRAF V600E</td>
<td>1) VBL, CS, MP</td>
<td>1.8</td>
<td>TRA</td>
<td>1.8</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>rash (DR)</td>
<td>-</td>
<td>ND</td>
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<tr>
<td>12</td>
<td>2.8/M</td>
<td>LCH</td>
<td>MS, RO-skin, PS, bone</td>
<td>MAP2K1Q56P</td>
<td>1) ARA-C 2) hydroxyurea, MXT</td>
<td>5.8</td>
<td>TRA</td>
<td>2.5</td>
<td>NAD +DI</td>
<td>N</td>
<td>n/a</td>
<td>loose stools</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>13</td>
<td>13.6/ M</td>
<td>LCH</td>
<td>MS, RO- bone, lungs</td>
<td>BRAF V600E</td>
<td>ARA-C</td>
<td>15.42</td>
<td>TRA</td>
<td>2.1</td>
<td>ADB</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>-</td>
<td>-</td>
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<tr>
<td>14</td>
<td>17.4/ M</td>
<td>LCH-ND</td>
<td>Isolated CNS</td>
<td>n/a</td>
<td>1) ARA-C cytarabine 2) CAFdA 3) MTX, rituximab</td>
<td>20.8</td>
<td>DAB, TRA</td>
<td>6.3</td>
<td>Improv. neurologic symptom, function</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>-</td>
<td>Low +</td>
</tr>
<tr>
<td>15</td>
<td>11.8/ F</td>
<td>RDD</td>
<td>LN, CNS</td>
<td>unknown</td>
<td>CS</td>
<td>11.9</td>
<td>TRA, DAB</td>
<td>2.8</td>
<td>ADS</td>
<td>N</td>
<td>n/a</td>
<td>rash (TRA)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>20.0/ F</td>
<td>RDD</td>
<td>skin, bone, LN</td>
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<td>1) VBL, CS 2) MTX 3) CAFdA 3) sirolimus, CS 4) ARA-C</td>
<td>31.0</td>
<td>TRA</td>
<td>0.3</td>
<td>ADP</td>
<td>N</td>
<td>23 mo</td>
<td>rash (DR)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

