BRIEF REPORT

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Clinical resistance associated with a novel *MAP2K1* mutation in a patient with Langerhans cell histiocytosis

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Abstract

Patients with Langerhans cell histiocytosis (LCH) harbor BRAF V600E and activating mutations of *MAP2K1/MEK1* in 50% and 25% of cases, respectively. We evaluated a patient with treatment-refractory LCH for mutations in the RAS-RAF-MEK-ERK pathway and identified a novel mutation in the *MAP2K1* gene resulting in a p.L98_K104 > Q deletion and predicted to be auto-activating. During treatment with the MEK inhibitor trametinib, the patient's disease showed significant progression. *In vitro* characterization of the MAP2K1 p.L98_K104 > Q deletion confirmed its effect on cellular activation of the ERK pathway and drug resistance.

KEYWORDS
Langerhans cell histiocytosis, MAP2K1, mutation

1 | INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare disorder characterized by the clonal expansion of Langerhans cells and is associated with a wide range of clinical symptoms.[1] Genomic analysis of patients with LCH have identified several functionally relevant mutations,[2] including BRAF V600E, which are present in over 50% of LCH patients,[3,4] *MAP2K1* (*MEK1*) mutations, which occur in about 25% of patients,[5–7] as well as mutations in *ARAF*,[8] and *MAP3K1*.[7] The identification of the BRAF V600E mutation in LCH has led to the application of targeted therapy,[9] in particular the use of vemurafenib.[10] *MAP2K1* mutations of LCH patients are activating mutations and mutually exclusive of the BRAF V600E mutation.[6] Mutations of genes in the RAS-RAF-MEK-ERK pathway in LCH suggest that targeted therapy with MEK inhibitors may benefit LCH patients. Studies of targeted therapy for *MAP2K1* using trametinib have been done for several cancer types[11,12] including histiocytosis.[13,14] Here, we describe a patient with LCH harboring a novel mutation in *MAP2K1*, who underwent treatment with trametinib resulting in disease progression, and subsequent functional analysis of the novel *MAP2K1* mutation.

2 | MATERIALS AND METHODS

2.1 | Mutation analysis

A sample of lymph node from a patient diagnosed with LCH was sent to Foundation Medicine (Cambridge, MA) for FoundationOne® sequencing analysis of a 315-gene panel.

2.2 | Cloning

The wild-type (MEK-WT) MEK1-GFP plasmid,[15] a gift from Rony Seger (Addgene plasmid #14746, Cambridge, MA), was used to create the *MEK1* mutant (MEK-MUT). Briefly, a double-stranded 44 bp oligonucleotide (Thermo Fisher; Waltham, MA) replicating the genomic deletion of the patient was substituted into the MEK-WT plasmid to generate the MEK-MUT version. Isolated clones of the MEK-MUT were sequence verified at the University of Arizona Genetics Core (Tucson, AZ).

