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Title page

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Running heads

cf*BRAF*-V600E in pediatric LCH

Type of article

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Authors' contribution

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, DJJ contributed to sample collection. MHH, LHY, and WTY made clinical contributions. ZR and LZG 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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Conflict of interests

The authors declare no potential conflicts of interest.

Data-sharing statement

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

Ethics approval statement

This study was approved by the Beijing Children's Hospital Institutional Review Board and was conducted in accordance with The Declaration of Helsinki.

Patient consent statement

Informed consent was obtained from the patient's guardians.

Trial registration

Chinese Clinical Trial Registry identifier: ChiCTR2000032844

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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 (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; Supplementary Figure S1). Twenty-eight (75.7%) had multisystem risk-organ-positive (MS RO^+) disease and nine (24.3%) had MS RO^- diseases; Baseline DAS 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 six months of maintenance chemotherapy (vindesine 3 mg/m² IV 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 nonactive 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 (PCs) were present pre-treatment in eight patients (7 diabetes insipidus, 1 cirrhosis); 11 new sequalae's 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 PCR (ddPCR). 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% (29/37). Median levels declined from 0.75% to 0.09% after 3 months of dabrafenib (P = 0.006) and continued to fall (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 (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 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 (Supplementary Figure S3A). Associations were consistent in MS RO^+ LCH across all time points, as well as in MS RO^- LCH at 3 months and during maintenance (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 patients (Nos. 4, 8, 9, 10) had a single low-level positive (0.05-0.19%) within one year that reverted to negative on subsequent tests; none relapsed. Of 12 relapses overall, six occurred after cessation; four relapsed around 3 months, three (Nos. 19, 20, 22) of whom had concomitant cf*BRAF*-V600E positivity (0.58%, 0.26%, and 3.26%, respectively), whereas two (Nos. 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 AUCs 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 and 0.05% during maintenance. Because the latter coincides with the assay LOD, its use should be considered provisional and confirmed in larger, multi-center 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 cfBRAF-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 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, multi-center prospective studies is required before clinical implementation.

References

[1] Rodriguez-Galindo C, Allen CE. Langerhans cell histiocytosis. Blood. 2020;135(16):1319-1331.

[2] Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood. 2010;116(11):1919-1923.

[3] Xiao Y, van Halteren AGS, Lei X, et al. Bone marrow-derived myeloid progenitors as driver mutation carriers in high- and low-risk Langerhans cell histiocytosis. Blood. 2020;136(19):2188-2199.

[4] Donadieu J, Larabi IA, Tardieu M, et al. Vemurafenib for Refractory Multisystem Langerhans Cell Histiocytosis in Children: An International Observational Study. J Clin Oncol. 2019;37(31):2857-2865.

[5] Eckstein OS, Visser J, Rodriguez-Galindo C, Allen CE, NACHO-LIBRE Study Group. Clinical responses and persistent BRAF V600E+ blood cells in children with LCH treated with MAPK pathway inhibition. Blood. 2019;133(15):1691-1694.

[6] Héritier S, Hélias-Rodzewicz Z, Lapillonne H, et al. Circulating cell-free BRAFV600E as a biomarker in children with Langerhans cell histiocytosis. Br J Haematol. 2017;178(3):457-467.

[7] Cui L, Zhang L, Ma HH, et al. Circulating cell-free BRAF V600E during chemotherapy is associated with prognosis of children with Langerhans cell histiocytosis. Haematologica. 2020;105(9):e444-447.

[8] Eder SK, Schwentner R, Ben Soussia P, et al. Vemurafenib acts as a molecular on-off switch governing systemic inflammation in Langerhans cell histiocytosis.

Blood Adv. 2022;6(3):970-975.

[9] Donadieu J, Piguet C, Bernard F, et al. A new clinical score for disease activity in Langerhans cell histiocytosis. Pediatr Blood Cancer. 2004;43(7):770-776.

[10] Donadieu J, Bernard F, van Noesel M, et al. Cladribine and cytarabine in refractory multisystem Langerhans cell histiocytosis: results of an international phase 2 study. Blood. 2015;126(12):1415-1423.

[11] Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-247.

