

Plasma cell-free MAP2K1 mutation levels are associated with progression or relapse in pediatric MAP2K1-mutated Langerhans cell histiocytosis

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Abstract

Langerhans cell histiocytosis (LCH) is a rare myeloid neoplasm driven by MAPK pathway alterations and marked clinical heterogeneity. *MAP2K1* mutations are among the most frequent driver alterations after *BRAF-V600E*. Still, the clinical value of plasma cell-free *MAP2K1* mutations (cf*MAP2K1*) remains unclear, partly because of the diversity of *MAP2K1* variants and the need for sensitive detection methods. We developed an amplicon-based targeted sequencing assay for cf*MAP2K1* detection and evaluated its clinical value in children with tissue-confirmed *MAP2K1*-mutated LCH. Compared to 127 contemporaneous patients with *BRAF-V600E*-mutated LCH, 82 *MAP2K1*-mutated patients were older, more frequently had single-system involvement, and had lower rates of risk-organ involvement and permanent consequences. Baseline cf*MAP2K1* was assessed in 67 patients and was detectable in 37 (55.2%). In the cfDNA-evaluable cohort, higher cf*MAP2K1* levels were associated with multisystem disease, age younger than two years, and lung involvement. cf*MAP2K1* was also related to later progression or relapse, with an area under the curve of 0.83 in ROC analysis. Using 0.1% as the cutoff, patients above this level had much lower 3-year progression-free survival than those below it (47.6% vs. 93.3%, $P < 0.001$). This cutoff retained prognostic significance in the multivariable model, together with multisystem disease. During first-line therapy, patients with detectable cf*MAP2K1* experienced progression or relapse more often than those who remained negative. In the trametinib-treated subgroup, rising or high-level cf*MAP2K1* was observed before or at progression/relapse in some patients. Overall, cf*MAP2K1* testing may provide useful molecular information for risk assessment and follow-up in pediatric *MAP2K1*-mutated LCH.

Key points

1. Baseline cf $\geq 0.1\%$ defined a subgroup of children with -mutated LCH with significantly inferior PFS.
2. Detectable cf during treatment was associated with more frequent progression or relapse, supporting its potential role in serial molecular monitoring.

Introduction

Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasm characterized by the accumulation of clonal CD1a-positive and CD207-positive dendritic cell in affected tissues (Allen et al. 2018; Allen and Rodriguez-Galindo 2020; Emile et al. 2016). Pediatric LCH exhibits significant clinical heterogeneity, presenting as either an isolated single-system lesion or multisystem disease. Despite advances in risk-adapted therapy that have improved survival, disease progression, relapse, and permanent sequelae continue to pose substantial clinical challenges (Abla 2023; Cui et al. 2023; Gadner et al. 2008; Gadner et al. 2013; Lin et al. 2025; Rigaud et al. 2016). Therefore, novel biomarkers are required to more accurately reflect disease activity and improve risk stratification and treatment monitoring.

Aberrant activation of the mitogen-activated protein kinase (MAPK) pathway is central to LCH pathogenesis(Jouenne et al. 2021; Kamionek et al. 2016). The identification of recurrent *BRAF*-V600E mutations has defined LCH as a clonal, MAPK-driven neoplastic disorder(Badalian-Very et al. 2010; Berres et al. 2014; Héritier et al. 2016). *MAP2K1* mutations represent one of the most frequent driver alterations in *BRAF*-V600E-negative LCH and are generally mutually exclusive with *BRAF* mutations(Alayed et al. 2016; Brown et al. 2014; Chakraborty et al. 2014; Zeng et al. 2017). Previous studies indicated that *MAP2K1*-mutated LCH may exhibit clinical features distinct from those with *BRAF*-V600E mutations(Hayase et al. 2020; Kemps et al. 2023; Yang et al. 2021). Nevertheless, tissue mutation status alone may not sufficiently reflect systemic disease burden or reliably predict disease progression and relapse.

Liquid biopsy utilizing circulating cell-free (cf) DNA provides a minimally invasive approach for tumor molecular profiling and disease monitoring(Alix-Panabières and Pantel 2021; Bettegowda et al. 2014; Wan et al. 2017). In pediatric LCH, circulating cf*BRAF*-V600E has been demonstrated to correlate with disease burden, treatment response, and prognosis(Cui et al. 2020; Cui et al. 2026; Héritier et al. 2017; Schwentner et al. 2018; Wang et al. 2021; Wang et al. 2024). By contrast, the clinical value of cf*MAP2K1* remains less well defined, particularly regarding its prognostic value at diagnosis and its utility for monitoring molecular persistence or relapse during therapy. This uncertainty may be due in part to the genetic heterogeneity of *MAP2K1* mutations. Consequently, highly sensitive sequencing techniques are needed to detect a broad spectrum of low-frequency cf*MAP2K1* mutations in plasma.

In this study, we established an amplicon-based targeted sequencing method for plasma cf*MAP2K1* detection. In a cohort of children with tissue-confirmed *MAP2K1*-mutated LCH, we compared clinical characteristics with those of a contemporaneous *BRAF*-V600E-positive cohort. We then evaluated the prognostic value of baseline cf*MAP2K1* levels and explored the utility of serial cf*MAP2K1* monitoring during first-line chemotherapy and trametinib treatment.

Material and Methods

Patients and cohorts

Children younger than 18 years with newly diagnosed LCH who were admitted to Beijing Children's Hospital from June 1, 2021, to June 30, 2024, were screened consecutively. Patients were eligible if LCH was confirmed by histopathology with positive CD1a and CD207 immunostaining, a *MAP2K1* mutation was identified in lesional tissue by sequencing, plasma samples were available for cf*MAP2K1* analysis, no antitumor treatment had been given before admission, and written informed consent had been obtained from legal guardians. The patient selection process is shown in Supplementary Fig. S1. Of 82 patients with tissue-confirmed *MAP2K1*-mutated LCH, 67 had plasma samples available for cf*MAP2K1* analysis.

This study was approved by the Institutional Review Board of Beijing Children's Hospital and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patients' guardians.

