Cell-free *BRAF*^{V600E} levels predict progression-free survival in children with Langerhans cell histiocytosis treated with dabrafenib and maintenance chemotherapy

Langerhans cell histiocytosis (LCH), a rare inflammatory myeloid neoplasm, predominantly affecting young children, exhibits constitutive MAPK pathway activation.[1] Approximately 80% of patients carry MAPK-pathway mutations, most commonly in *BRAF*.[2] Myeloid progenitors harboring *BRAF* mutations drive the pathology of LCH and circulate in both high- and low-risk patients.[3] Although MAPK-targeted agents produce rapid clinical improvement in refractory or recurrent LCH, they fail to eradicate the mutated clone, leading to high relapse rates after treatment discontinuation.[4,5] The long-term effects of BRAF/MEK inhibitors in children remain incompletely defined, underscoring the need to better assess progression/relapse risk.

Circulating cell-free (cf) *BRAF*^{V600E} is a promising biomarker and has been linked to reactivation risk in pediatric LCH.[6,7] However, the peripheral blood levels of *BRAF*^{V600E} did not consistently track with clinical response during MAPK inhibition.[8] To examine how longitudinal cf*BRAF*^{V600E} dynamics relate to outcomes under targeted therapy, we analyzed the data from the BCH-LCH-Dab trial (*Chinese Clinical Trial Registry Identifier: ChiCTR2000032844*) - a prospective study of dabrafenib plus maintenance chemotherapy in relapsed/refractory *BRAF*^{V600E}-positive pediatric LCH. We enrolled 37 consecutive eligible patients between November 2016 and December 2019 (median age, 2.2 years; range, 0.3-5.3 years; *Online Supplementary Figure S1*). Twenty-eight patients (75.7%) had multisystem risk-organ-positive (MS RO$^+$) disease and nine (24.3%) had MS RO$^-$ diseases; baseline disease activity score medians were 8 (range, 1-20) and 3 (range 1-6), respectively.[9]

Patients received oral dabrafenib (2 mg/kg twice daily) for 12 months, followed by 6 months of maintenance chemotherapy (vindesine 3 mg/m² intravenous every 3 weeks; prednisone 40 mg/m² orally days 1-5 every 3 weeks; 6-mercaptopurine 50 mg/m² orally daily). Treatment responses were classified longitudinally as non-active disease (NAD), active disease - better (ADB), active disease - stable (ADS), or active disease - worse (ADW).[10] The primary endpoint was objective response rate (ORR) including complete response (CR) and partial response (PR).[11,12] Secondary endpoints were progression-free survival (PFS) and overall survival (OS). This study was approved by the Beijing Children's Hospital Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the guardians of the patients involved.

The ORR was 81.1% (95% confidence interval [CI]: 67.8-94.3%), including 16 CR and 14 PR; seven were non-responders (6 RO$^+$, 1 RO$^-$). With a median follow-up of 72.9 months, two patients died of liver cirrhosis, eight experienced disease progression during dabrafenib, and 12 relapsed after remission (Figure 1). Permanent consequences (PC) were present pre-treatment in eight patients (7 diabetes insipidus, 1 cirrhosis); eleven new sequalaes occurred post-treatment (3 neurodegeneration, 1 diabetes insipidus, 1 cirrhosis, 1 growth delay). The 5-year OS rate was 94.5±3.8%; PFS rate 45.3±8.3%; and relapse/reactivation rate 42.2±0.9%.

Blood samples were collected prospectively at baseline (before dabrafenib); during dabrafenib treatment at 1, 3, 6, and 9-12 months; during maintenance at 3-6 months; and 3 months to 5 years after treatment cessation. Additional samples were obtained at progression, relapse, or onset of PC. Plasma cfDNA was extracted and normalized to a 10 ng-input for *BRAF*^{V600E} detection by droplet digital polymerase chain reaction. The assay limit of detection (LOD) was 0.05%, established by serial dilutions of Tru-Q7 reference DNA (Horizon Discovery) into wild-type genomic donor DNA. An analysis of 231 plasma samples from 37 patients revealed that cf*BRAF*^{V600E} was detected at baseline in 78.4% of patients (29/37). Median levels declined from 0.75% to 0.09% after 3 months of dabrafenib (P=0.006) and continued to fall (*Online Supplementary Figure S2A*). At 9 to 12 months, 50% of patients had detectable cf*BRAF*^{V600E} (median 0.04%); 46.4% were positive during maintenance (median 0). Among 18 patients who progressed or relapsed, 77.8% (14/18) were cf*BRAF*^{V600E}-positive (median 0.64%), not significantly different from baseline (P=0.614). At PC, cf*BRAF*^{V600E} was mostly negative, with only 20.0% (2/10) testing positive. Categorical analysis showed similar trends (*Online Supplementary Figure S2B*).