[12] Karri V, Lin H, Velazquez J, et al. Clinical, radiological and molecular responses to combination chemotherapy with MAPK pathway inhibition in relapsed and refractory Langerhans cell histiocytosis. Br J Haematol. 2024;204(5):1882-1887.

[13] Evseev D, Osipova D, Kalinina I, et al. Vemurafenib combined with cladribine and cytarabine results in durable remission of pediatric BRAF V600E-positive LCH. Blood Adv. 2023;7(18):5246-5257.

[14] Lei J, Wang W, Lin D, et al. Vemurafenib combined with chemotherapy achieved sustained remission in pediatric LCH: a multi-center observational study. J Cancer Res Clin Oncol. 2024;150(1):12.

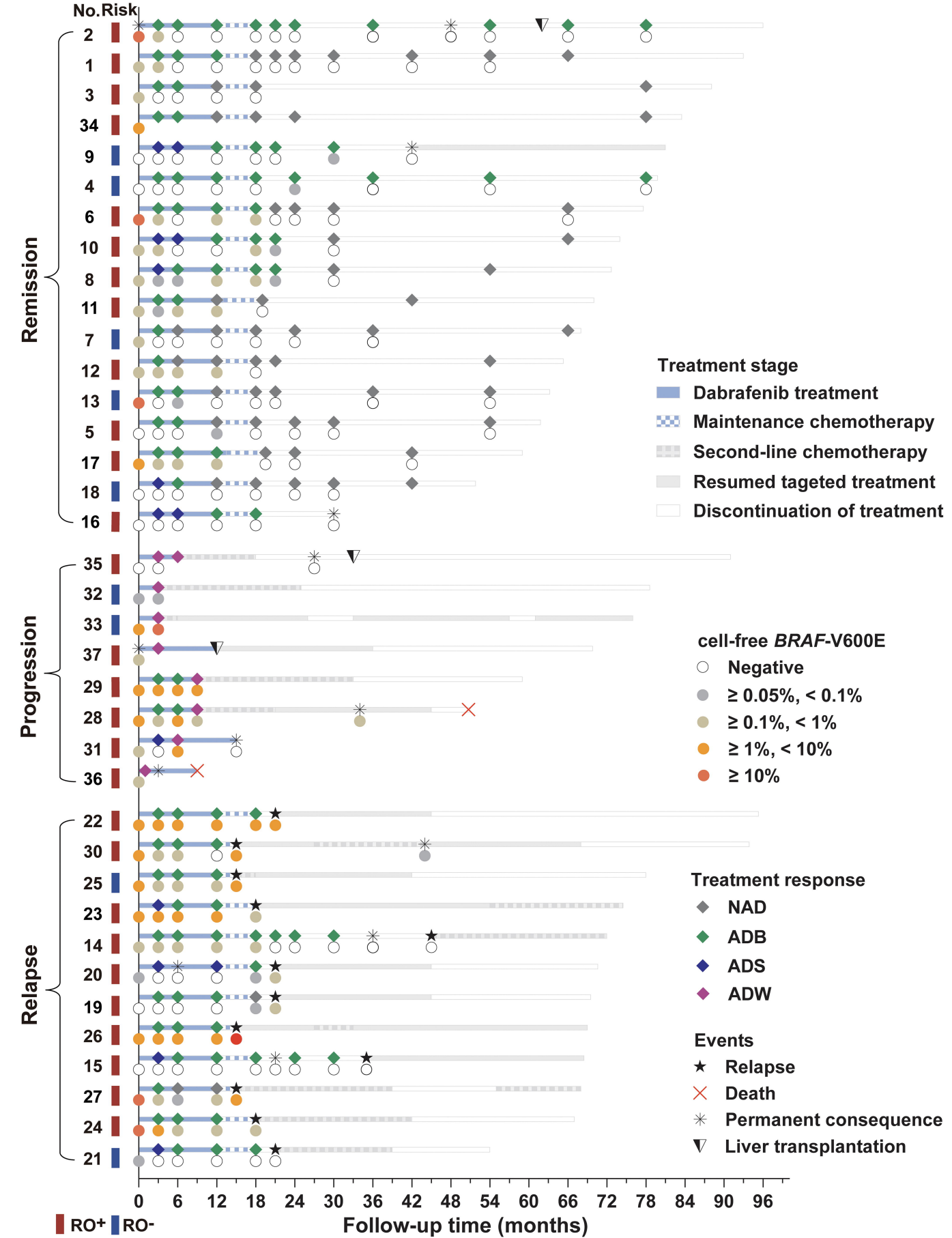
[15] Gadner H, Minkov M, Grois N, et al. Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. Blood. 2013;121(25):5006-5014.

