Efficacy of MEK inhibition in patients with histiocytic neoplasms

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Histiocytic neoplasms are a heterogeneous group of clonal haematopoietic disorders that are marked by diverse mutations in the mitogen-activated protein kinase (MAPK) pathway1–2. For the 50% of patients with histiocytosis who have BRAFV600 mutations3–5, RAF inhibition is highly efficacious and has markedly altered the natural history of the disease6,7. However, no standard therapy exists for the remaining 50% of patients who lack BRAFV600 mutations. Although ERK dependence has been hypothesized to be a consistent feature across histiocytic neoplasms, this remains clinically unproven and many of the kinase mutations that are found in patients who lack BRAFV600 mutations have not previously been biologically characterized. Here we show ERK dependency in histiocytes through a proof-of-concept clinical trial of cobimetinib, an oral inhibitor of MEK1 and MEK2, in patients with histiocytoses. Patients were enrolled regardless of their tumour genotype. In parallel, MAPK alterations that were identified in treated patients were characterized for their ability to activate ERK. In the 18 patients that we treated, the overall response rate was 89% (90% confidence interval of 73–100). Responses were durable, with no acquired resistance to date. At one year, 100% of responses were ongoing and 94% of patients remained progression-free. Cobimetinib treatment was efficacious regardless of genotype, and responses were observed in patients with ARAF, BRAF, RARF, NRAS, KRAS, MEK1 (also known as MAP2K1) and MEK2 (also known as MAP2K2) mutations. Consistent with the observed responses, the characterization of the mutations that we identified in these patients confirmed that the MAPK pathway mutations were activating. Collectively, these data demonstrate that histiocytic neoplasms are characterized by a notable dependence on MAPK signalling—and that they are consequently responsive to MEK inhibition. These results extend the benefits of molecularly targeted therapy to the entire spectrum of patients with histiocytosis.

On the basis of the success of targeting BRAFV600 in two histiocytic neoplasms (Erdheim–Chester disease and Langerhans cell histiocytosis), we and others attempted to identify potential genomic drivers of disease in patients that do not possess this mutation. These genomic studies of histiocytoses have identified a considerable diversity of both previously characterized and newly identified alterations that involve multiple components of the MAPK pathway8–13. Many of the recurrent mutations in histiocytic neoplasms occur in genes for MAPK-pathway proteins—such as ARAF, RAF1, MEK1 and MEK2—that are rarely, if ever, mutated in other malignancies. Consequently, many of these mutations have not previously been biologically characterized. Consistent with the underlying hypothesis that these non-BRAFV600 mutations are likely to drive histiocytic neoplasms, drugs that inhibit both the MEK1 and MEK2 kinases, which are immediately downstream of BRAF, have previously been shown in case reports to evoke responses in patients with non-BRAFV600 mutations14–16. Despite these findings, the true extent and durability of these responses, as well as the safety of MEK1 and MEK2 inhibition across a wide range of histologic and molecular subsets of histiocytosis, remains unknown.

To formally evaluate the therapeutic potential of MEK1 and MEK2 inhibition in histiocytic neoplasms, we conducted a phase II study of cobimetinib in adult patients with histiocytoses of any mutational status (ClinicalTrials.gov identifier NCT01959326). Consistent with common clinical practice for response assessment in these disorders17–19, the primary end point was response rate (complete response + partial response) as determined by fluorodeoxyglucose positron emission tomography (PET). To provide additional corroboration of treatment efficacy, a key secondary end point included the response by computed tomography and/or magnetic resonance imaging (MRI), according to the ‘Response Evaluation Criteria in Solid Tumours’ (RECIST) version 1.120. Simultaneously, we used this clinical trial as a platform to perform real-time, patient-driven discovery of MAPK-pathway alterations through biological characterization of the alterations that were identified by comprehensive profiling of patient samples.

We enrolled and treated a total of 18 patients (Extended Data Table 1), who had a variety of histiocytic neoplasms that included Erdheim–Chester disease (n = 12 patients), Langerhans cell histiocytosis (n = 2), Rosai–Dorfman disease (n = 2) and mixed histiocytosis (n = 2). Eighty-nine per cent (16 out of 18) of patients had received at least 1 previous therapy and 56% (10 out of 18) had received 2 or more previous therapies. Five patients (28%) had an Eastern Cooperative Oncology Group performance status of ≥2.