2.3 | Cell assays

HEK293A cells (Thermo Fisher) were grown in DMEM (Corning, Manassas, VA) supplemented with 10% FBS (Atlas Biologicals, Ft. Collins,
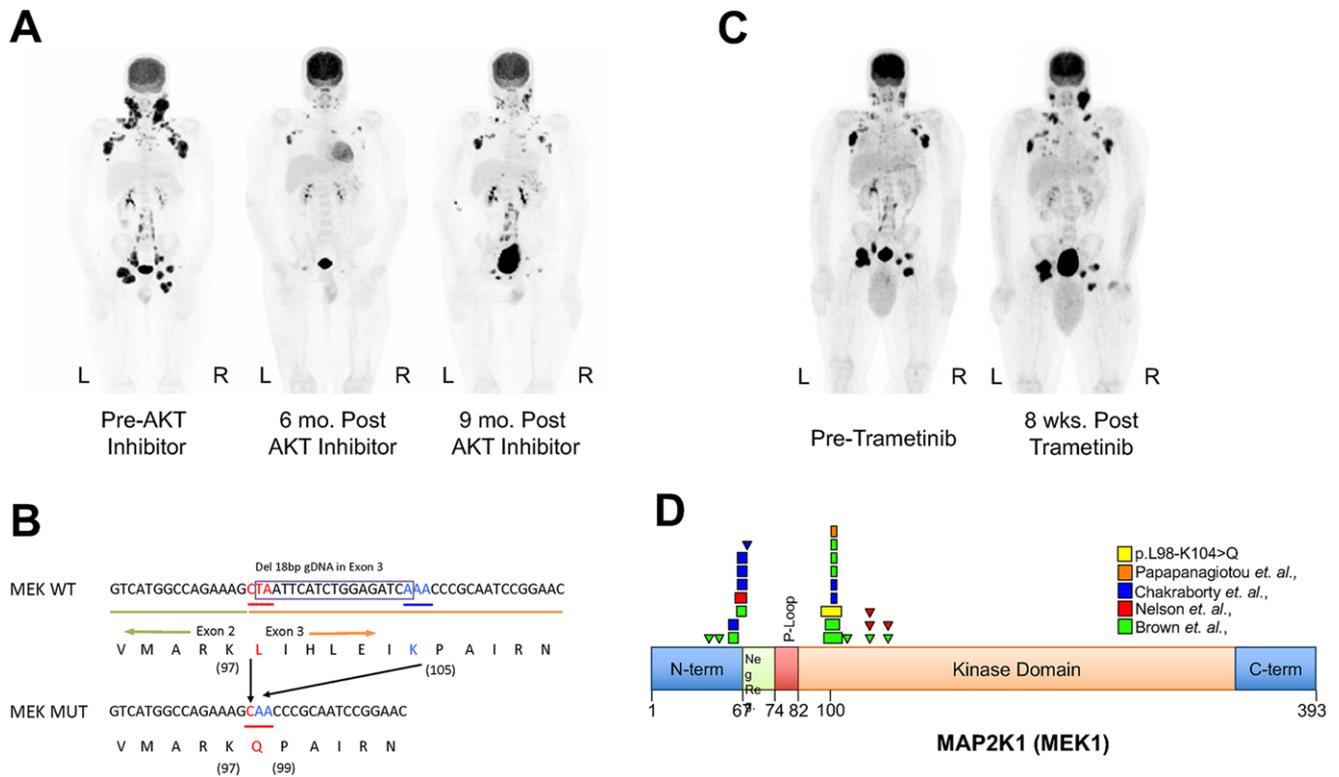
FIGURE 1 Identification of a novel mutation in *MAP2K1* (*MEK1*) in a patient with Langerhans cell histiocytosis (LCH). A, PET scan of LCH patient prior to treatment with afuresertib and subsequent scans, at both 6 and 9 months post AKT inhibitor treatment, showing partial response. Written informed consent was provided for publication of the images. B, Sequencing analysis of a sample from the LCH patient indicates an amino acid deletion/change (L98_K104 > Q) due to a 18 bp deletion in exon 3 of *MAP2K1* (*MEK1*). This region of the MEK protein corresponds to the p-loop binding site for phosphorylation of serines 217/222 (p-MEK). C, PET scan of the LCH patient prior to treatment with trametinib and 8 weeks post trametinib treatment. D, Mutations in MAP2K1 protein that have been identified in LCH patient samples

CO) and 2 mM L-Glutamine (Thermo Fisher) at 5% CO_2 and 37°C. HEK293A cells were transiently transfected with purified MEK-WT or MEK-MUT plasmids using Xtremegene-HP (Roche Life Sciences, Indianapolis, IN). At 48 hr, cells were treated with the inhibitors: trametinib; U0126; MK-2206; or SCH772984 (all drugs from Selleckchem, Houston, TX) for 1 hr. Cells were collected for protein analysis. Stable expressing cells were generated through Neomycin selection using 0.4 mg/ml G418 (Thermo Fisher).

2.4 | Western blot/AlphaLISA

Cellular lysates from transfected cells were normalized for equal loading on gels and AlphaLISA (PerkinElmer; Waltham, MA) kit. Gels were transferred to PVDF membrane (Bio-Rad, Hercules, CA) and probed with antibodies against MEK/p-MEK; ERK/p-ERK; AKT/p-AKT; or COX IV (Cell Signaling Technology, Danvers, MA). Blots were developed using ECL (Thermo Fisher) and imaged. AlphaLISA assay for p-ERK (PerkinElmer) was analyzed as per manufacturer's instruction.