Risk stratification and treatment

Patients were classified as single-system LCH (SS LCH) or multisystem LCH (MS LCH) according to the number of organs or systems involved. MS LCH was further categorized as risk organ-positive (RO⁺) or risk organ-negative (RO⁻) based on liver, spleen, and/or hematopoietic system involvement (Haupt et al. 2013). First-line chemotherapy was administered according to the BCH-LCH 2014 protocol, as previously reported (Cui, et al. 2023). Briefly, treatment consisted of one or two 6-week induction courses with vinblastine and corticosteroids, followed by maintenance therapy with vinblastine, prednisone, and 6-mercaptopurine, for a total duration of 12 months. Trametinib was given to patients with *MAP2K1*-mutated MS LCH who were intolerant to standard chemotherapy or developed disease progression or relapse during or after first-line therapy. Trametinib was administered orally at 0.025mg/kg once daily for 12 months, followed by a 6- to 12- month maintenance phase with stepwise dose reduction. Treatment duration was individualized based on disease status. Treatment response was evaluated according to the International LCH Study Group criteria (Gadner, et al. 2013).

Tissue and plasma mutation analysis

Mutation analysis of lesional tissue was performed using a targeted next-generation sequencing panel, as previously described (Wang et al. 2025). Genomic DNA was extracted from diagnostic formalin-fixed paraffin-embedded tissue samples. The panel covered MAPK pathway-related genes and was used for molecular classification. Libraries were sequenced on an Illumina NovaSeq 6000 platform. Sequencing data were aligned to the human reference genome, and variants, including single-nucleotide variants and insertions/deletions, were identified using a standard bioinformatic pipeline.

Peripheral blood was collected in cfDNA blood collection tubes (CoWin Biosciences) at diagnosis and during follow-up. Plasma was isolated by two-step centrifugation at 1,000 × g for 10 minutes and 16,000 × g for 5 minutes. cfDNA was extracted with the QIAamp Circulating Nucleic Acid Kit (Qiagen), stored at -80°C, and quantified using Qubit. Samples with cfDNA concentrations ≥ 0.1 ng/μL were subjected to sequencing. cf*MAP2K1* mutations in hotspot regions of exons 2 and 3 were detected by amplicon-based targeted sequencing (Fig. 1). Primer sequences, PCR conditions, library preparation, sequencing procedures, bioinformatic analysis, and quality-control criteria are described in the Supplementary Methods. In the comparator cohort, cf*BRAF*-V600E was measured by digital PCR, as previously reported (Cui, et al. 2020).

Statistical analysis

Continuous variables were compared using the Mann-Whitney U test or Kruskal-Wallis test, as appropriate, and categorical variables were compared using Fisher's exact test. Operating characteristic (ROC) curves were used to assess the ability of cfMAP2K1 levels to distinguish patients with subsequent progression or relapse. Progression-free survival (PFS) was calculated from the start of treatment to disease progression, relapse, or death from any cause, or last follow-up. Patients without an event were censored at the last follow-up. Cox proportional hazards model was used for prognostic analyses, and hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. Variables with $P < 0.05$ in univariate analysis were entered into the multivariate Cox regression model. Statistical analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA) and R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). The final follow-up date was March 31, 2026.

Results

Clinical characteristics of patients with MAP2K1-mutated LCH

Eighty-two children with *MAP2K1*-mutated LCH were included in this cohort, with a male predominance (55 boys and 27 girls). At diagnosis, SS disease accounted for most cases (60/82, 73.2%), while 20 patients (24.4%) had MS RO⁻ LCH and only two (2.4%) had MS RO⁺ LCH. Compared with 127 the contemporaneous *BRAF*-V600E cohort ($n = 127$), the *MAP2K1* cohort had an older age at diagnosis (median, 6.1 vs. 3.3 years; $P < 0.001$), a higher proportion of SS LCH (73.2% vs. 46.5%), and a lower proportion of MS RO⁺ disease (2.4% vs 18.9%; $P < 0.001$). Liver, spleen, skin, and pituitary involvement were less frequent in patient with *MAP2K1* mutations (all $P < 0.05$), whereas bone involvement was more frequent ($P = 0.004$). Unifocal bone lesion ($P = 0.036$) and appendicular skeleton involvement ($P = 0.020$) were also more commonly observed in *MAP2K1*-mutated LCH (Fig. 2).

Of the 82 patients, 60 received vinblastine-prednisone-based first-line therapy, 10 received upfront trametinib, and 12 were managed with observation alone. Among those initially treated with first-line therapy, three were later switched to trametinib after disease progression or relapse. After a median follow-up of 34.9 months, nine patients (11.0%) developed disease progression and 12 (14.6%) relapses. The estimated 3-year PFS was $76.1 \pm 4.8\%$, which did not significantly differ from that of the *BRAF*-V600E-positive cohort ($68.0\% \pm 4.3\%$, $P = 0.313$). Permanent consequences were recorded in only one patient (1.2%) with *MAP2K1*-mutated LCH, significantly fewer than in the *BRAF*-V600E-positive cohort ($P = 0.002$; Fig. 2). Clinical characteristics and outcomes were similar between patients with *MAP2K1* mutations in exons 2 and 3 (Supplementary Table S2).

Prognostic Value of Baseline cf MAP2K1 Levels

Baseline plasma cfMAP2K1 was assessed at diagnosis in 67 children with tissue-confirmed *MAP2K1*-mutated LCH (Fig. 3A). cfMAP2K1 was detectable in 37 patients (55.2%), with a median level of 0.13% (range, 0.008% – 6.98%). Detection rates were similar between patients with exon 2 and exon 3 mutations (54.5% vs. 58.3%, $P = 1.000$). cfMAP2K1 levels were higher in patients with MS LCH than in

those with SS LCH ($P = 0.010$). Higher levels were also observed in children younger than two years ($P = 0.033$) and in patients with lung involvement ($P < 0.001$; Fig. 3B).

cfMAP2K1 levels were associated with subsequent progression or relapse. ROC analysis showed an association between baseline cfMAP2K1 and later clinical events, with an area under the curve (AUC) of 0.83 (95%CI 0.69–0.97, $P < 0.001$; Fig. 4A). Patients were then stratified by baseline cfMAP2K1 levels into four groups: $< 0.01\%$, $\geq 0.01\%$ to $< 0.1\%$, $\geq 0.1\%$ to $< 1\%$, and $\geq 1\%$. Compared with patients with cfMAP2K1 $< 0.01\%$, those with levels $\geq 0.01\%$ to $< 0.1\%$ did not have an increased risk of progression or relapse (HR, 0.9; 95% CI, 0.4–9.4; $P = 0.896$). By contrast, the risk was higher in patients with cfMAP2K1 $\geq 0.1\%$ to $< 1\%$ (HR, 7.1; 95% CI, 1.4–35.5; $P = 0.016$) and was highest in those with cfMAP2K1 $\geq 1\%$ (HR, 15.1; 95% CI, 2.9–78.2; $P = 0.001$; Fig. 4B).