At the time of the pre-planned primary efficacy analysis, which was performed using a data cut-off of 25 April 2018, the overall response rate was 89% (90% one-sided confidence interval of 73–100) as determined by the PET response criteria (Fig. 1a). Overall, 72% of the patients (13 out of 18) had a complete response, 17% (3 out of 18) had a partial response, 6% (1 out of 18) experienced stable disease, none had progressive disease and 6% (1 out of 18) could not be evaluated owing to early withdrawal for clinical deterioration (Extended Data Table 2). All patients, including the patient who could not be evaluated, were accounted for in our analysis, according to the protocol. The patient who could not be evaluated was counted as not responding to treatment. Responses occurred at all sites of disease, including the central nervous system—a site that is associated with higher morbidity and mortality21. According to RECIST version 1.1 (judged on the basis of computed tomography and/or MRI), the overall response rate was 64% (90% one-sided confidence interval of 44–100) in the 14 out of 18 patients who...
were evaluable by these criteria (Extended Data Fig. 1). The median time to best response, according to PET response criteria, was 3.2 months (range of 1.6–15.9 months, Fig. 1b). At the time of data analysis, 50% (9 out of 18) of patients remained on protocol therapy and an additional 17% (3 out of 18)—all in the complete-response category—had elected to withdraw and continue to receive MEK inhibitory therapy off-label.

The median duration of response and the median progression-free survival had not yet been reached after a median follow-up of 11.9 months (range of 4.6–26.4 months) (Fig. 1c, Extended Data Fig. 2). No responding patient has progressed to date. Among those who discontinued treatment for any reason, the median time on treatment was 11.9 months (range of 1.6–23.7 months). At one year, 100% of responses were ongoing and 94% of patients remained progressions-free.

The adverse events observed are shown in Extended Data Tables 3, 4. Overall, the safety profile was consistent with previous studies of cobimetinib.22,23 Of the 18 treated patients, 10 (56%) had their cobimetinib dose reduced at least once. Adverse events leading to dose reduction included ejection-fraction decrease in five patients, rash in two patients, diarrhea in two patients, and fatigue and thrombocytopenia (each in one patient). In all cases, patients who had their doses reduced had their best response maintained at the lower dose. One patient permanently discontinued cobimetinib owing to an adverse event (central retinal vein occlusion). Another patient with extensive baseline histiocytic lung involvement died of pneumonia—deemed to be unrelated to the treatment with cobimetinib—before the first assessment of response.

To identify the MAPK-pathway alterations that were present in each patient, we performed a variety of sequencing assays according to tissue availability (Extended Data Table 5). In some cases, MAPK mutations were defined on the basis of sequencing that was performed at an external commercial laboratory. At least one mutation that involved the MAPK pathway was identified in 83% (15 out of 18) of patients. Patients had a variety of MAPK-pathway mutations involving BRAF ($n = 5$ with BRAF$^{V600E}$, $n = 1$ with non-V600 BRAF), MEK1 ($n = 4$), KRAS ($n = 3$), ARAF ($n = 2$), RAF1 ($n = 1$), MEK2 ($n = 1$) and NRAS ($n = 1$). Two patients had mutations that involved more than one gene in the MAPK pathway. Three patients had an unknown mutational status, including one case in which sequencing of two separate biopsies failed owing to low tumour cellularity. Responses to cobimetinib were observed across genotypes, as well as in two of the three patients with unknown mutations (Fig. 1a). Although several alterations detected here (including several in BRAF, ARAF and MEK1) are known to be recurrent in histiocytoses, RAF1 mutations have not previously been described in histiocytoses, and the specific RAF1, MEK2 and BRAF mutations that we identify here have not, to our knowledge, been functionally characterized in any setting (Extended Data Fig. 3). We therefore evaluated the transforming potential, ability to activate ERK and responsiveness to cobimetinib in vitro for each of these three mutations. In each case, introducing the MEK2$^{R149Q}$, BRAF$^{V600E, T491delinsK}$ (which leads to a replacement of Asn 486 to Thr 491 with a single lysine residue) or RAF1$^{K666N}$ mutations robustly led to cytokine independence in Ba/F3 cells (a mouse IL-3-dependent, pro-B cell line) and activated ERK signalling, whereas wild-type MEK2, RAF1 and BRAF did not (Fig. 2a–f). Moreover, the expression of each mutation sensitized Ba/F3 cells to cobimetinib, whereas parental Ba/F3 cells remain relatively insensitive to cobimetinib (Fig. 2g). These in vitro responses were consistent with the clinical responses that were seen in the trial in patients who bear these same alleles (Fig. 1a, b, 2a, c, e).

In this study, the selective inhibitor of MEK1 and MEK2 cobimetinib had marked and durable activity in adults with histiocytic neoplasms. Responses to cobimetinib were observed across histiocytosis subtypes and tumour genotypes, although—consistent with the expectations for a study that mandated adult patients with either refractory or multi-organ disease—the majority (67%) of patients we treated had Erdheim–Chester disease. Therefore, some caution is warranted when applying these findings to all patients with histiocytosis. The efficacy of cobimetinib across MAPK genotypes here is notable given that the single-agent efficacy of MEK1 and MEK2 inhibitors in other solid tumours with alterations to the MAPK pathway has generally been disappointing.24 Indeed, previous work has shown that RAS mutations vary in their dependence on MEK1 and MEK2.25,26 Similarly, preclinical studies have previously suggested that several MEK1 and MEK2 mutations, including some that were observed in the patients we treated, would confer resistance to MEK1 and MEK2 inhibition.27,28 Importantly, responses to cobimetinib were not only nearly universal but also durable; in fact, no acquired resistance has been observed to date. This finding suggests that histiocytic neoplasms may lack the ability to adapt to tonic MEK1 and MEK2 inhibition, and that cobimetinib may markedly alter the natural history of these disorders. It is noteworthy that a similar efficacy and durability of response has previously been observed with vemurafenib in the subset of histiocytic neoplasms that contain BRAF$^{V600E}$ mutations.