LCH= Langerhans Cell Histiocytosis, JXG= juvenile xanthogranuloma, RDD= Rosai-Dorfman disease, CNS = central nervous system, Dx= diagnosis, BM= bone marrow, LN= lymph node, PS=Pituitary Stalk, MS= multisystem, RO+/-= Risk organ positive/negative, ITx= Inhibitor treatment, VBL= Vinblastine, CS= corticosteroid, EPEG=etoposide CTX=cyclophosphamide, CAFdA=clofarabine, ARA-C= cytarabine, MP= mercaptopurine. MTX=Methotrexate, DAB=Dabrafenib, TRA=Trametinib, NAD = no active disease; NAD + DI= No active disease with residual diabetes insipidus (DI) N/A = not applicable; ND = not done, DR= Dose reduction
TABLE 2 Trametinib and/or Dabrafenib as First-line Therapy. Disease classifications, mutations, and response to trametinib and or dabrafenib in patients who did not receive prior therapy for their histiocytic disorder.
<table>
<thead>
<tr>
<th>Pt #</th>
<th>Age (yrs)/Sex</th>
<th>Dx</th>
<th>Disease Classification/Site</th>
<th>Genetic Mutation</th>
<th>Age ITx Start (yrs)</th>
<th>Inhib -itor</th>
<th>ITx (yrs)</th>
<th>Response at follow-up</th>
<th>Trial off ITx (Y/N)</th>
<th>Time to Recurr. off therapy</th>
<th>Adverse Effects</th>
<th>PB BRAF-V600E RT-PCR</th>
<th>ddPCR Histiotrak</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>0.6/F</td>
<td>LCH</td>
<td>MS, RO+ skin, spleen, liver, LN</td>
<td>BRAF V600E</td>
<td>1.1</td>
<td>DAB, TRA (PP)</td>
<td>5.0</td>
<td>NAD</td>
<td>Y</td>
<td>18 mo***</td>
<td>none</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>1.7/F</td>
<td>LCH</td>
<td>MS, RO+ bone, spleen, lungs, LN, BM</td>
<td>BRAF V600E</td>
<td>1.7</td>
<td>TRA</td>
<td>1.7</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>0.17/M</td>
<td>LCH</td>
<td>MS, RO+ skin, BM, liver, GI tract</td>
<td>BRAF V600E</td>
<td>0.2</td>
<td>TRA</td>
<td>1.8</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>0.3/M</td>
<td>LCH</td>
<td>MS, RO-skin, bone</td>
<td>BRAF V600E</td>
<td>0.7</td>
<td>DAB</td>
<td>4.4</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>0.7/M</td>
<td>LCH</td>
<td>MS, RO-skin, bone</td>
<td>BRAF V600E</td>
<td>0.7</td>
<td>TRA</td>
<td>2.9</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>36.0/F</td>
<td>LCH</td>
<td>MS, RO,- CNS, soft tissue, CNS</td>
<td>BRAF N486_P490 deletion</td>
<td>45.0</td>
<td>TRA</td>
<td>6.5</td>
<td>NAD, resolution of DI, improved cognition</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>23</td>
<td>3.8/F</td>
<td>LCH</td>
<td>SS, unifocal bone</td>
<td>BRAF V600E</td>
<td>3.9</td>
<td>TRA, DAB</td>
<td>2.1</td>
<td>NAD</td>
<td>Y</td>
<td>N</td>
<td>hair loss with TRA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>8.7/F</td>
<td>LCH</td>
<td>SS, unifocal bone</td>
<td>BRAF V600E</td>
<td>8.8</td>
<td>DAB</td>
<td>2.0</td>
<td>NAD</td>
<td>Y</td>
<td>N</td>
<td>nausea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>3.0/F</td>
<td>LCH</td>
<td>SS- unifocal bone</td>
<td>BRAF L485_P490&gt;F</td>
<td>3.1</td>
<td>TRA</td>
<td>1.1</td>
<td>NAD</td>
<td>Y</td>
<td>N</td>
<td>none</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>26</td>
<td>9.3/F</td>
<td>LCH</td>
<td>SS, unifocal bone</td>
<td>BRAF V600E</td>
<td>9.4</td>
<td>TRA</td>
<td>0.3</td>
<td>NAD</td>
<td>Y</td>
<td>N</td>
<td>rash, hair loss, grey hair (stopped)</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td>13.6/M</td>
<td>LCH</td>
<td>SS, multifocal bone</td>
<td>BRAF V600E</td>
<td>13.7</td>
<td>DAB</td>
<td>4.0</td>
<td>NAD</td>
<td>Y</td>
<td>N</td>
<td>none</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>12.3/M</td>
<td>LCH</td>
<td>SS- multifocal bone</td>
<td>BRAF V600E</td>
<td>12.4</td>
<td>TRA</td>
<td>1.8</td>
<td>NAD</td>
<td>Y</td>
<td>4 mo</td>
<td>rash, abdominal pain (DR)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29</td>
<td>12.1/M</td>
<td>LCH</td>
<td>SS, multifocal bone</td>
<td>unknown</td>
<td>12.7</td>
<td>TRA</td>
<td>0.3</td>
<td>NAD</td>
<td>Y</td>
<td>N</td>
<td>none</td>
<td>N/A</td>
<td>-</td>
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<tr>
<td>30</td>
<td>15.8/M</td>
<td>LCH</td>
<td>Pituitary Stalk</td>
<td>n/a</td>
<td>15.9</td>
<td>TRA</td>
<td>3.2</td>
<td>Decrease size + DI</td>
<td>N</td>
<td>n/a</td>
<td>rash (DR)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>10.5/F</td>
<td>LCH</td>
<td>Isolated-CNS PS, cerebellar changes</td>
<td>n/a</td>
<td>10.6</td>
<td>TRA</td>
<td>3.4</td>
<td>stable size of PS lesion +DI</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>32</td>
<td>4.3/M</td>
<td>LCH</td>
<td>Isolated-CNS PS, cerebellar changes</td>
<td>**BRAF V600E</td>
<td>4.3</td>
<td>TRA</td>
<td>3.4</td>
<td>decrease in size of PS lesion +DI</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>33</td>
<td>0.3/M</td>
<td>JXG</td>
<td>JXG skin, liver, spleen, BM</td>
<td>TFG-RET fusion</td>
<td>0.3</td>
<td>TRA</td>
<td>1.3</td>
<td>NAD</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>34</td>
<td>6.6/M</td>
<td>JXG</td>
<td>skin, CNS</td>
<td>GAB2-BRAFusion</td>
<td>6.7</td>
<td>TRA</td>
<td>3.9</td>
<td>NAD + DI</td>
<td>N</td>
<td>n/a</td>
<td>none</td>
<td>N/A</td>
<td>N/A</td>
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</tbody>
</table>