Median cf*BRAF*^{V600E} values did not differ between responders and non-responders at any single time point (all P>0.05). Categorically, non-responders had a higher prevalence of cf*BRAF*^{V600E} ≥ 1% at 6 months (100% *vs*. 13.8%; P=0.039). Receiver-operating-characteristic (ROC) analysis showed increasing prognostic discrimination of cf*BRAF*^{V600E}: baseline area under the curve (AUC) was 0.551 (P=0.594), 3 months 0.705 (P=0.042), 6 months 0.790 (P=0.006), and 9-12 months 0.719 (P=0.042). Accuracy was highest during maintenance (AUC=0.854; P=0.002) (Figure 2A). In Cox analysis, cf*BRAF*^{V600E} ≥1% during dabrafenib and ≥0.05% during maintenance were associated with increased risk of progression/relapse (Figure 2B). Kaplan-Meier analysis using these phase-specific cut-offs showed lower PFS with higher cf*BRAF*^{V600E} (*Online Supplementary Figure S3A*). As-

sociations were consistent in MS RO^{+} LCH across all time points, as well as in MS RO^{-} LCH at 3 months and during maintenance (*Online Supplementary Figure S3B*).

Additionally, changes of cf*BRAF*^{V600E} from baseline to 3 months were informative of PFS (P<0.001; Figure 3A). Patients negative at both time points had the best outcomes. A ≥50% reduction was associated with better PFS than <50% reduction (P=0.009). Increases in cf*BRAF*^{V600E} were associated with the worst prognosis. All six patients with <50% reduction or an increase ultimately progressed or relapsed. Conversion status at 3 months was likewise prognostic (P=0.004; Figure 3B): remaining negative or converting to negative was favorable; persistent positivity or conversion to positive was unfavorable.

Post-cession cf*BRAF*^{V600E} monitoring was available for 12 of 17 patients in remission, covering 3 months to 5 years. Four