Overall, our data demonstrate that treatment with cobimetinib results in consistent and durable responses across clinical and genetic subtypes of histiocytic neoplasms, which represents an area of
previously unmet medical need. These findings further suggest that histiocytic neoplasms are collectively characterized by dependence on MAPK-pathway signalling and, consequently, are responsive to the inhibition of MEK1 and MEK2.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1012-y.

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Fig. 2 | Characterization of activating mutations in MEK2, RAF1 and BRAF and their dependence on ERK signalling in histiocytoses.
a. Coronal PET, and fused PET and computed tomography, imaging of femurs that show femoral lesions that are characteristic of Erdheim–Chester disease (ECD). These images are of a patient with Erdheim–Chester disease with the MEK2(Y134H) mutation, and were taken before and during treatment with cobimetinib. b. Western blot (left) and number of viable cells (right) after IL-3 withdrawal of Ba/F3 cells that stably express an empty vector, wild-type BRAF or mutant BRAF(N486_T491delinsK). The average of n = 3 biological replicates ± s.d. is plotted. P values were calculated using two-way ANOVA. c. Axial fused PET and computed tomography imaging showing scalp lesions (arrow) before and during treatment with cobimetinib in a patient with Langerhans cell histiocytosis (LCH) with the BRAF(N486_T491delinsK) mutation. d. Western blot (left) and number of viable cells (right) following IL-3 withdrawal of Ba/F3 cells that stably express an empty vector, wild-type MEK2 or mutant MEK2(Y134H). The average of n = 3 biological replicates ± s.d. is plotted. P values were calculated using two-way ANOVA. e. Axial fused PET and computed tomography imaging showing sacral lesions (arrow) before and during treatment with cobimetinib, in a patient with mixed histiocytosis with the RAF1(K106N) mutation. f. Western blot (left) and number of viable cells (right) following IL-3 withdrawal of Ba/F3 cells that stably express an empty vector, wild-type RAF1 or mutant RAF1(K106N). The average of n = 3 biological replicates ± s.d. is plotted. P values were calculated using two-way ANOVA. g. Half-maximal inhibitory concentration (IC50) of cells from b, d and e to 72 h of treatment with cobimetinib. Each experiment was performed with n = 3 biological replicates, and the average ± s.d. is plotted. P values were calculated using an ordinary one-way ANOVA. ***P < 0.001, ****P < 0.0001.

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Author contributions E.L.D., O.A.-W. and D.M.H. designed the study. M.K., L.B. and H.C. processed all patient material for isolation of DNA and RNA for genomic analyses. B.H.D. cloned all cDNA and performed in vitro studies of their effects on cell growth and signalling under the supervision of N.R. and O.A.-W. B.H.D. also analysed genomic data under the supervision of O.A.-W. J.B. collected data and samples for processing by M.K., L.B. and H.C. E.L.D., J.H.F., R.R., M.L. and L.A.B. enrolled and treated the patients. E.D. and A.I. performed statistical analyses. G.A.U. and R.J.Y. evaluated all radiographic studies. E.L.D., B.H.D., N.O., A.D., O.A.-W. and D.M.H. analysed the clinical and laboratory data. E.L.D., B.H.D., O.A.-W. and D.M.H. prepared the manuscript with help from all co-authors. The study was designed jointly by E.L.D., B.H.D., O.A.-W. and D.M.H. The investigators collected and analysed the data. All authors had access to the data, and E.L.D., B.H.D., O.A.-W. and D.M.H. wrote the first draft of the manuscript. All the authors were involved in the data analysis and manuscript preparation. All the authors vouch for the completeness and accuracy of the data and analyses, and for the adherence to the study protocols. All the authors made the decision to submit the manuscript for publication.

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METHODS

The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Patients. Eligible patients had a histiocytic neoplasm, were aged 16 or older, had an Eastern Cooperative Oncology Group performance-status score of 0 to 3 (on a scale from 0 to 5, with higher scores indicating greater disability) and had adequate major organ function. Patients were required to have one of the following: (1) multi-system disease; (2) disease that was recurrent or refractory to standard therapies; or (3) single-organ system disease that was deemed unlikely to benefit from conventional therapies (for example, central nervous system or cardiac involvement). Patients with tumours that were wild type for BRAFV600 were eligible. Patients with BRAFV600 mutations were eligible only in the setting of (1) intolerance or resistance to previous BRAF inhibitor therapy or (2) the inability to access BRAF inhibitor therapy. Complete eligibility criteria are available in the study protocol (see Supplementary Information).