Because the risk increase was mainly observed at levels of 0.1% or higher, 0.1% was used as the threshold for further prognostic stratification. Patients with cfMAP2K1 $\geq 0.1\%$ had inferior PFS compared with those with levels $< 0.1\%$, with estimated 3-year PFS rates of $47.6 \pm 10.9\%$ and $93.3 \pm 3.7\%$, respectively ($P < 0.001$). Baseline cfMAP2K1 $\geq 0.1\%$ was associated with an increased risk of progression or relapse (HR, 9.9; 95% CI 2.8–35.8; $P < 0.001$; Fig. 4C).

A comparable pattern was seen in the tissue-confirmed BRAF-V600E cohort ($n = 123$). Patients with baseline cfBRAF-V600E $\geq 0.1\%$ had inferior PFS compared with those with lower levels ($55.2 \pm 6.2\%$ vs. $85.4 \pm 5.1\%$; $P < 0.001$), with a corresponding increase in the risk of progression or relapse (HR 3.5, 95% CI 1.5–7.9; $P = 0.003$; Fig. 4C). Together, the cfMAP2K1 and cfBRAF-V600E analyses support an association between higher baseline circulating mutation burden and adverse clinical outcomes in pediatric LCH.

Among 46 patients who initially received first-line therapy and had baseline cfMAP2K1 testing, univariate analysis showed that baseline cfMAP2K1 $\geq 0.1\%$, age younger than two years, multisystem disease, and lung involvement were associated with inferior PFS (Supplementary Table S3). In the multivariable Cox model, only multisystem disease (HR, 5.2; $P = 0.036$) and baseline cfMAP2K1 $\geq 0.1\%$ (HR, 5.5; $P = 0.027$) remained independently associated with worse PFS (Fig. 4D).

Monitoring of cf MAP2K1 during follow-up

Serial cfMAP2K1 monitoring during first-line therapy was available for 54 patients, although samples were not collected at every predefined time point for all cases. During treatment, cfMAP2K1 became detectable at least once in five patients, four of whom subsequently developed progression or relapse (80.0%; Fig. 5A). The only patient without a subsequent event, Patient No.30, showed transient cfMAP2K1 positivity at week 6 (0.14%), which became undetectable by week 12 and remained negative thereafter; this patient remained in continuous remission two years after chemotherapy discontinuation (Fig. 5B). Among the remaining 49 patients with persistently undetectable cfMAP2K1, 10 (20.4%) developed progression or relapse, whereas most remained event-free. Detectable cfMAP2K1 during first-

line therapy was therefore associated with a higher incidence of progression or relapse than persistently undetectable *cfMAP2K1* (80.0% vs. 20.4%, $P = 0.013$).

Serial *cfMAP2K1* monitoring was also performed in 11 patients treated with trametinib. Four patients had detectable *cfMAP2K1* during treatment. In patients No.9 and N0.10, *cfMAP2K1* levels increased before relapse, and both showed high-level positivity ($> 0.1\%$) at the time of progression or relapse (Fig. 6A). In contrast, patients No.63 and No.81 showed transient or low-level *cfMAP2K1* positivity without subsequent progression or relapse (Fig. 6B). Patient No.63 became *cfMAP2K1*-negative after trametinib dose reduction and remained negative after treatment discontinuation. Patient No.81 became negative at six months, showed low-level positivity again at 12 months while continuing full-dose trametinib, and remained in sustained remission for two years. Thus, in this small trametinib-treated subgroup, disease progression or relapse was accompanied by rising or high-level *cfMAP2K1* in some patients, whereas isolated low-level positivity was not necessarily followed by clinical deterioration and should be interpreted alongside the overall disease assessment.

Discussion

In this study, plasma *cfMAP2K1* showed clinical relevance in children with tissue-confirmed *MAP2K1*-mutated LCH. Higher baseline *cfMAP2K1* levels, particularly at the 0.1% threshold, were associated with higher-risk clinical features and inferior PFS, and remained significant in multivariable analysis. Serial monitoring also provided additional information during treatment: *cfMAP2K1* positivity during first-line therapy was more common in patients who later developed progression or relapse, and rising or high-level *cfMAP2K1* during trametinib treatment was observed before or at relapse in some patients.

Our data also emphasize the clinical heterogeneity of MAPK-driven LCH. Compared with children with *BRAF*-V600E-mutated LCH, patients with *MAP2K1*-mutated disease were older and more often presented with SS disease and skeletal involvement, whereas MS RO⁺ disease and systemic organ involvement were less frequent. This distribution is in line with previous reports suggesting that *BRAF*-V600E-positive LCH is more closely linked to disseminated disease, treatment resistance, and permanent consequences, while *MAP2K1*-mutated LCH tends to show more frequent bone involvement and less frequent RO involvement (Berres, et al. 2014; Hayase, et al. 2020; H  ritier, et al. 2016; Kemps, et al. 2023; Yang, et al. 2021). Genomic and single-cell studies have also supported biological diversity across MAPK-altered histiocytic neoplasms (Diamond et al. 2016; Halbritter et al. 2019; Kamionek, et al. 2016; Milne et al. 2022; Nelson et al. 2015). Nevertheless, PFS was not significantly different between the *MAP2K1*-mutated and *BRAF*-V600E cohorts in our study. This findings argues against considering *MAP2K1*-mutated LCH as uniformly low risk and highlights the need for additional biomarkers to identify patients prone to progression or relapse.