Figure legends

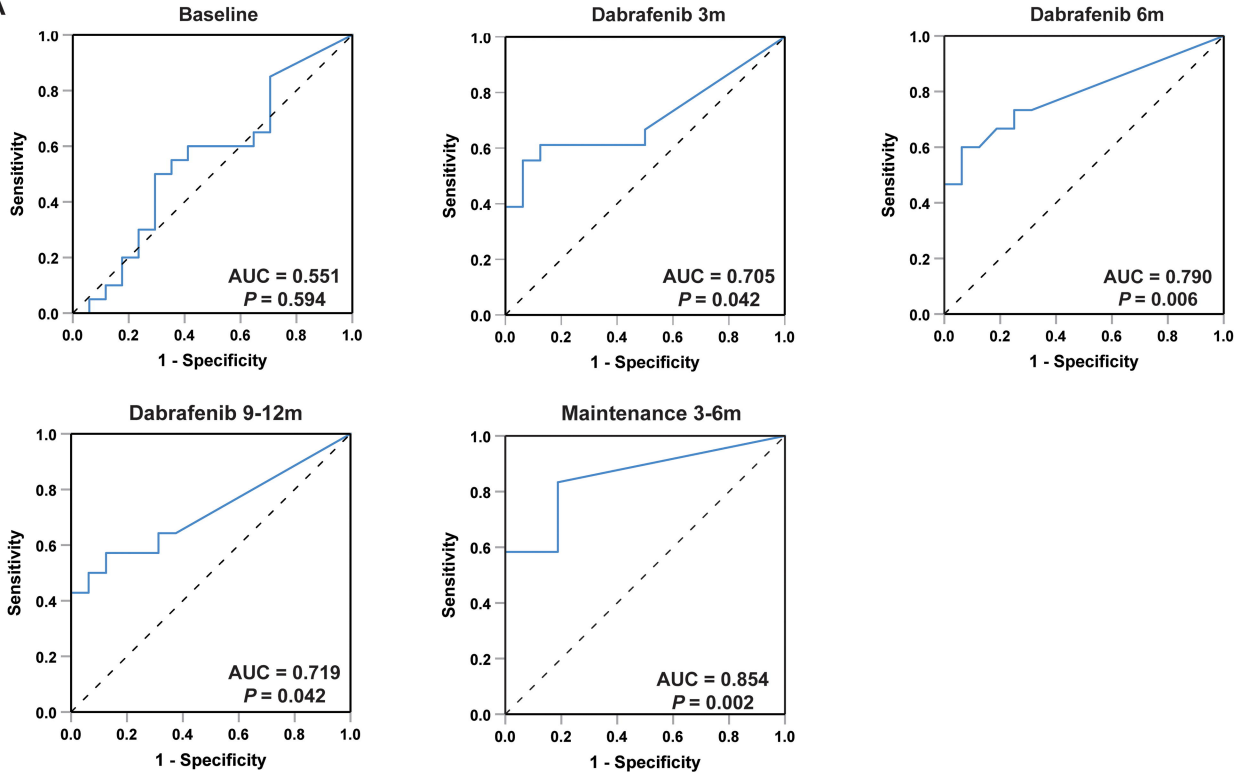
Figure 1 A swimmer plot depicting the duration since the initiation of dabrafenib treatment, alongside cf*BRAF*-V600E status, treatment response, and clinical outcomes of patients. Abbreviations: RO, risk organ; NAD, nonactive disease; ADB, active disease-better; ADS, active disease-stable; ADW, active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

Figure 2 Predictive significance of cell-free (cf) *BRAF*-V600E during dabrafenib and maintenance treatment. (A) ROC curve for 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.