The protocol was approved by the institutional review board at Memorial Sloan Kettering Cancer Center, complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent.

Study design and treatment. The primary end point was the overall response rate (complete responses + partial responses) by PET response criteria (see Supplementary Information). These criteria were previously used for analysis of vemurafenib efficacy in BRAFV600-mutant histiocytic disorders, a dataset that formed the basis of regulatory authorization for this indication in the United States.1 PET responses were investigator-assessed by a radiologist with dual board certification in diagnostic radiology and nuclear medicine (G.A.U.). Secondary end points included the duration of response and progression-free survival based on PET criteria, safety and overall response rate according to RECIST version 1.1. Patients received cobimetinib at a starting dose of 60 mg daily for 21 days of each 28-day cycle.

Study assessments. Tumour assessments were performed by means of PET and computed tomography, MRI or clinical measurement with calipers (in the case of multi-system lesions) at baseline, every 8 weeks for 6 months and every 16 weeks thereafter until disease progression. Adverse events were assessed from the date that informed consent was obtained until at least 28 days after the final dose of cobimetinib was administered. Adverse events were classified and graded according to the Common Terminology Criteria for Adverse Events, version 4.0 (https://evs.nci.nih.gov/ftp1/CTCAE/About.html).

Study oversight. Data, safety and quality monitoring were performed by the Memorial Sloan Kettering Cancer Center Data and Safety Monitoring Committee. All data elements were de-identified by removing identifying characteristics and replacing them with code numbers 1 through 18.

Statistical analysis. All the analyses were conducted in accordance with the pre-specified statistical plan as outlined in the protocol, unless otherwise indicated (see Supplementary Information). The primary analysis presented here was performed at the time the first 18 consecutively enrolled patients to receive cobimetinib were evaluable for response, or discontinued protocol therapy (Extended Data Fig. 4). Patients who discontinued therapy without a post-baseline tumour assessment were considered to be non-responsive to treatment. Assuming a binary end point of PET response (complete response or partial response, versus neither), we estimated that a sample of 18 patients would provide the study with 90% power to test the hypothesis that the response rate is promising (defined as 35% or higher) against a non-promising rate of 10% or lower. Using an exact, one-sample test for binomial proportion—with type I error = 0.10 and type II error = 0.10—the above rates provided a sample size of 18 patients; if at least 4 out of 18 responses were observed then this would be considered a positive study (that is, conclude against a non-promising rate of 10% or lower. Using an exact, one-sample test to test the hypothesis that the response rate is promising (defined as 35% or higher) was used as controls. Mutual exclusions were closed into the MSCV-IRE5-GFP backbone, and checked by digestion and sequencing.

Western blotting. Antibody phospho-p44/42 MAPK (ERK1 and ERK2) (Thr202/Tyr204) (no. 9101), anti-p44/42 MAPK (ERK1 and ERK2) (137F5) (no. 4695), anti-MEK1 and MEK2 (47E6) (no. 9126), anti-BRAF (D976S) (no. 14814), anti-CRAF (D4B3) (no. 53745), as well as the secondary antibodies anti-rabbit IgG–HRP (no. 7076) and anti-mouse IgG–HRP (no. 7074) were purchased from Cell Signaling Technology. Anti-β-actin (A5441) was purchased from Sigma-Aldrich. Cell lysates were prepared in RIPA buffer supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Equal amounts of protein, as measured by the Bradford protein assay, were loaded in 4–12% Bis-Tris NuPage gradient gels (Life Technologies), and transferred electrophoretically on a polyvinylidene difluoride 0.45-m membrane. Membranes were blocked for 1 h at room temperature in 5% bovine serum albumin (BSA) in TBST before being incubated overnight at 4°C with the primary antibodies. All primary antibodies were diluted 1:1,000 in 5% BSA in TBST, except anti-β-actin, which was diluted 1:5,000 in 5% BSA in TBST. After three washes of 10 min in TBST, secondary antibodies were diluted 1:2,000 in 5% BSA in TBST and incubated for 1 h at room temperature. After another three washes in TBST, detection of the signal was achieved by incubating the membrane on an ECL solution from Millipore and exposure on autoradiography films from Du Pont

Drug studies. Cobimetinib was purchased from Selleckchem. Drug studies were conducted in vitro using fluorescence-activated cell-sorted, DAPI eGFP Ba/F3 cells that stably expressed the MIGII-empty vector, MIGII-MEK2(Y134H), MIGII-RAF1(K106N) and MIGII-BRAF(N486_T491delinsK) mutations and expressed them in Ba/F3 cells. Murine stem-cell virus-based expression vectors with GFP and the full-length MAP2K2, RAF1 and BRAF wild type were used as controls. Mutational constructs were cloned into the MSCV-IRE5-GFP backbone, and checked by digestion and sequencing.