Baseline *cfMAP2K1* added information that could not be obtained from tissue mutation status alone. Tissue sequencing was essential for defining the driver alteration, but it represented only the sampled lesion and did not measure the circulating mutation burden. In our cohort, higher *cfMAP2K1* levels were

found in children with multisystem disease, age younger than two years, or lung involvement. This suggests that the plasma signal reflected the clinical extent of disease rather than the mere presence of a tissue *MAP2K1* mutation. Patients with baseline cf*MAP2K1* $\geq 0.1\%$ had poorer PFS, and this association was retained in the multivariable analysis. Similar observations from cf*BRAF*-V600E studies in pediatric LCH suggest that circulating MAPK-mutant DNA reflects disease activity across different genetic subtypes(Cui, et al. 2020; Cui, et al. 2026; H  ritier, et al. 2017; Schwentner, et al. 2018; Wang, et al. 2021; Wang, et al. 2024). For children with tissue-confirmed *MAP2K1*-mutated LCH, baseline cf*MAP2K1* testing may therefore be used as an adjunct to conventional clinical risk assessment, particularly for selecting patients who may require closer molecular and clinical follow-up.

During first-line therapy, patients with detectable cf*MAP2K1* were more likely to experience later progression or relapse, whereas most patients with persistently negative results remained event-free. This observation is consistent with previous cf*BRAF*-V600E studies, in which persistent or recurrent circulating mutation signals during treatment were linked to residual active disease and inferior prognosis(Cui, et al. 2020; Cui, et al. 2026; H  ritier, et al. 2017; Lee et al. 2017; Schwentner, et al. 2018; Wang, et al. 2021; Wang, et al. 2024). However, transient low-level positivity followed by clearance, as seen in one patient in our cohort, indicates that a single low-level positive result should not be overinterpreted. During trametinib treatment, cf*MAP2K1* increases were seen before or at progression/relapse in two patients, whereas isolated low-level positivity occurred in patients who remained in remission. MAPK pathway inhibitors have shown substantial activity in LCH and other histiocytic neoplasms, but clinical improvement does not necessarily imply complete eradication of mutation-positive cells or molecular residual disease(Abla 2023; Cournoyer et al. 2024; Cui, et al. 2026; Diamond et al. 2018; Diamond et al. 2019; Donadieu et al. 2019; Eckstein et al. 2019; Eder et al. 2022; Goyal et al. 2022; Hyman et al. 2015; Ouyang et al. 2026; Whitlock et al. 2023). Thus, the trend and level of cf*MAP2K1*, rather than positivity alone, are likely to be more informative and should be interpreted together with clinical evaluation and imaging findings,

From a technical standpoint, cf*MAP2K1* detection is more complex than cf*BRAF*-V600E detection. *BRAF*-V600E is a single recurrent hotspot mutation and can be quantified using allele-specific assays such as digital PCR. By contrast, *MAP2K1* alterations are heterogeneous, including single-nucleotide variants and in-frame deletions clustered mainly in exon 2 and 3(Brown, et al. 2014; Chakraborty, et al. 2014). A sequencing-based approach is therefore more suitable for detecting diverse low-frequency cf*MAP2K1* variants in plasma. The amplicon-based targeted sequencing assay used in this study enabled simultaneous detection and quantification of multiple hotspot variants. However, low-frequency cfDNA sequencing is sensitive to preanalytical and analytical factors, including plasma processing, cfDNA input, amplification bias, sequencing depth, sequencing errors, and variant-calling thresholds(Abbosh et al. 2017; Cohen et al. 2018; Corcoran and Chabner 2018; Dawson et al. 2013; Diehl et al. 2008; Forshew et al. 2012; Heitzer et al. 2018; Murtaza et al. 2013; Newman et al. 2016; Phallen et al. 2017; Schmitt et al. 2012; Siravegna et al. 2017; Tie et al. 2022). Future multicenter studies are needed to standardize assay performance, reporting thresholds, and quality-control criteria for cf*MAP2K1* testing.

Several limitations need to be taken into account when interpreting these results. This was a single-center study, and although the cohort was sizeable for pediatric *MAP2K1*-mutated LCH, only a small number of patients has MS RO⁺ disease. Baseline plasma was not available for every tissue-confirmed case, and serial samples were missing at some planned time points, which limited a more detailed assessment of cf*MAP2K1* kinetics. The trametinib-monitored subgroup was also small, with variability in treatment setting and disease status. Another limitation is that cf*MAP2K1* was analyzed only in plasma. Paired peripheral blood mononuclear cells were not tested, leaving open the possibility that mutation-positive cellular reservoirs were missed. Longer follow-up is needed to evaluate late relapse and delayed sequelae, including neuroendocrine dysfunction and neurodegeneration.

In conclusion, plasma cf*MAP2K1* was detectable in more than half of children with tissue-confirmed *MAP2K1*-mutated LCH and was associated with disease extent and selected high-risk clinical features. A baseline threshold of 0.1% identified patients with an increased risk of progression or relapse and remained independently associated with inferior PFS after adjustment for clinical factors. During follow-up, detectable cf*MAP2K1* during first-line therapy was more frequent in patients who later developed progression or relapse, and rising or high-level cf*MAP2K1* during trametinib treatment was observed before or at relapse in some cases. These findings suggest that plasma cf*MAP2K1* testing may complement tissue genotyping in pediatric *MAP2K1*-mutated LCH by capturing circulating mutation burden at diagnosis and tracking molecular changes during treatment. Future prospective multicenter studies are warranted to validate these findings and clarify how cf*MAP2K1* monitoring should be integrated into risk-adapted treatment and follow-up strategies.

Declarations

Conflict of Interest Disclosures

The authors declare no conflicts of interest.

Authorship Contributions

ZZJ and WCJ analyzed the data and wrote the manuscript. WD, ZYZ, LHY LW, and WTY made clinical contributions. LWJ, DJJ, and TRJ contributed to sample collection. WJ was involved in the data analysis. LZG, CL, and ZR designed the research, supervised the study, and revised the paper. All authors were involved in the final approval of the paper.

Ethical approval

This study was approved by the Institutional Review Board of Beijing Children's Hospital and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patients' guardians.

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Author Contribution

ZZJ and WCJ analyzed the data and wrote the manuscript. WD, ZYZ, LHY LW, and WTY made clinical contributions. LWJ, DJJ, and TRJ contributed to sample collection. WJ was involved in the data analysis. LZG, CL, and ZR designed the research, supervised the study, and revised the paper. All authors were involved in the final approval of the paper.

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Data availability

The de-identified datasets that support the findings of this study are available from the corresponding authors upon reasonable request and subject to institutional approvals and a data use agreement.