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

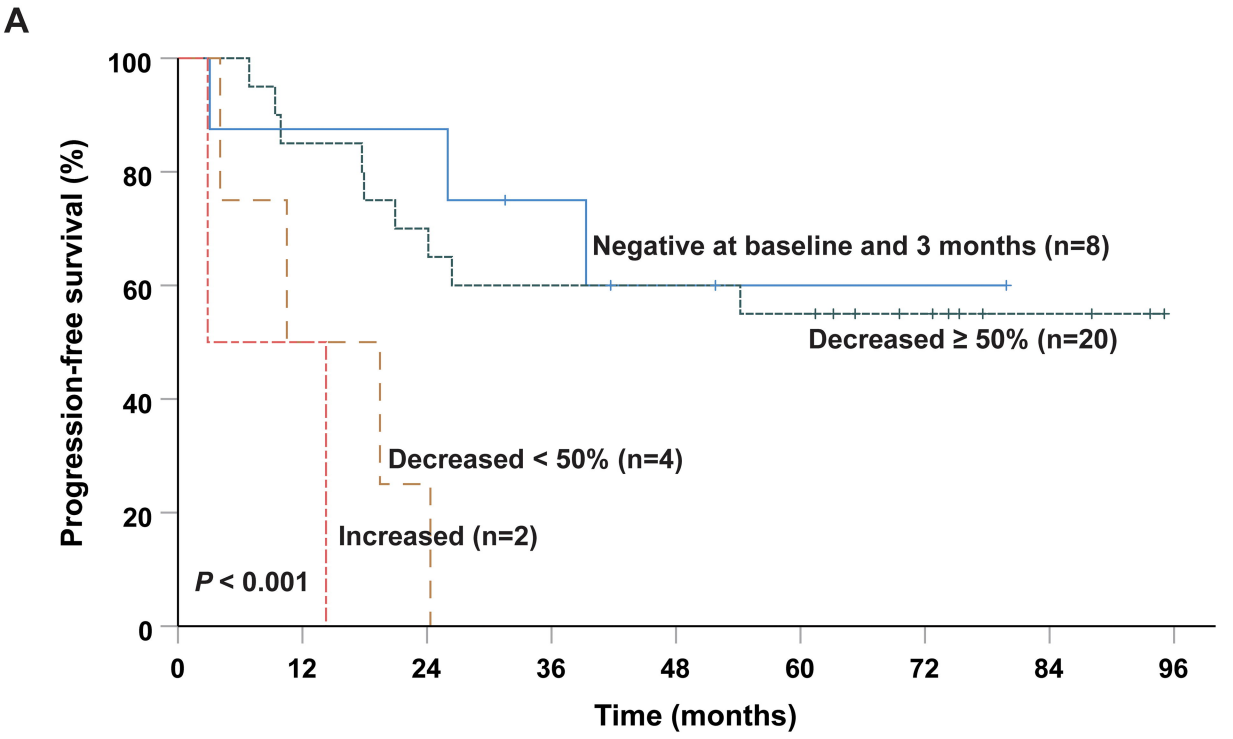

No.Risk
Remission
2
1
3
34
9
4
6
10
8
11
7
12
13
5
17
18
16
Progression
35
32
33
37
29
28
31
36
Relapse
22
30
25
23
14
20
19
26
15
27
24
21
Treatment stage
Dabrafenib treatment
Maintenance chemotherapy
Second-line chemotherapy
Resumed tageted treatment
Discontinuation of treatment
cell-free BRAF-V600E
Negative
≥ 0.05%, < 0.1%
≥ 0.1%, < 1%
≥ 1%, < 10%
≥ 10%
Treatment response
NAD
ADB
ADS
ADW
Events
Relapse
Death
Permanent consequence
Liver transplantation
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96
Follow-up time (months)
RO+
RO-