Western blotting. Antibody phospho-p44/42 MAPK (ERK1 and ERK2) (Thr202/Tyr204) (no. 9101), anti-p44/42 MAPK (ERK1 and ERK2) (137F5) (no. 4695), anti-MEK1 and MEK2 (47E6) (no. 9126), anti-BRAF (D976S) (no. 14814), anti-CRAF (D4B3) (no. 53745), as well as the secondary antibodies anti-rabbit IgG–HRP (no. 7076) and anti-mouse IgG–HRP (no. 7074) were purchased from Cell Signaling Technology. Anti-β-actin (A5441) was purchased from Sigma-Aldrich. Cell lysates were prepared in RIPA buffer supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Equal amounts of protein, as measured by the Bradford protein assay, were loaded in 4–12% Bis-Tris NuPage gradient gels (Life Technologies), and transferred electrophoretically on a polyvinylidene difluoride 0.45-m membrane. Membranes were blocked for 1 h at room temperature in 5% bovine serum albumin (BSA) in TBST before being incubated overnight at 4°C with the primary antibodies. All primary antibodies were diluted 1:1,000 in 5% BSA in TBST, except anti-β-actin, which was diluted 1:5,000 in 5% BSA in TBST. After three washes of 10 min in TBST, secondary antibodies were diluted 1:2,000 in 5% BSA in TBST and incubated for 1 h at room temperature. After another three washes in TBST, detection of the signal was achieved by incubating the membrane on an ECL solution from Millipore and exposure on autoradiography films from Du Pont

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All datasets generated and/or analysed during the current study, including patient-level clinical data as well as all sequencing data have been deposited and are publicly available in the BioPortal for Cancer Genomics under the accession code ‘HistiocytosisCobimetinib (MSK, Nature 2019)’ (https://www.bioportal.org/study?id=histiocytosis_cobi_msks_2019).

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Extended Data Fig. 1 | Waterfall plot of maximum change in tumour size by RECIST after treatment with cobimetinib in patients with histiocytosis. The upper and lower dotted lines represent cut-offs for progressive disease and partial response, respectively. Colours of bars indicate the genomic alteration present. Notations above bars indicate the specific mutation. One patient (marked by an asterisk) had undergone previous BRAF inhibitor therapy that was discontinued owing to intolerance. One patient (marked by a dagger) died owing to underlying disease. n = 14 patients in total.
Extended Data Fig. 2 | PET-defined duration of response. This graph depicts the duration of response according to PET criteria in the 16 patients that responded to treatment, beginning with date of initial response.
Extended Data Fig. 3 | Histopathology of histiocytoses with activating mutations in MEK2, RAF1 and BRAF treated in this study. a, Protein diagram (top) and histological images (bottom) from a patient with Erdheim–Chester disease with a MAP2K2\textsuperscript{Y134H} mutation. b, Protein diagram (top) and histological images (bottom) from a patient with non-Langerhans cell histiocytosis with a RAF1\textsuperscript{K106N} mutation. c, Protein diagram (top) and histological images (bottom) from a patient with Langerhans cell histiocytosis with a BRAF\textsuperscript{N486_T491delinsK} mutation.
Extended Data Fig. 4 | Study CONSORT diagram. This diagram shows the flow of patients through all phases of study participation from enrolment and follow-up through to data analysis.
Extended Data Table 1 | Demographic and clinical characteristics for the 18 patients

| Characteristic                                                                 | Value, N (%)          |
|-------------------------------------------------------------------------------|-----------------------|
| **Age, Median (range) year**                                                  | 51.9 (18.3-79.5)      |
| **Sex**                                                                       |                       |
| Male                                                                          | 13 (72)               |
| Female                                                                        | 5 (28)                |
| **Histiocytosis type**                                                        |                       |
| Erdheim-Chester disease                                                       | 12 (67)               |
| Rosai-Dorfman disease                                                         | 2 (11)                |
| Langerhans cell histiocytosis                                                 | 2 (11)                |
| Mixed histiocytosis                                                           | 2 (11)                |
| **Mitogen-activated protein kinase mutation**                                 |                       |
| BRAF V600E                                                                    | 4 (22)                |
| N486_Q491delinsK                                                              | 1 (6)                 |
| MEK1 Q56P                                                                     | 1 (6)                 |
| P105_L107del                                                                  | 1 (6)                 |
| P124L                                                                        | 1 (6)                 |
| P124Q                                                                        | 1 (6)                 |
| ARAF S225V                                                                    | 1 (6)                 |
| RAF1 K106N                                                                   | 1 (6)                 |
| MEK2 Y134H                                                                   | 1 (6)                 |
| KRAS R149G                                                                   | 1 (6)                 |
| >1 mutation                                                                 |                       |
| BRAF V600E; NRAS G12D; KRAS G13C                                              | 1 (6)                 |
| KRAS G12R; ARAF P216A                                                         | 1 (6)                 |
| Unknown                                                                      | 3 (17)                |
| **ECOG performance-status score**                                            |                       |
| 0                                                                             | 5 (28)                |
| 1                                                                             | 8 (44)                |
| 2                                                                             | 2 (11)                |
| 3                                                                             | 3 (17)                |
| **Central nervous system involvement**                                       |                       |
| Yes                                                                           | 9 (50)                |
| No                                                                            | 9 (50)                |
| **No. of prior systemic therapies**                                           |                       |
| 0                                                                             | 2 (11)                |
| 1                                                                             | 6 (33)                |
| 2                                                                             | 8 (44)                |
| ≥3                                                                            | 2 (11)                |
| **Prior systemic therapy**                                                    |                       |
| Any prior therapy                                                             | 16 (89)               |
| Immunosuppression<sup>1</sup>                                                   | 12 (67)               |
| Cytotoxic chemotherapy<sup>2</sup>                                             | 9 (50)                |
| Interferon-alpha                                                             | 5 (28)                |
| Kinase inhibitor<sup>3</sup>                                                   | 3 (18)                |