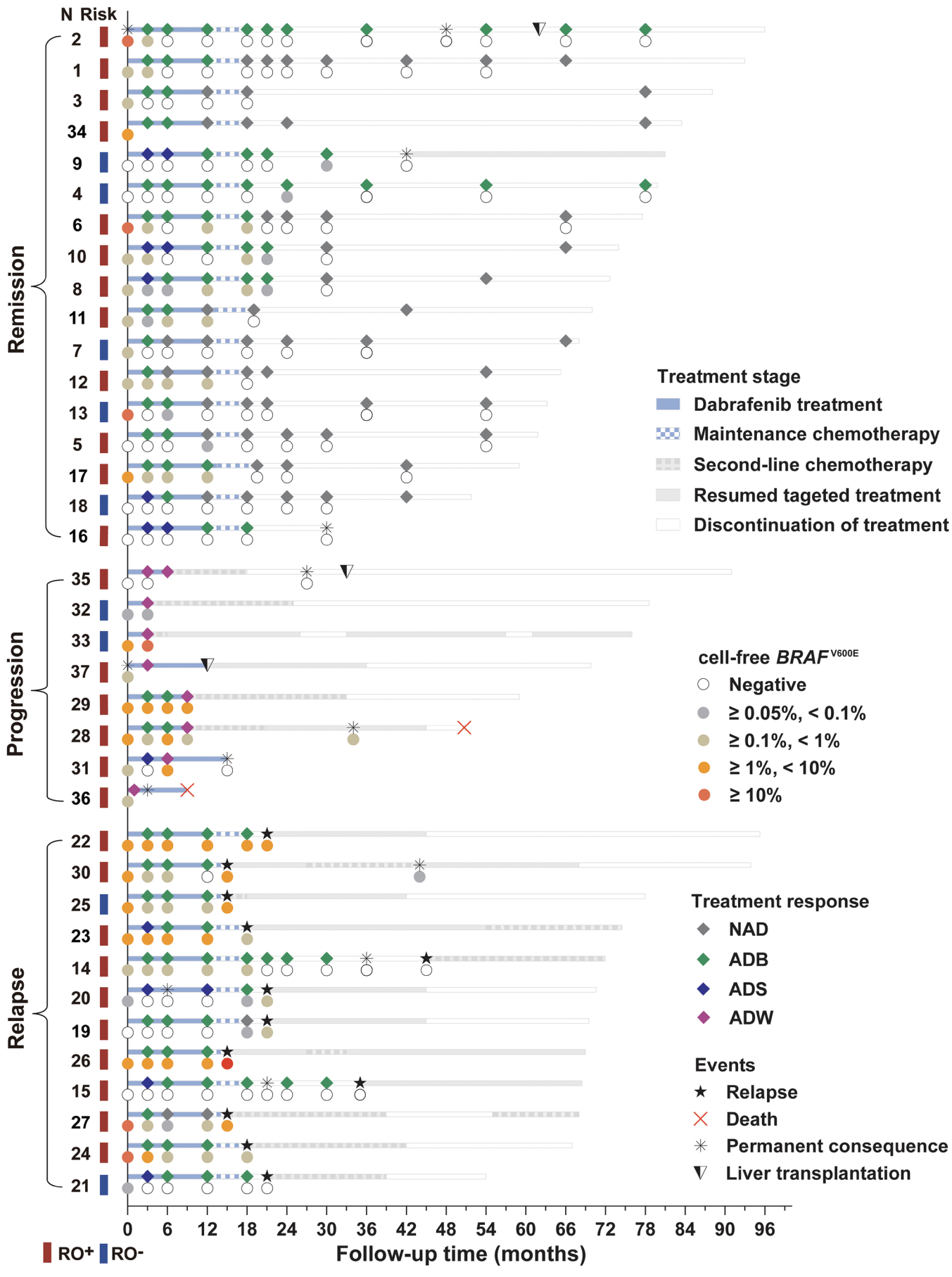

Figure 1. A swimmer plot depicting the duration since the initiation of dabrafenib treatment, alongside cell-free *BRAF*^{V600E} status, treatment response, and clinical outcomes of patients. RO: risk organ; NAD: non-active disease; ADB: active disease - better; ADS: active disease - stable; ADW: active disease - worse.