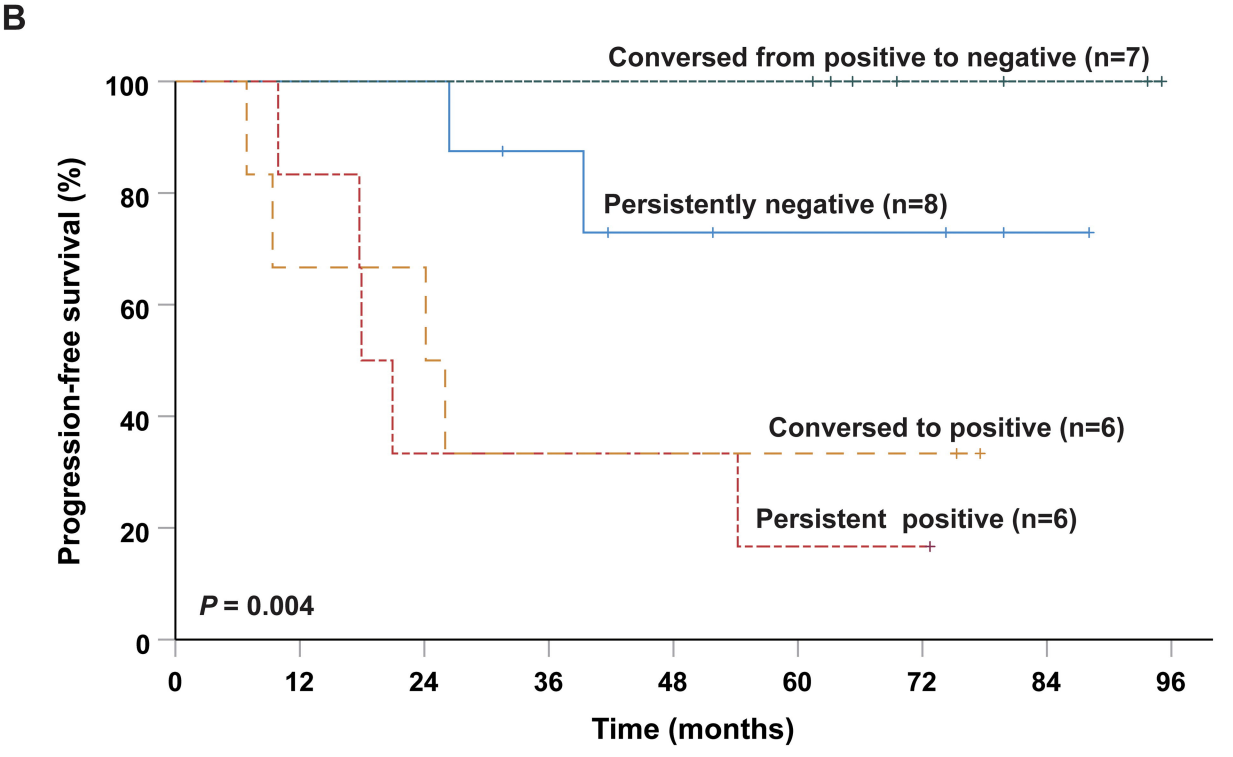
A

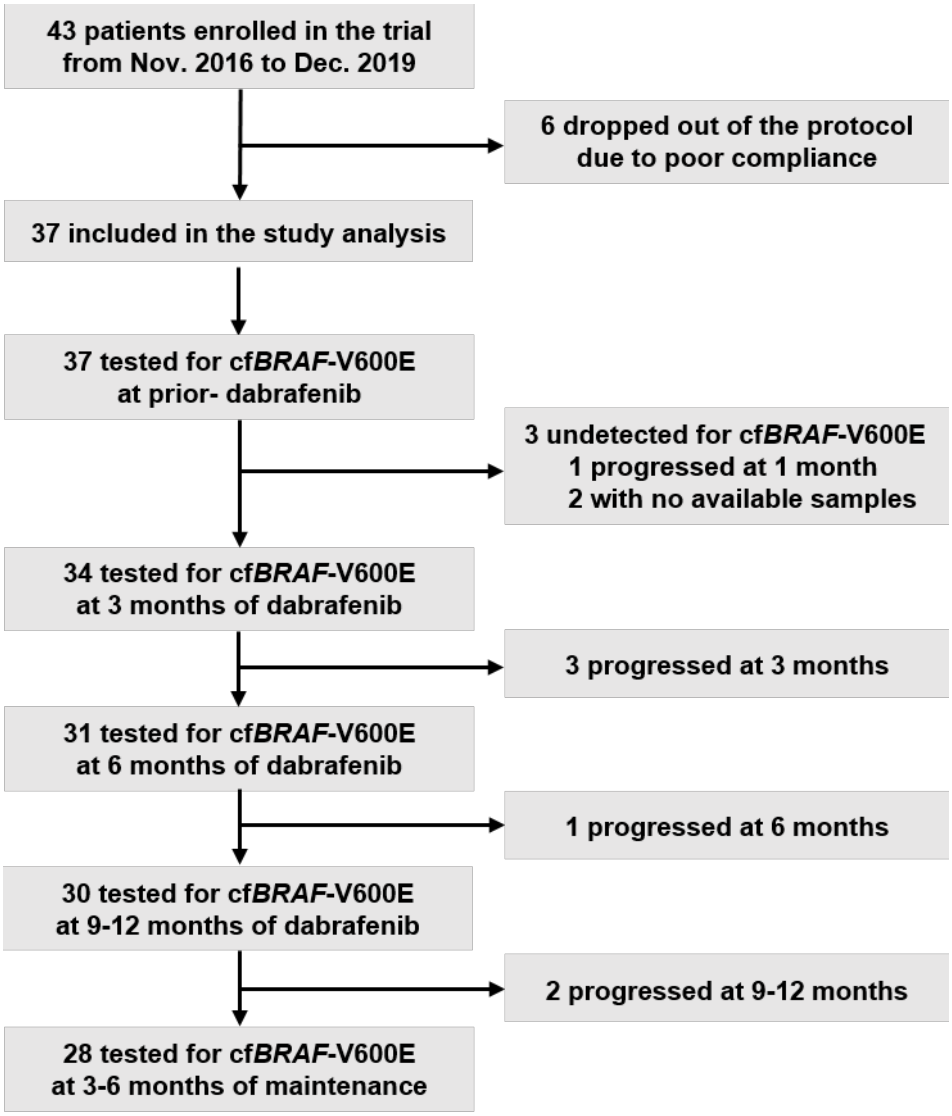


B

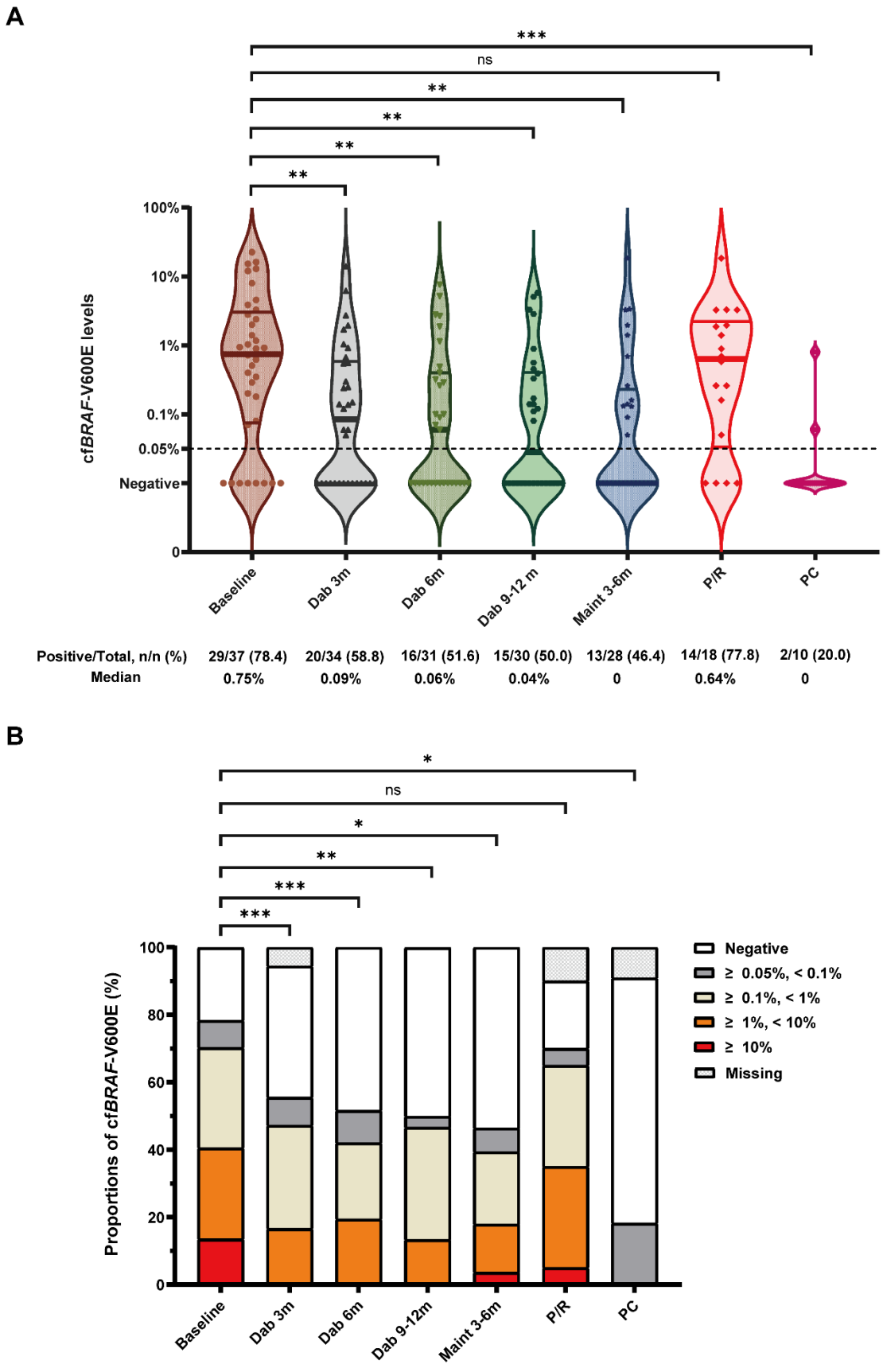
Groups	n	HR (95% CI)	P value
cfBRAF-V600E at baseline			
< 0.05%	8	Reference	
≥ 0.05%, < 0.1%	3	4.079 (0.811-20.504)	0.088
≥ 0.1%, < 1%	11	1.023 (0.227-4.605)	0.976
≥ 1%, < 10%	10	3.573 (0.930-13.732)	0.064
≥ 10%	5	1.065 (0.177-6.402)	0.945
cfBRAF-V600E at dabrafenib 3m			
< 0.05%	14	Reference	
≥ 0.05%, < 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
cfBRAF-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
cfBRAF-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
cfBRAF-V600E at maintenance 3-6m			
< 0.05%	15	Reference	
≥ 0.05%, < 1%	8	6.696 (1.288-34.802)	0.024
≥ 1%*	5	-	


A
100
80
60
40
20
0
Progression-free survival (%)
Negative at baseline and 3 months (n=8)
Decreased ≥ 50% (n=20)
Decreased < 50% (n=4)
Increased (n=2)
P < 0.001
0
12
24
36
48
60
72
84
96
Time (months)


B
Conversed from positive to negative (n=7)
Persistently negative (n=8)
Conversed to positive (n=6)
Persistent positive (n=6)
100
80
60
40
20
0
Progression-free survival (%)
P = 0.004
0
12
24
36
48
60
72
84
96
Time (months)