3 | CASE DESCRIPTION

A 16-year-old male was diagnosed with multifocal LCH primarily involving lymph nodes. The patient was treated with LCH-III therapy[16]

and initially responded to treatment. One year later, the patient presented with painful right-sided neck swelling and a biopsy of the lesion confirmed recurrence of LCH. The patient was then treated with the oral pan-AKT inhibitor afuresertib as part of a clinical trial.[17] The patient responded to afuresertib therapy and completed a total of 30 weeks of treatment (Figure 1A). Several months later, the patient relapsed and was treated with cytarabine 150 mg/m²/day for a 5-day course. After two courses, the patient did not show a good response, and vincristine and steroids were added to the therapy. Afterward, the patient received two cycles of clofarabine, after which he was lost to follow-up. Three years after his initial diagnosis, the patient presented again with LCH symptoms and had cancer-related gene mutation analysis performed at Foundation Medicine. The genomic analysis identified a novel mutation in *MAP2K1* (*MEK1*): an 18 bp deletion c293_310del in exon 3 resulting in a p.L98_K104 > Q in-frame deletion (Figure 1B). Based upon the gene target, the patient received the MEK inhibitor, trametinib, 2 mg once daily. PET-CT whole body evaluation after 8 weeks of treatment showed progressive disease (Figure 1C). Treatment with trametinib was discontinued, and the patient was further treated with a combination of clofarabine and cytarabine for three cycles for disease control. Thereafter, the patient's disease was in control to obtain a haploidentical transplant, and more than a year after transplant the patient was doing well.
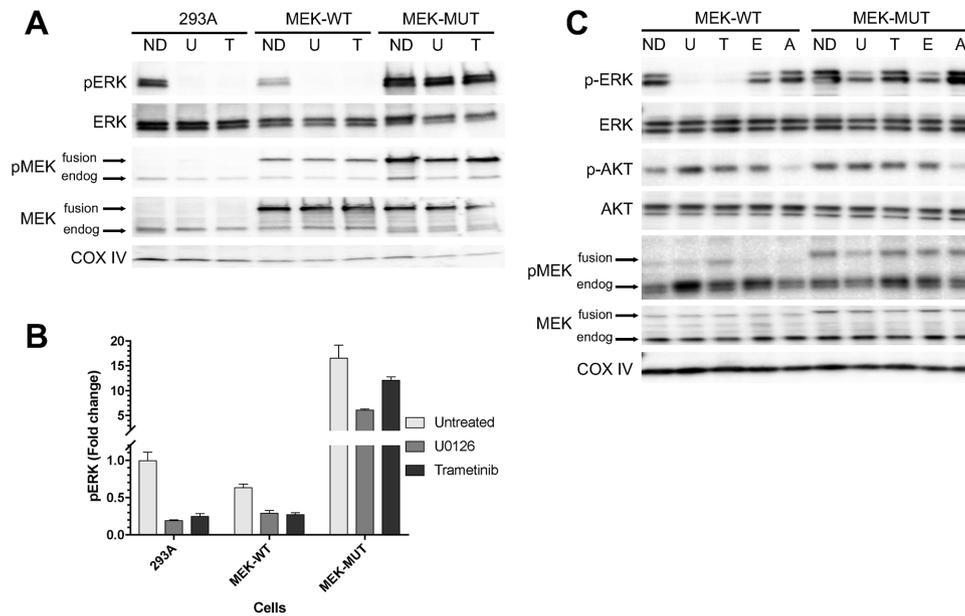
FIGURE 2 Functional analysis of the L98_K104 > Q mutation of *MAP2K1* (*MEK1*). A, Western blot analysis of untransfected HEK293A cells (293A), HEK293A transiently transfected with wild-type *MAP2K1* (MEK-WT) and HEK293A transiently transfected with *MAP2K1* containing the L98_K104 > Q mutation (MEK-MUT) treated with 100 nM trametinib (T), or 30 μM U0126 (U) for 1 hr. Lysates from each treatment were analyzed for protein expression using antibodies to p-ERK, total ERK, pMEK, and total MEK. Antibody to COX IV was used as a loading control. Western blot panel shows increased expression of the MEK-WT and MEK-MUT transfectants. The p-MEK-MUT has a two- to threefold increase versus p-MEK-WT resulting in a significant activation of p-ERK in the MUT samples. For 293A and MEK-WT, both U0126 and trametinib show loss of activation of p-ERK by inhibiting p-MEK expression. The MEK-MUT form shows no sensitivity to either MEK inhibitor. B, AlphaLISA analysis of p-ERK activation levels. Cell lysates (5 μg protein/sample) used in the western blots were analyzed for p-ERK levels using a double antibody AlphaLISA assay (PerkinElmer) and results normalized to untreated 293A cell lysate. Similar to the western blot results, the AlphaLISA shows p-ERK activation in the 293A and MEK-WT samples are completely inhibited by both U0126 and trametinib. The transient MEK-MUT showed no sensitivity to trametinib compared to no drug treatment, while showing partial inhibition by U0126. C, Analysis of MEK-MUT activity in stably expressing cells. Cells transfected with either MEK-WT or MEK-MUT were grown in media containing G418 for generating stable expressing cells. Stably expressing MEK-WT or MEK-MUT were treated with no drugs (ND), 30 μM U0126 (U), 100 nM trametinib (T), the ERK inhibitor SCH772984 (E) at 200 nM, and the AKT inhibitor MK-2206 2HCl (A) at 100 nM. Western blot analysis of p-ERK expression showed a similar pattern to that of the transient samples except for decreased expression levels of transfected MEK and transfected p-MEK. The MEK inhibitors U0126 and trametinib inhibited p-ERK activation in MEK-WT cells but not in MEK-MUT cells as with the transient transfected cells