A

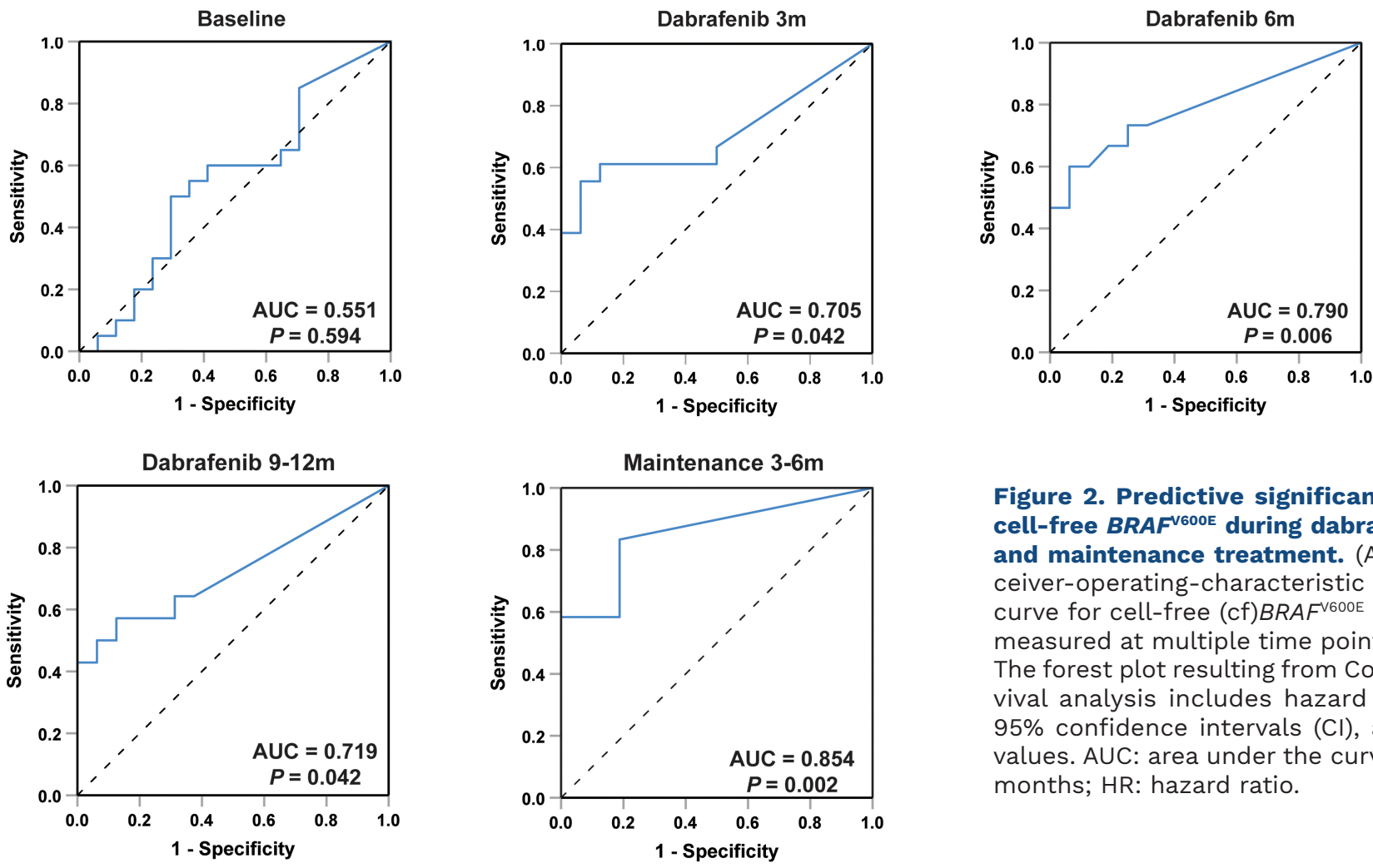

Figure 2. Predictive significance of cell-free *BRAF*^{V600E} during dabrafenib and maintenance treatment. (A) Receiver-operating-characteristic (ROC) curve for cell-free (cf)*BRAF*^{V600E} levels measured at multiple time points. (B) The forest plot resulting from Cox survival analysis includes hazard ratio, 95% confidence intervals (CI), and *P* values. AUC: area under the curve; m: months; HR: hazard ratio.

B

Groups	N	HR (95% CI)	P value
cf*BRAF*^{V600E} at baseline			
< 0.05%	8	Reference	
≥ 0.05%, < 0.1%	3	4.079 (0.811-20.504)	0.088
≥ 0.1%, < 1%	11	1.023 (0.227-4.605)	0.976
≥ 1%, < 10%	10	3.573 (0.930-13.732)	0.064
≥ 10%	5	1.065 (0.177-6.402)	0.945
cf*BRAF*^{V600E} at dabrafenib 3m			
< 0.05%	14	Reference	
≥ 0.05%, < 0.1%	3	0.843 (0.101-7.031)	0.873
≥ 0.1%, < 1%	11	1.093 (0.332-3.603)	0.656
≥ 1%	6	5.394 (1.595-18.244)	0.008
cf*BRAF*^{V600E} at dabrafenib 6m			
< 0.05%	15	Reference	
≥ 0.05%, < 0.1%	3	1.458 (0.162-13.086)	0.736
≥ 0.1%, < 1%	7	2.843 (0.708-11.417)	0.141
≥ 1%	6	17.483 (4.153-73.595)	< 0.001
cf*BRAF*^{V600E} at dabrafenib 9-12m			
< 0.05%	15	Reference	
≥ 0.05%, < 1%	11	1.531 (0.441-5.315)	0.502
≥ 1%	4	9.026 (2.148-37.928)	0.003
cf*BRAF*^{V600E} at maintenance 3-6m			
< 0.05%	15	Reference	
≥ 0.05%, < 1%	8	6.696 (1.288-34.802)	0.024
≥ 1%*	5	-	

patients (patient number: 4, 8, 9, 10) had a single low-level positive (0.05-0.19%) within 1 year that reverted to negative on subsequent tests; none relapsed. Of 12 relapses overall, six occurred after cessation; four relapsed around 3 months, three (patient number: 19, 20, 22) of whom had concomitant cf*BRAF*^{V600E} positivity (0.58%, 0.26%, and 3.26%, respectively), whereas two (patient number: 14, 15) later relapses (27 and 17 months) remained negative both off therapy and at relapse (Figure 1).