<sup>1</sup>Corticosteroids, anakinra, sirolimus, infliximab, intravenous immunoglobulin and/or rituximab.

<sup>2</sup>Methotrexate, cytarabine, cladribine, 6-mercaptopurine, vinblastine, lenalidomide, cyclophosphamide and/or etoposide.

<sup>3</sup>Vemurafenib and/or dasatinib.
### Extended Data Table 2 | Overall response rate

| Response                          | PET Response (N=18) | RECIST Response (N=14) |
|-----------------------------------|---------------------|------------------------|
| Overall response rate, % (90% one-sided Confidence Interval) | 89 (73-100)         | 64 (44-100)            |
| Best Response, N (%)              |                     |                        |
| Complete                          | 13 (72)             | 2 (14)                 |
| Partial                           | 3 (17)              | 7 (50)                 |
| Stable                            | 1 (6)               | 4 (29)                 |
| Progressive                       | 0 (0)               | 0 (0)                  |
| Not Evaluable                     | 1 (6)               | 1 (7)                  |

This table demonstrates the overall response rate, and best response (complete, partial, stable, progressive and non-evaluable), using both PET response and RECIST version 1.1 criteria.
### Extended Data Table 3 | Safety of cobimetinib in phase 2 study in patients with histiocytosis

| Event                                | Grade 1/2(%) | Grade 3/4 (%) | All (%) |
|--------------------------------------|--------------|---------------|---------|
| Rash*                                | 15 (83)      | 0 (0)         | 15 (83) |
| Diarrhea                             | 11 (61)      | 2 (11)        | 13 (72) |
| Creatine phosphokinase elevation     | 10 (56)      | 1 (6)         | 11 (61) |
| Hypomagnesemia                       | 10 (56)      | 0 (0)         | 10 (56) |
| Alkaline phosphatase increased       | 9 (50)       | 0 (0)         | 9 (50)  |
| AST/ALT elevation                    | 8 (44)       | 0 (0)         | 8 (44)  |
| Nausea                               | 7 (39)       | 0 (0)         | 7 (39)  |
| Anemia                               | 4 (22)       | 2 (11)        | 6 (33)  |
| Dry skin                             | 5 (28)       | 0 (0)         | 5 (28)  |
| Infection†                           | 5 (28)       | 0 (0)         | 5 (28)  |
| Vomiting                             | 5 (28)       | 0 (0)         | 5 (28)  |
| Abdominal disturbance                | 4 (22)       | 0 (0)         | 4 (22)  |
| Edema limbs                          | 4 (22)       | 0 (0)         | 4 (22)  |
| Fatigue                              | 4 (22)       | 0 (0)         | 4 (22)  |
| Hyponatremia                         | 2 (11)       | 2 (11)        | 4 (22)  |
| Anorexia                             | 3 (17)       | 0 (0)         | 3 (17)  |
| Hypoalbuminemia                      | 3 (17)       | 0 (0)         | 3 (17)  |
| Hypocalcemia                         | 3 (17)       | 0 (0)         | 3 (17)  |
| Pruritus                             | 3 (17)       | 0 (0)         | 3 (17)  |
| Serum amylase increased              | 2 (11)       | 1 (6)         | 3 (17)  |
| White blood cell decreased           | 3 (17)       | 0 (0)         | 3 (17)  |
| Hypokalemia                          | 0 (0)        | 2 (11)        | 2 (11)  |
| Lipase increased                     | 1 (6)        | 1 (6)         | 2 (11)  |
| Lymphocyte count decreased           | 0 (0)        | 1 (6)         | 1 (6)   |
| Retinal vein occlusion               | 0 (0)        | 1 (6)         | 1 (6)   |

The adverse events listed here are those that were attributed by investigators as related to cobimetinib, and which occurred in at least 15% of patients (regardless of grade) or at any frequency (for grade ≥ 3).

*Combines the following terms: rash acneiform and rash maculo-papular.