4 | RESULTS AND DISCUSSION

In order to evaluate the function of the novel *MAP2K1* mutation, we duplicated the p.L98_K104 > Q mutation using an expression construct for GFP-MEK.[15] HEK293A cells were transiently transfected with either the wild-type GFP-MEK construct (MEK-WT), the p.L98_K104 > Q mutant (MEK-MUT), or left untransfected and were treated with MEK inhibitors U0126 or trametinib. Both MEK inhibitors were able to decrease p-ERK expression in untransfected cells and MEK-WT-expressing cells, but not in the MEK-MUT cells (Figure 2A). MEK-MUT cells also expressed much higher levels of p-ERK than either MEK-WT or untransfected HEK293A cells using an AlphaLISA p-ERK assay (Figure 2B). Furthermore, both MEK inhibitors greatly decreased p-ERK levels in untransfected and MEK-WT-expressing HEK293A cells, while having a mild effect on MEK-MUT cells. Transiently transfected cells were further grown in selection media to establish cells stably overexpressing either MEK-WT or MEK-MUT. These cells were similarly treated with MEK inhibitors U0126 and trametinib as well as the ERK inhibitor SCH772984 and the AKT inhibitor MK-2206. The MEK inhibitors decreased expression of p-ERK in the stably express-

ing MEK-WT cells but not in the stably expressing MEK-MUT cells (Figure 2C). Taken together, these functional studies indicate that the *MAP2K1* p.L98_K104 > Q mutation leads to increased p-ERK activation that is unaffected by MEK inhibitors.

The p.L98_K104 > Q mutation in *MAP2K1* has not been previously described but it is found in a region of the *MAP2K1* gene where several other activating mutations have been found[5,6,18,19] (Figure 1D). *In vitro* functional studies show that the MEK inhibitor U0126 can inhibit ERK activation resulting from these mutations in this region of *MAP2K1*.[6] Another study showed that the p.F53_Q58 > L, p.Q58_E62del, and p.C121S/G128V mutations in *MAP2K1* constituently activated ERK and were susceptible *in vitro* to MEK inhibitors including trametinib.[20] Interestingly, a recent report describes the complete remission of an LCH patient with an *MAP2K1* p.E102-I103del mutation treated with trametinib.[14] The described *MAP2K1* p.E102-I103del deletion involves six bases that fall inside the p.L98_K104 > Q deletion and has been shown to be an activating mutation.[5,6] The difference in the response of these two patients, having similar but not identical *MAP2K1* mutations, to trametinib could be due to several factors including the structural change in MAP2K1 protein that the larger

p.L98_K104 > Q deletion would have on MAP2K1 activity or trametinib binding. The expression of the *MAP2K1* p.L98_K104 > Q mutation identified in this LCH patient leads to hyper-activated p-MAP2K1 and increases activation of p-ERK. As seen in these studies, and consistent with the clinical results, this activating *MAP2K1* mutation is not responsive to inhibition with MEK inhibitors, including trametinib. Further functional investigation of these *MAP2K1* mutations is needed. Furthermore, it would be of interest to understand the role that the previous therapeutic treatment with afuresertib may have played in the development of this particular resistance-associated mutation. These results emphasize the importance of the functional assessment of genomic data in assigning treatment for patients with LCH or other cancers, as well as provide evidence for choice of inhibitor in patients with this or similar mutations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Harmon CM, Brown N. Langerhans cell histiocytosis: a clinicopathologic review and molecular pathogenetic update. *Arch Pathol Lab Med.* 2015;139:1211–1214.