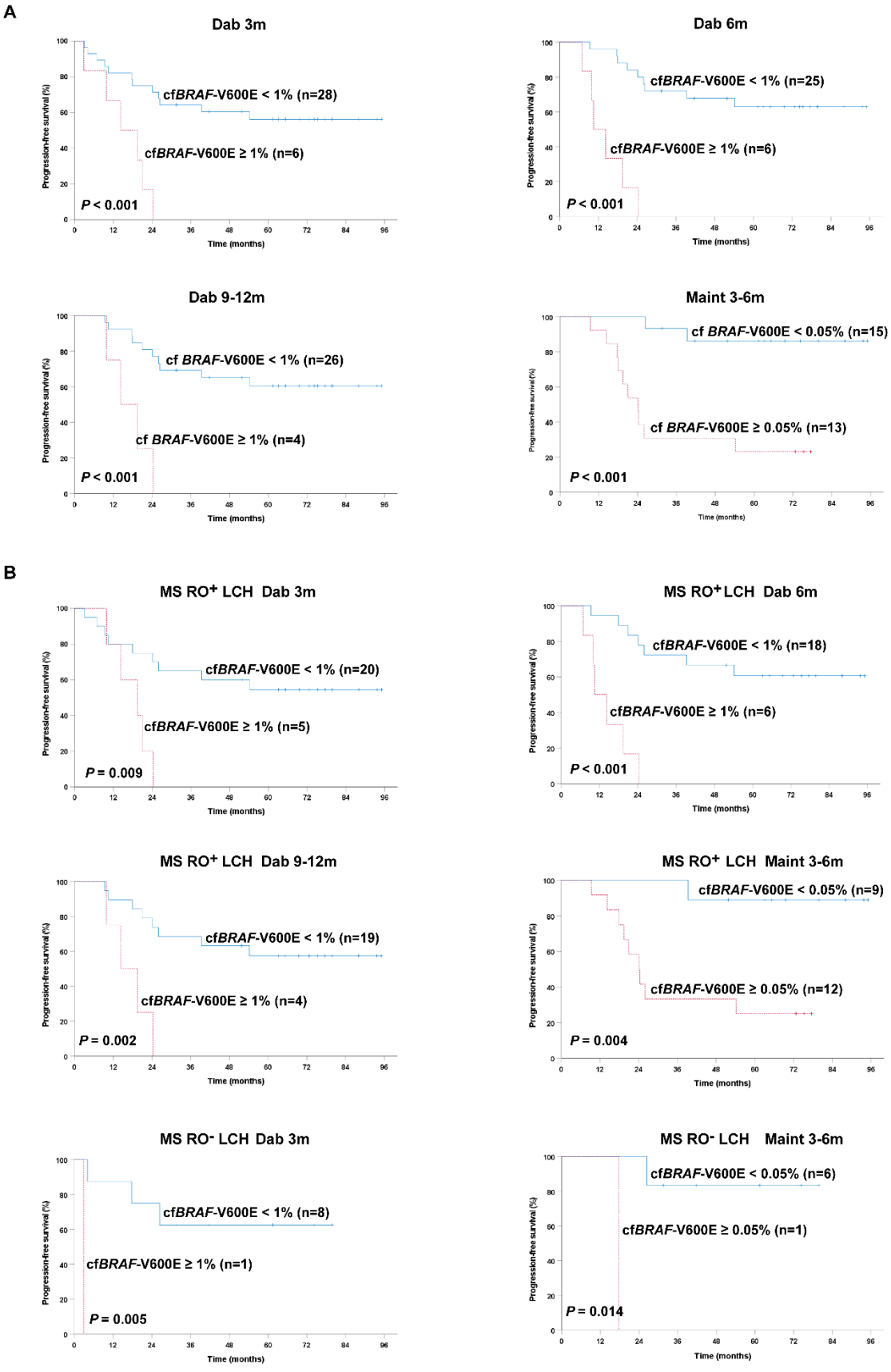


Supplementary Figure S1 The flow diagram of the patients analyzed and excluded.



Supplementary Figure S2 Levels of cf*BRAF*-V600E during treatment of dabrafenib and

maintenance, and at the time of events. (A) Data is presented as a violin plot, showcasing medians and quantiles. (B) A Bar plot displays the percentages of cf*BRAF*-V600E as a categorical variable. *P* values: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant, $P > 0.05$. Abbreviations: Dab, dabrafenib; Maint, maintenance; P/R, progression/relapse; PC, permanent consequence.



Supplementary Figure S3 Kaplan–Meier curves illustrating progression-free survival

(PFS) based on levels of cf*BRAF*-V600E. (A) Comparison of PFS at various time points during treatment. (B) Comparison of PFS in patients with MS RO+ or MS RO- LCH. (Note: The comparison of PFS according to cf*BRAF*-V600E at 6 months and 9-12 months in MS RO- patients is not presented here due to a lack of patients with cfBRAF-V600E ≥ 1% in this group)