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Figures

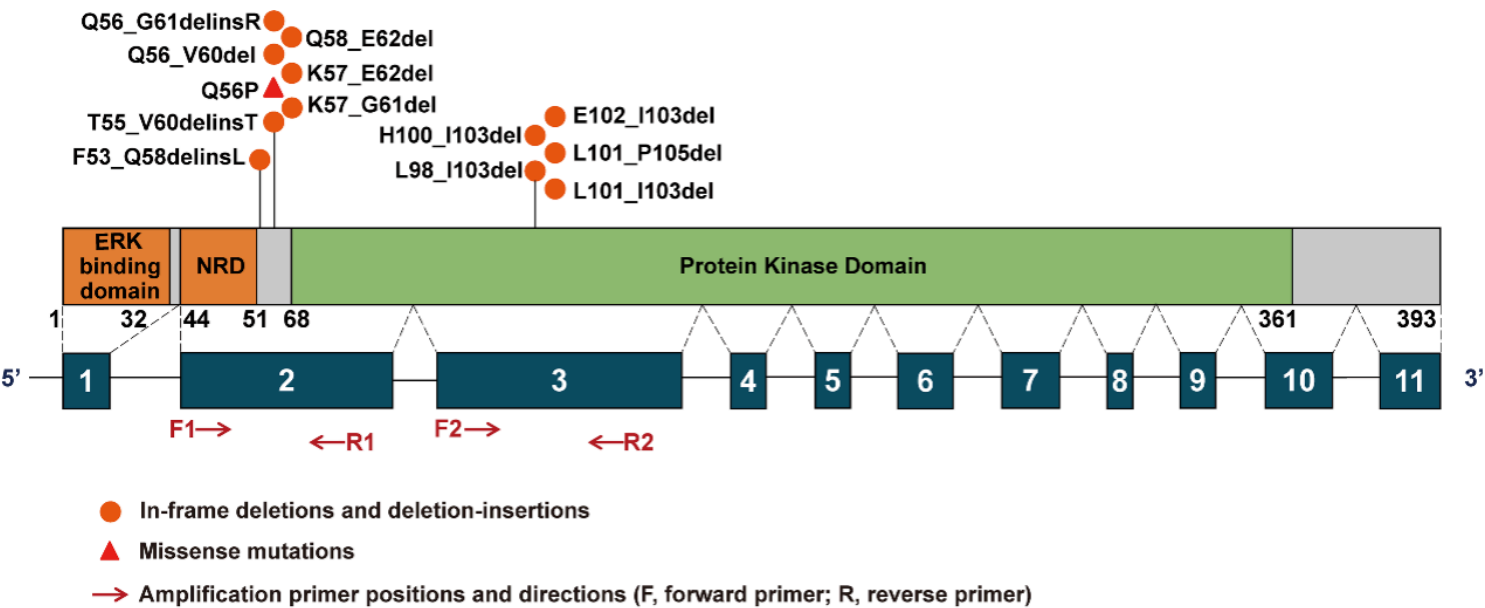


Figure 1

Schematic representation of *MAP2K1* mutations in exon2 and 3 and primer positions used for cf*MAP2K1* detection by amplicon-based targeted sequencing