*Combines the following terms: bladder infection, bronchial infection, oral infection, tooth infection, upper respiratory infection, urinary tract infection and infections (other).
Extended Data Table 4 | Safety regardless of attribution

| Adverse Event                             | Grade 1/2, N (%) | Grade ≥3, N (%) | All, N (%) |
|-------------------------------------------|------------------|----------------|------------|
| Hyperglycemia                             | 15 (83)          | 1 (6)          | 16 (89)    |
| Infection                                 | 14 (78)          | 1 (6)          | 15 (83)    |
| Rash                                      | 15 (83)          | -              | 15 (83)    |
| Hypoalbuminemia                           | 14 (78)          | -              | 14 (78)    |
| Diarrhea                                  | 11 (61)          | 2 (11)         | 13 (72)    |
| Anemia                                    | 9 (50)           | 2 (11)         | 11 (61)    |
| CPK increased                             | 10 (56)          | 1 (6)          | 11 (61)    |
| Hypomagnesemia                            | 11 (61)          | -              | 11 (61)    |
| Alkaline phosphatase increased            | 10 (56)          | -              | 10 (56)    |
| Hypermagnesia                             | 10 (56)          | -              | 10 (56)    |
| AST/ALT increased                         | 9 (50)           | 1 (6)          | 10 (56)    |
| Platelet count decreased                  | 8 (44)           | 2 (11)         | 10 (56)    |
| White blood cell decreased                | 7 (39)           | 1 (6)          | 8 (44)     |
| Edema limbs                               | 7 (39)           | -              | 7 (39)     |
| Hypocalcemia                              | 5 (28)           | 2 (11)         | 7 (39)     |
| Lipase increased                          | 5 (28)           | 2 (11)         | 7 (39)     |
| Nausea                                    | 7 (39)           | -              | 7 (39)     |
| Dry skin                                  | 6 (33)           | -              | 6 (33)     |
| Fatigue                                   | 5 (28)           | -              | 5 (28)     |
| Hyperkalemia                              | 4 (22)           | 1 (6)          | 5 (28)     |
| Hypoglycemia                              | 5 (28)           | -              | 5 (28)     |
| Hyponatremia                              | 2 (11)           | 3 (17)         | 5 (28)     |
| Neutrophil count decreased                | 4 (22)           | 1 (6)          | 5 (28)     |
| Serum amylase increased                   | 4 (22)           | 1 (6)          | 5 (28)     |
| Vomiting                                  | 5 (28)           | -              | 5 (28)     |
| Blood bilirubin increased                 | 4 (22)           | -              | 4 (22)     |
| Constipation                              | 4 (22)           | -              | 4 (22)     |
| Dyspnea                                   | 3 (17)           | 1 (6)          | 4 (22)     |
| Gastrointestinal symptoms                 | 4 (22)           | -              | 4 (22)     |
| Lymphocyte count decreased                | -                | 4 (22)         | 4 (22)     |
| Pain                                      | 3 (17)           | 1 (6)          | 4 (22)     |
| Anorexia                                  | 3 (17)           | -              | 3 (17)     |
| Creatinine increased                      | 3 (17)           | -              | 3 (17)     |
| Dry mouth                                 | 3 (17)           | -              | 3 (17)     |
| Hypertriglyceridemia                      | 3 (17)           | -              | 3 (17)     |
| Hypokalemia                               | 1 (6)            | 2 (11)         | 3 (17)     |
| INR increased                             | 2 (11)           | 1 (6)          | 3 (17)     |
| Pruritus                                  | 3 (17)           | -              | 3 (17)     |

The adverse events listed here are those that occurred in at least 15% of patients, regardless of grade or investigator attribution. Infection combines the following terms: bladder infection, bronchial infection, oral infection, tooth infection, upper respiratory infection, urinary tract infection and infections (other). Rash combines: rash acneiform and rash maculo-papular.
## Extended Data Table 5 | Genomic testing methodology

| Study ID | Histiocytosis | MAPK Pathway Mutation | Assays      |
|----------|---------------|------------------------|-------------|
| 1        | ECD           | ARAF S225V             | WES, TES    |
| 2        | RDD           | No mutation identified | None        |
| 3        | ECD           | KRAS R149G             | WES, TES    |
| 4        | RDD           | No mutation identified | WES, TES, TRS |
| 5        | ECD           | BRAF V600E             | PCR         |
| 6        | ECD           | BRAF V600E             | TES, TRS, cfDNA |
| 7        | ECD           | BRAF V600E             | PCR         |
| 8        | ECD           | BRAF V600E             | PCR         |
| 9        | LCH           | BRAF N486_T491del      | WES, TES, TRS |
| 10       | Mixed Histiocytosis | RAF1 K106N         | WES, TES, TRS |
| 11       | Mixed Histiocytosis | MEK1 P124L         | WES, TES, TRS, Sequenom |
| 12       | ECD           | MEK1 P124Q             | WES, TES, TRS |
| 13       | ECD           | MEK1 Q56P              | WES, TES    |
| 14       | ECD           | MEK1 P105_I107del     | WES, TES    |
| 15       | ECD           | MEK2 Y134H             | WES, TES, cfDNA |
| 16       | ECD           | KRAS G12R / ARAF P216A | TES         |
| 17       | LCH           | BRAF V600E / NRAS G12D / KRAS G13C | TES |
| 18       | ECD           | No mutation identified | WES, TES    |