** identified by ddPCR as no tissue available due to isolated CNS involvement *** Restarted 18 months after trialing off therapy when noted to be ddPCR+ in peripheral blood, Dx= diagnosis, LCH = Langerhans Cell Histiocytosis, JXG= juvenile xanthogranuloma, BM= bone marrow, LN= lymph node, PS= Pituitary Stalk, SS= single system disease, MS= multisystem, RO+/- = Risk organ positive/negative, ITx= Inhibitor treatment, DAB= Dabrafenib, TRA=Trametinib. NAD = no active disease; NAD + DI= No active disease with residual diabetes insipidus (DI) N/A = not applicable; ND = not done, DR= Dose reduction
**TABLE 3** Summary of patient demographics, inhibitor treatment, disease classification, mutation status

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22 (65)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (35)</td>
</tr>
<tr>
<td>Total</td>
<td>34 (100)</td>
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**Inhibitor Treatment**

<table>
<thead>
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<th>Inhibitor Treatment</th>
<th>n (%)</th>
</tr>
</thead>
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<tr>
<td>Dabrafenib</td>
<td>11 (32)</td>
</tr>
<tr>
<td>Trametinib</td>
<td>20 (59)</td>
</tr>
<tr>
<td>Both</td>
<td>3 (9)</td>
</tr>
</tbody>
</table>

**Disease Classification**

<table>
<thead>
<tr>
<th>Disease Classification</th>
<th>n (%)</th>
</tr>
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<tbody>
<tr>
<td>LCH</td>
<td>30 (88)</td>
</tr>
<tr>
<td>SS</td>
<td>7 (20.5)</td>
</tr>
<tr>
<td>MS, RO -</td>
<td>7 (20.5)</td>
</tr>
<tr>
<td>MS, RO +</td>
<td>12 (35)</td>
</tr>
<tr>
<td>Isolated CNS or PS</td>
<td>4 (12)</td>
</tr>
<tr>
<td>RDD</td>
<td>2 (6)</td>
</tr>
<tr>
<td>JXG</td>
<td>2 (6)</td>
</tr>
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</table>

**Mutation**

<table>
<thead>
<tr>
<th>LCH</th>
<th>BRAF V600E</th>
<th>23 (68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRAF L485</td>
<td>1 (3)</td>
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<tr>
<td></td>
<td>BRAF N486</td>
<td>1 (3)</td>
</tr>
<tr>
<td></td>
<td>MAP2K1 Q56P</td>
<td>1 (3)</td>
</tr>
<tr>
<td></td>
<td>Unable to biopsy *</td>
<td>3 (8)</td>
</tr>
<tr>
<td></td>
<td>Unknown **</td>
<td>1 (3)</td>
</tr>
<tr>
<td>JXG</td>
<td>GAB2-BRAF</td>
<td>1 (3)</td>
</tr>
<tr>
<td>RDD</td>
<td>TFG-RET fusion</td>
<td>1 (3)</td>
</tr>
<tr>
<td></td>
<td>Unknown **</td>
<td>1 (3)</td>
</tr>
<tr>
<td></td>
<td>Unknown ***</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