Recent reports suggest that combining MAPK inhibitors with chemotherapy may achieve sustained remissions in children with LCH.[13,14] In our relapsed/refractory, *BRAF*^{V600E}-positive cohort treated with dabrafenib plus maintenance chemotherapy, the 5-year relapse/reactivation estimate was 42.2%. By contrast, the randomized LCH-III trial in newly diagnosed MS LCH with RO involvement reported a 5-year reactivation risk of 27% after 12 months of vinblastine/prednisone-based therapy.[15] Given differences in the populations, therapy, and study designs, these rates are not directly comparable; nevertheless, the contrast supports ongoing efforts to refine the intensity, composition, and duration of combination regimens to further reduce relapse risk.

ROC analysis indicated increasing discrimination of cf*BRAF*^{V600E} over time, from non-predictive at baseline to clinically informative in this cohort during maintenance therapy. These performance estimates should be interpreted in light of the small, single-center, non-randomized design and will require external validation. Although earlier time-point AUC of 0.705-0.719 reached statistical significance, the ~30% misclassification risk argued for integrating clinical parameters rather than rely on cf*BRAF*^{V600E} alone. The phase-specific thresholds differed - 1% during dabrafenib

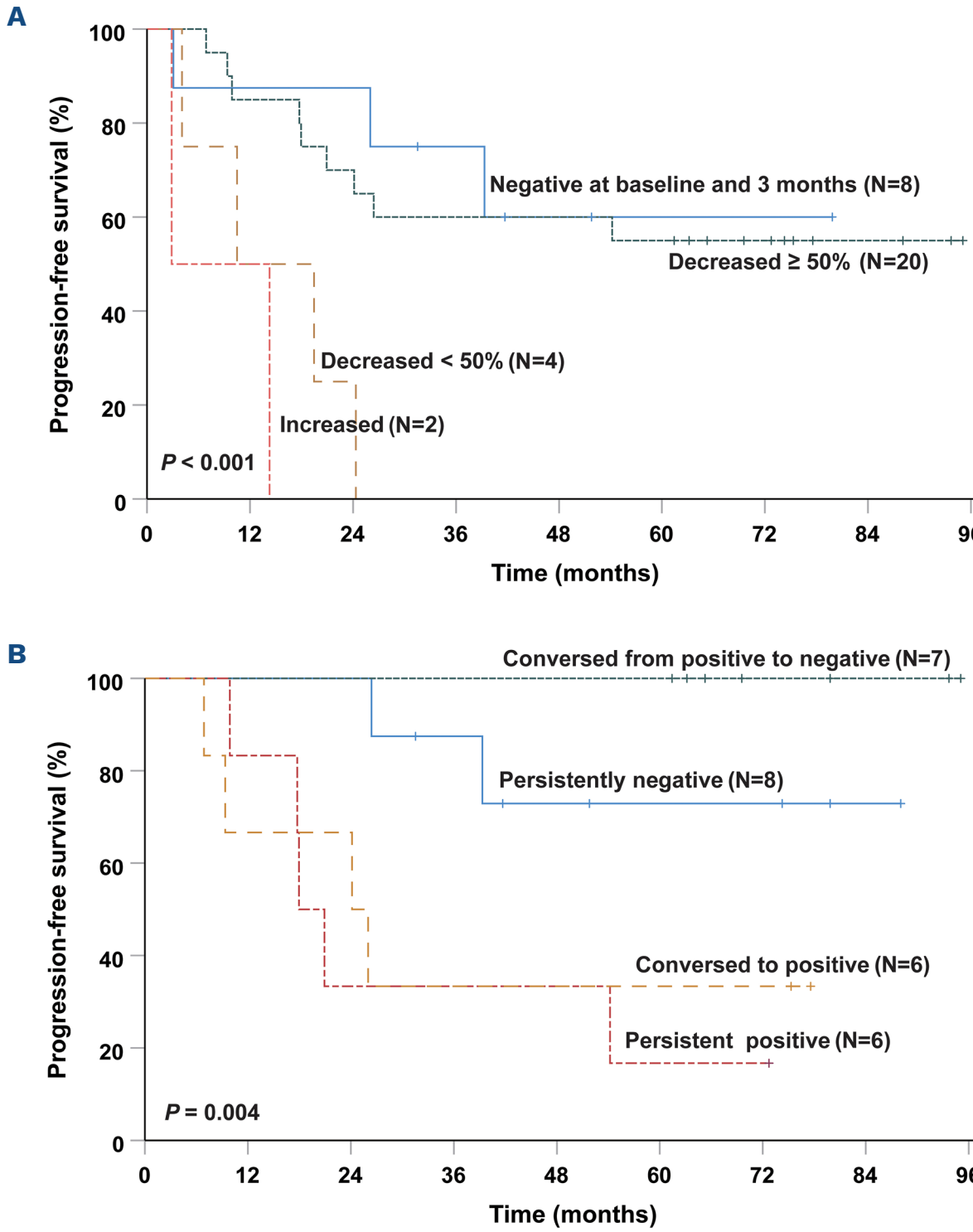

Figure 3. Kaplan-Meier curves depicting progression-free survival based on dynamic changes in cell-free *BRAF*^{V600E}. (A) Based on dynamics of cell-free (cf)*BRAF*^{V600E} levels from baseline to 3 months of dabrafenib treatment. (B) According to changes in cf*BRAF*^{V600E} from 3 months of dabrafenib to the maintenance period.

and 0.05% during maintenance. Because the latter coincides with the assay LOD, its use should be considered provisional and confirmed in larger, multicenter studies that account for pre-analytical variables and platform heterogeneity.

Early declines in cf*BRAF*^{V600E} may index treatment responsiveness to dabrafenib, whereas absolute thresholds may reflect residual disease burden. It is important to note the potential contradiction in the prognostic implications of these two metrics. The limited size of our cohort restricted our ability to perform multivariate modeling. Larger studies are necessary to validate whether combining these metrics further enhances prognostication. Until then, clinical interpretation should consider both metrics in context. A significant reduction in cf*BRAF*^{V600E} may mitigate the risk associated with temporarily high absolute levels, whereas a lack of a meaningful decline could outweigh any favorable absolute values observed at time points.