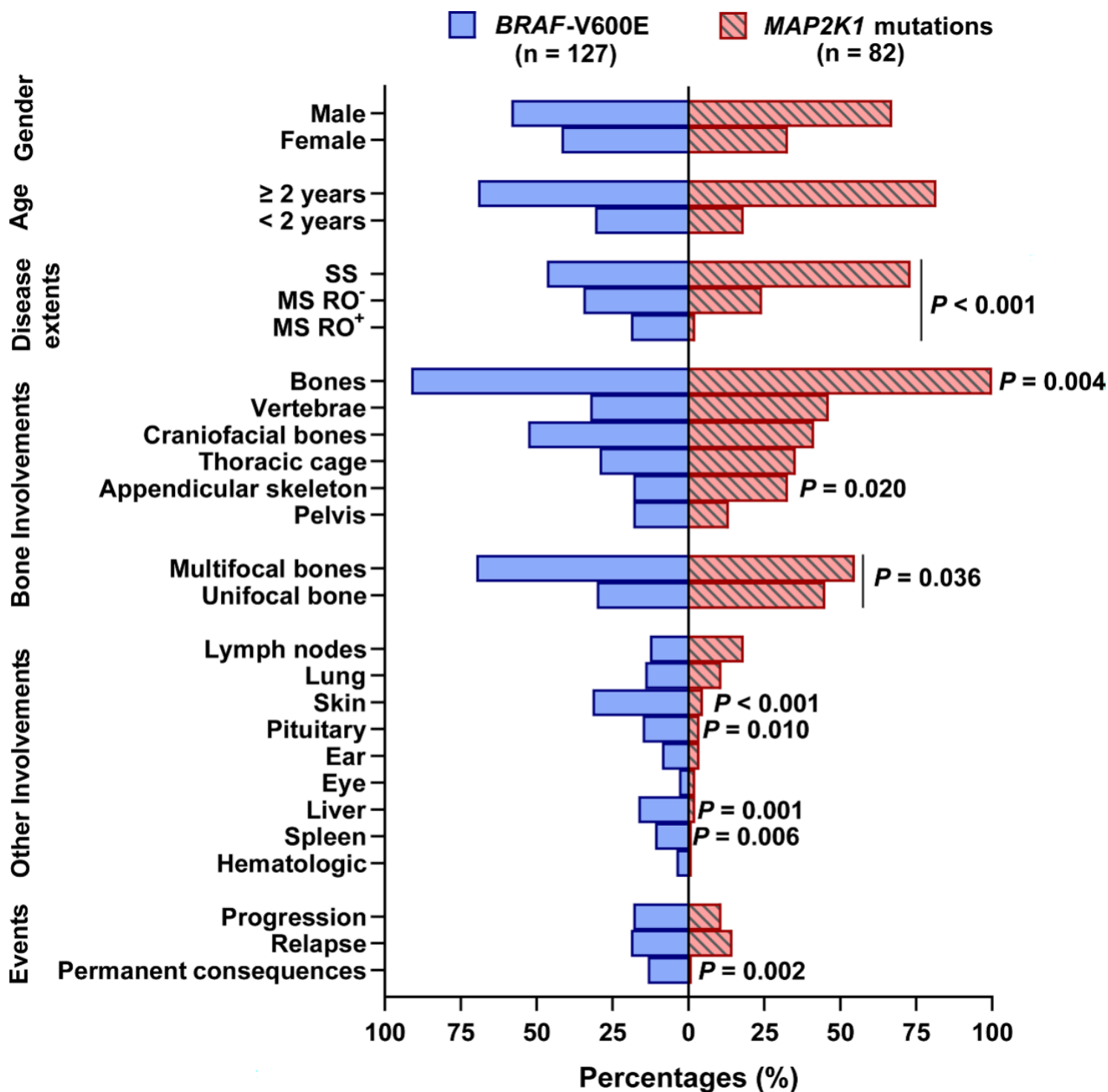


Figure 2

Comparison of clinical characteristics and outcomes between children with *BRAF*-V600E-mutated and *MAP2K1*-mutated LCH

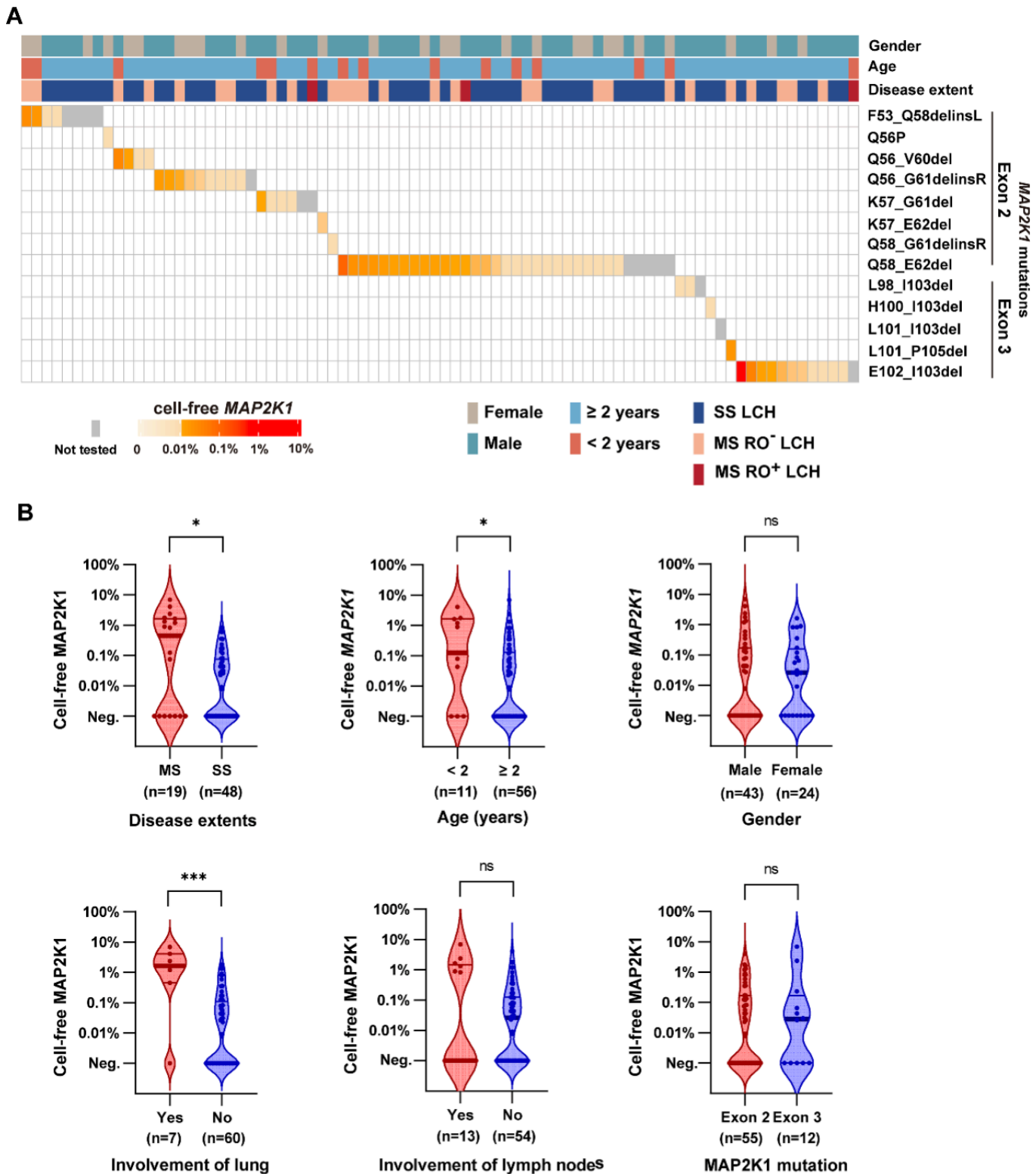


Figure 3

Baseline plasma cfMAP2K1 levels in children with tissue-confirmed MAP2K1-mutated LCH. **A** Distribution of baseline plasma cfMAP2K1 levels according to clinical features and tissue MAP2K1 mutation sites; **B** Comparison of cfMAP2K1 levels among subgroups stratified by disease extent, age, gender, lung involvement, lymph node involvement, and mutation exon. SS, single-system; MS, multisystem; RO, risk organ; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