LCH = Langerhans Cell Histiocytosis, JXG = Juvenile Xanthogranuloma, RDD = Rosai Dorfman Disease, SS = single system, MS = multisystem, RO +/- = risk organ positive/negative, CNS = central nervous system, PS = pituitary stalk
* Unable to biopsy due to location (e.g. CNS, PS)
** NGS not approved by insurance for patients 15 and patient 29 (VE1 IHC negative)
*** no mutations identifiable by NGS (patient 16)
FIGURE LEGENDS

Figure 1. **Response to inhibitor therapy in patients with relapsed/refractory disease.** A. Graphical summary of all responses achieved in patients with refractory/recurrent disease. Response categories are represented by the segments of the doughnut plot. (NAD = no active disease, ADS = active disease stable, ADB = active disease better, DI = diabetes insipidus) B. Post-contrast brain MRI of a 7-year-old with a history of recurrent LCH when he developed sudden onset diabetes insipidus (upper panel). The arrow points to thickened infundibulum. He was treated with trametinib and repeat MRI 6 weeks later (lower panel) showed normal thickness of the enhancing infundibulum (red arrow).

Figure 2. **Isolated pituitary-stalk disease diagnosed by HistioTrak.** A. Post contrast brain MRI of a 4-year-old boy with sudden onset central diabetes insipidus, showing infundibular infiltration (arrow). Extensive evaluation was negative for histiocytosis or germ cell tumor. A biopsy of the pituitary-stalk was deemed unsafe. B. Follow-up MRI a few months later showed worsening of pituitary-stalk thickening (arrow). Treatment was initiated with trametinib. C. Repeat MRI 3 months after initiation of trametinib showed resolution of pituitary-stalk infiltration (arrow). D. HistioTrak on peripheral blood mononuclear DNA revealed presence of BRAFV600E (positive droplets circled).

Figure 3. **Response to inhibitor as first-line therapy.** A. Graphical summary of all responses achieved in patients treated with inhibitor as first-line therapy. Response categories are represented by the segments of the doughnut plot. (NAD = no active disease, DI = diabetes insipidus) B. Positron Emission Tomography (PET) of a 20-month-old female at diagnosis (left panel) with mixed histiocytosis with features of LCH and JXG, demonstrating extensive disease including FDG avid lesions of the calvarium, chest wall, vertebrae, pelvis, and lower extremities, as well as splenomegaly and profound lymphadenopathy involving the neck, chest, abdomen.
and pelvis. Biopsy was positive for BRAF-V600E. Repeat imaging (right panel) following 8 weeks of therapy with trametinib, with marked interval decrease in size and FDG avidity of bony lesions, decrease in splenomegaly and marked improvement in lymphadenopathy throughout. C. Positron Emission Tomography (PET, upper and middle row) and computed tomography (CT, bottom row) of a 12-year-old male at diagnosis (left frames in each row) of multifocal bone LCH demonstrating a large bony lesion involving the L1 vertebral body, with SUVmax value of 15. Repeat imaging (right frames) performed following 6 weeks of therapy with trametinib showing minimal FDG uptake and marked improvement of the vertebral lesion.
A

Outcomes in Relapsed/Refractory Disease (n=16)

- NAD: 6 (38%)
- NAD with residual DI/cirrhosis: 1 (6%)
- ADS (RDD): 1 (6%)
- CNS Improved: 1 (6%)
- ADB
- Progression (RDD): 6 (38%)

B

[Images of brain scans with annotations]
Figure 3

A. Outcomes in patients treated with inhibitors as first-line therapy (n=18)

- NAD: 72%
- CNS Improved: 17%
- NAD + DI: 5%
- CNS Stable: 6%
- Others: 3% (1 case each)

B. Imaging studies showing (top left) before and (top right) after treatment.

C. PET scans highlighting changes post-treatment (red arrows indicate new lesions).
Supplemental Figure: Graph depicting changes in PET-CT SUVmax values (vertical axis) in patients at the start of treatment and at their first follow-up scans (time interval in weeks on horizontal axis). Each line represents change in SUVmax for a single patient.