2. Diamond EL, Durham BH, Haroche J, et al. Diverse and targetable kinase alterations drive histiocytic neoplasms. *Cancer Discov.* 2016;6: 154–165.

3. Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. *Blood.* 2010;116: 1919–1923.

4. Mehes G, Irsai G, Bedekovics J, et al. Activating BRAF V600E mutation in aggressive pediatric Langerhans cell histiocytosis: demonstration by allele-specific PCR/direct sequencing and immunohistochemistry. *Am J Surg Pathol.* 2014;38:1644–1648.

5. Brown NA, Furtado LV, Betz BL, et al. High prevalence of somatic MAP2K1 mutations in BRAF V600E-negative Langerhans cell histiocytosis. *Blood.* 2014;124:1655–1658.

6. Chakraborty R, Hampton OA, Shen X, et al. Mutually exclusive recurrent somatic mutations in MAP2K1 and BRAF support a central role for ERK activation in LCH pathogenesis. *Blood.* 2014;124:3007–3015.

7. Nelson DS, van Halteren A, Quispel WT, et al. MAP2K1 and MAP3K1 mutations in Langerhans cell histiocytosis. *Genes Chromosomes Cancer.* 2015;54:361–368.

8. Nelson DS, Quispel W, Badalian-Very G, et al. Somatic activating ARAF mutations in Langerhans cell histiocytosis. *Blood.* 2014;123:3152–3155.

9. Abla O, Weitzman S. Treatment of Langerhans cell histiocytosis: role of BRAF/MAPK inhibition. *Hematology Am Soc Hematol Educ Program.* 2015;2015:565–570.

10. Haroche J, Cohen-Aubart F, Emile JF, Donadieu J, Amoura Z. Vemurafenib as first line therapy in BRAF-mutated Langerhans cell histiocytosis. *J Am Acad Dermatol.* 2015;73:e29–e30.

11. Falchook GS, Lewis KD, Infante JR, et al. Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. *Lancet Oncol.* 2012;13:782–789.

12. Kim KB, Kefford R, Pavlick AC, et al. Phase II study of the MEK1/MEK2 inhibitor trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. *J Clin Oncol.* 2013;31:482–489.

13. Nordmann TM, Juengling FD, Recher M, et al. Trametinib after disease reactivation under dabrafenib in Erdheim–Chester disease with both BRAF and KRAS mutations. *Blood.* 2017;129:879–882.

14. Papapanagiotou M, Griewank KG, Hillen U, et al. Trametinib-induced remission of an MEK1-mutated Langerhans cell histiocytosis. *JCO Clin Cancer Inform.* 2017;1:1–5.

15. Yao Z, Flash I, Raviv Z, et al. Non-regulated and stimulated mechanisms cooperate in the nuclear accumulation of MEK1. *Oncogene.* 2001;20:7588–7596.

16. Gadner H, Minkov M, Grois N, et al. Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. *Blood.* 2013;121:5006–5014.

17. Arceci RJ, Allen CE, Dunkel IJ, et al. A phase IIa study of afuresertib, an oral pan-AKT inhibitor, in patients with Langerhans cell histiocytosis. *Pediatr Blood Cancer.* 2017;64:e26325.

18. Alayed K, Medeiros LJ, Patel KP, et al. BRAF and MAP2K1 mutations in Langerhans cell histiocytosis: a study of 50 cases. *Hum Pathol.* 2016;52:61–67.

19. Zeng K, Ohshima K, Liu Y, et al. BRAFV600E and MAP2K1 mutations in Langerhans cell histiocytosis occur predominantly in children. *Hematol Oncol.* 2017;35:845–851.

20. Lee LH, Gasilina A, Roychoudhury J, et al. Real-time genomic profiling of histiocytoses identifies early-kinase domain BRAF alterations while improving treatment outcomes. *JCI Insight.* 2017;2:e89473.