RDD, Rosai–Dorfman disease; CR, complete response; PR, partial response; SD, stable disease; TES, targeted exon sequencing; TRS, targeted RNA sequencing; cfDNA, cell-free DNA; PCR, polymerase chain reaction; WES, whole-exome sequencing; NE, not evaluable; NA, not applicable.
### Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

### Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| Item | Confirmed |
|------|-----------|
| The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement | Yes |
| An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly | Yes |
| The statistical test(s) used AND whether they are one- or two-sided | Yes |
| Only common tests should be described solely by name; describe more complex techniques in the Methods section. | Yes |
| A description of all covariates tested | Yes |
| A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons | Yes |
| A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) | Yes |
| For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted | Yes |
| Give P values as exact values whenever suitable. | Yes |
| For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings | Yes |
| For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes | Yes |
| Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated | Yes |
| Clearly defined error bars | Yes |
| State explicitly what error bars represent (e.g. SD, SE, CI) | Yes |

Our web collection on statistics for biologists may be useful.

### Software and code

Policy information about availability of computer code

| Data collection | Not applicable |
|-----------------|----------------|
| Data analysis   | R, version 3.5.0 |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All datasets generated during and/or analyzed during the current study, including patient-level clinical data and laboratory data utilized to construct all figures has been included with this submission as source data files.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | A sample of 18 patients was estimated to provide the study with 90% power to test the hypothesis that the response rate is promising (defined as 35% or higher) against a non-promising rate of 10% or lower. Using an exact, one-sample test for binomial proportion, with Type I error=10% and Type II error=10%, the above rates provided a sample size of 18 patients; if at least 4/18 responses were observed then this would be considered a positive study (i.e. conclude that the true response rate is >10%). Ruling out a lower limit of 10% for the overall response rate was considered to be clinically meaningful in light of the poor response to treatment in the context of multi-system or refractory histiocytosis. |
| Data exclusions | No data was excluded. |
| Replication | Clinical data elements were not replicated as it is not possible for a clinical trial. Western blots, growth curves, and Cell titer glows were performed in biological triplicate and were the data were reliably reproduced. |
| Randomization | No randomization was performed. |
| Blinding | No blinding was performed as this was a single-arm, open-label, non-randomized, phase II clinical trial. The investigators were not blinded to allocation or outcome assessments in the laboratory experiments. |

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | Involved in the study |
| ❑ | Unique biological materials |
| ❑ | Antibodies |
| ❑ ❑ | Eukaryotic cell lines |
| ❑ | Palaeontology |
| ❑ ❑ | Animals and other organisms |
| ❑ ❑ | Human research participants |
| n/a | Involved in the study |
| ❑ | ChiP-seq |
| ❑ | Flow cytometry |
| ❑ | MRI-based neuroimaging |

Antibodies

Antibodies used

Anti-phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204) (no. 9101), anti-p44/42 MAPK (ERK1/2) (137F5) (no. 4695), anti-MEK1/2 (47E6) (no. 9126), anti-B-Raf (D9T6S) (no. 14814), anti-c-Raf (D4B3J) (no. 53745), as well as the secondary antibodies anti-rabbit IgG-HRP (no. 7076) and anti-mouse IgG-HRP (no. 7074) were purchased from Cell Signaling Technology. Anti-β-Actin (A5441) was purchased from Sigma-Aldrich®.

Validation

Each antibody described above has been extensively utilized in prior literature and established for specificity using inhibitors against the target proteins as well as genetic validation.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Ba/F3 cells were purchased from DSMZ.

Authentication

The cell lines utilized in this study underwent authentication by STR profiling.

Mycoplasma contamination

The cell lines utilized in this study was negative for Mycoplasma contamination.
Human research participants

Policy information about studies involving human research participants

Population characteristics

Eligible patients had a histiocytic neoplasm, were age 16 or older, had an Eastern Cooperative Oncology Group performance-status score of 0 to 3 (on a scale from 0 to 5, with higher scores indicating greater disability), and had adequate major organ function. Patients were required to have one of the following: (1) multi-system disease, (2) disease that was recurrent or refractory to standard therapies, or (3) single-organ system disease deemed unlikely to benefit from conventional therapies (for example, central nervous system or cardiac infiltration). Patients with tumors that were wild type for BRAF V600 were eligible. Patients with BRAF V600-mutations were eligible only in the setting of (1) intolerance or resistance to prior BRAF inhibitor therapy, or (2) the inability to access BRAF inhibitor therapy. Complete eligibility criteria are available in the study protocol.

Recruitment

Patients with the diagnosis of histiocytosis were recruited from the outpatient ambulatory care clinics at Memorial Sloan Kettering Cancer Center regardless of race, gender, ethnicity or other characteristics.