Off-therapy cf*BRAF*^{V600E} monitoring may add context to risk after treatment cessation. Positivity shortly after cessation may coincide with early events, whereas sustained negativity does not exclude late reactivation. Overall, cf*BRAF*^{V600E} alone to forecast late relapse appeared limited in our cohort. Given the small numbers and irregular sampling, these observations are exploratory and support standardized off-therapy time points in larger cohorts. The baseline negativity of cf*BRAF*^{V600E} in 21.6% of tissue-confirmed cases likely reflected prior therapies reducing the tumor burden below detection. Among patients negative at baseline who later relapsed, potential explanations include selection of non-V600E subclones, clonal evolution leading to the emergence of other mutations, or immune-mediated suppression of cfDNA shedding. These hypotheses require validation through studies that pair cfDNA with tissue samples. Neurodegenerative LCH events were few in our cohort (N=3), precluding definitive conclusions about the predictive role of cf*BRAF*^{V600E}. Future work incorporation cerebrospinal fluid with serum biomarkers is warrants.

In this single-center cohort of relapsed/refractory pediatric LCH, longitudinal cf*BRAF*^{V600E} dynamics were associated with PFS under dabrafenib plus maintenance chemotherapy. These observations are hypothesis-generating and may inform individualized management and future trial design. Confirmation in adequately powered, multicenter prospective studies is required before clinical implementation.

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Disclosures

No conflicts of interest to disclose.

Contributions

CL analyzed the data and wrote the manuscript. WD, ZYZ, and YJF were involved in data analysis. WCJ and ZZJ performed the experiments. LWJ, ZQ, GJ, FZS, WWQ and DJJ contributed to sample collection. MHH, LHY and WTY made clinical contributions. ZR and LZG designed the research, supervised the study, and revised the manuscript. All authors were involved in the final approval of the mauscript.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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