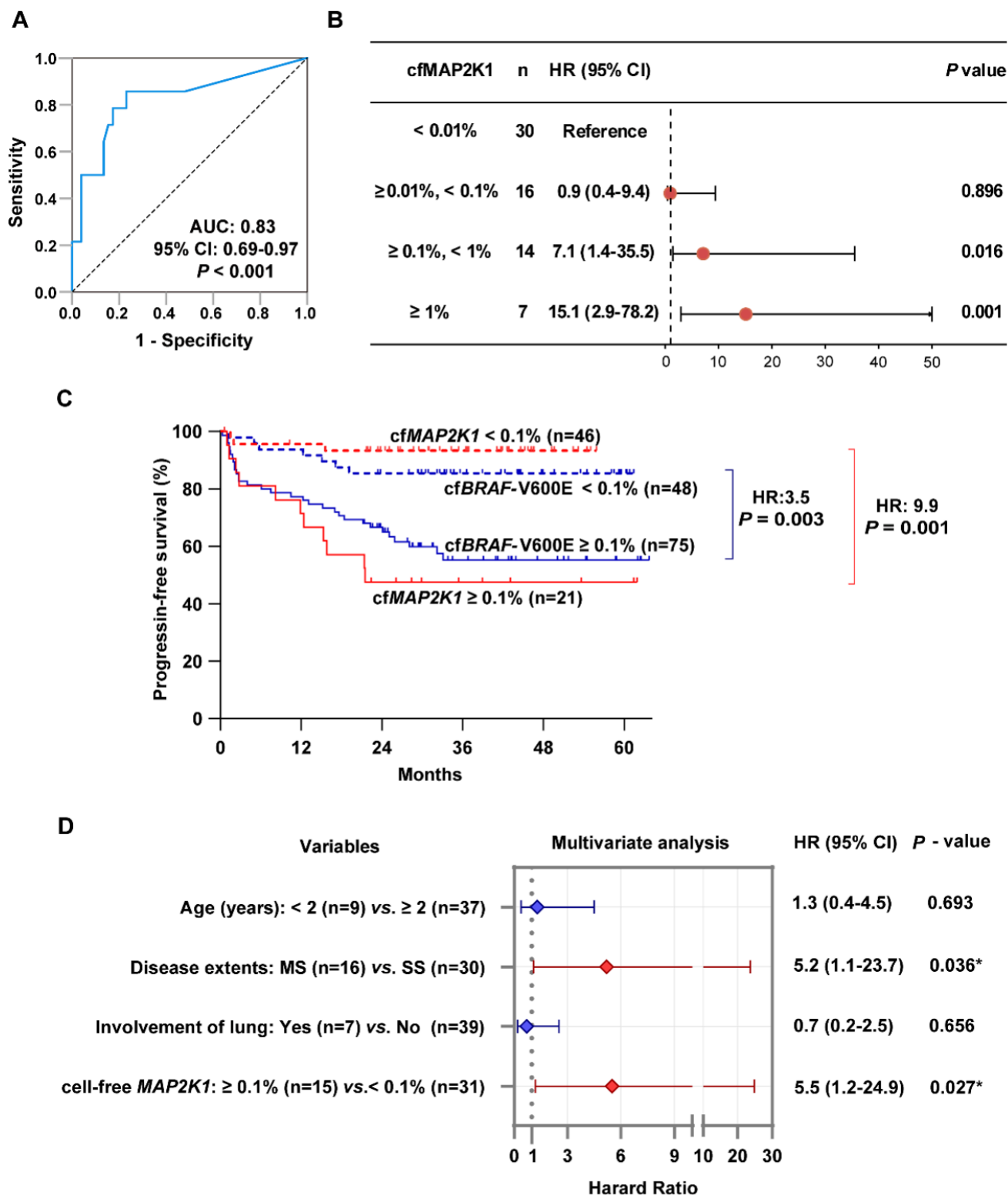


Figure 4

Prognostic significance of baseline cfMAP2K1 levels in children with MAP2K1-mutated LCH. **A** ROC curve evaluating the association between cfMAP2K1 levels and subsequent progression or relapse; **B** Hazard ratios for progression or relapse according to different baseline cfMAP2K1 burden groups; **C** Kaplan-Meier analysis of PFS according to baseline cfMAP2K1 levels in the MAP2K1-mutated cohort and cfBRAF-V600E levels in the BRAF-V600E-mutated cohort; **D** Multivariable Cox regression analysis for PFS

in patients with *MAP2K1*-mutated LCH who received first-line therapy. ROC, receiver operating characteristic; AUC, area under the curve; HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; SS, single-system; MS, multisystem

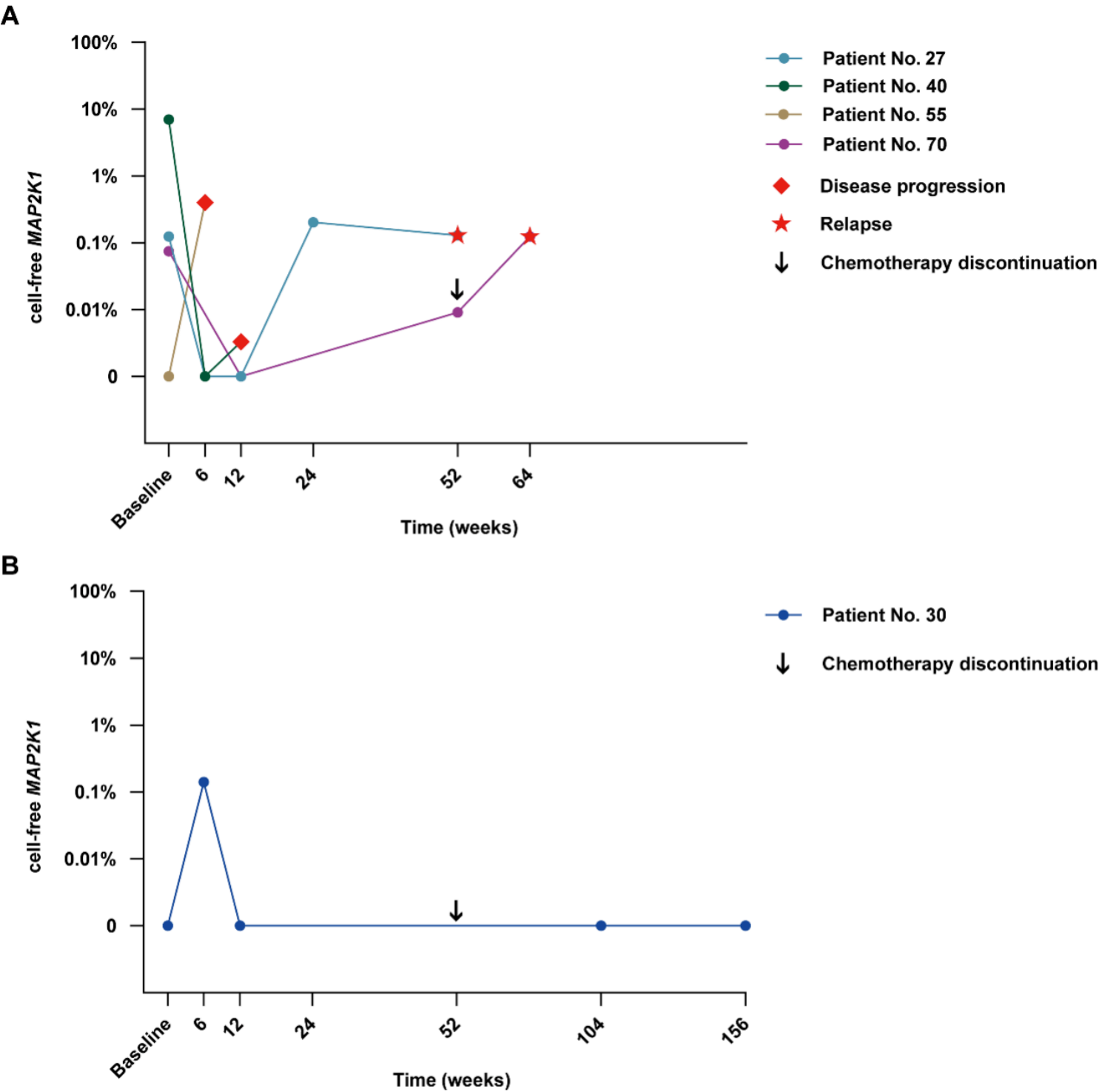


Figure 5

Monitoring of plasma cf*MAP2K1* levels during first-line therapy in children with *MAP2K1*-mutated LCH. **A** Dynamic changes in cf*MAP2K1* levels in patients with detectable cf*MAP2K1* during first-line therapy who subsequently developed disease progression or relapse; **B** A patient with transient cf*MAP2K1*

positivity during first-line therapy who remained in continuous remission after chemotherapy discontinuation. Red diamonds indicate disease progression, red stars indicate relapse, and black arrows indicate chemotherapy discontinuation

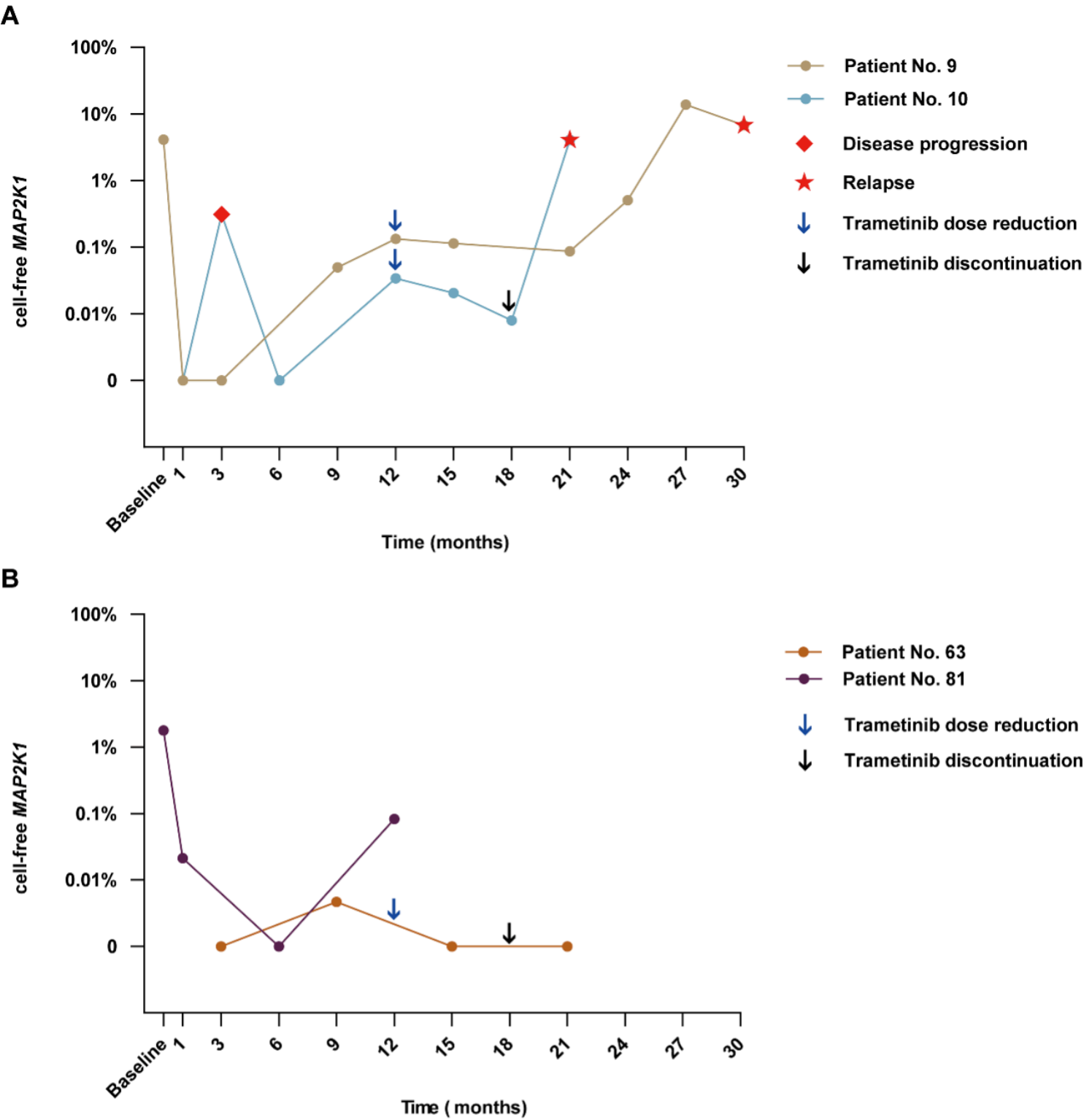


Figure 6

Serial monitoring of plasma cfMAP2K1 levels during trametinib treatment in children with MAP2K1-mutated LCH. **A** Dynamic changes in cfMAP2K1 levels in patients with increasing or high-level cfMAP2K1

before or at disease progression/relapse during trametinib treatment; **B** Patients with transient or low-level *cfMAP2K1* positivity who remained in sustained remission. Red diamonds indicate disease progression, red stars indicate relapse, blue arrows indicate trametinib dose reduction, and black arrows indicate trametinib discontinuation

Supplementary Files

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