Liquid Biopsy Monitoring in *BRAF* V600E–Mutated Patients With Non–Small Cell Lung Cancer Treated With Dabrafenib Plus Trametinib: The Prospective, Multicenter LiBRA Study (GOIRC-03-2020)

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ABSTRACT

PURPOSE Dabrafenib plus trametinib is a standard first-line treatment for *BRAF* V600E–mutated non–small cell lung cancer (NSCLC). The LiBRA study aimed to explore the role of liquid biopsy in detecting and monitoring *BRAF* V600E mutation, assessing its potential to predict treatment response and emerging resistance.

MATERIALS AND METHODS This prospective multicenter study enrolled patients with *BRAF* V600E–mutated NSCLC treated with first-line dabrafenib plus trametinib. Plasma samples were collected at baseline (t0), after 4 weeks (t1), and longitudinally until disease progression (PD). *BRAF* V600E was monitored by digital droplet PCR (ddPCR). Next-generation sequencing (NGS) was performed at t0 and PD to identify resistance mechanisms.

RESULTS Forty patients were enrolled. Dabrafenib plus trametinib achieved an overall response rate of 42.5%, with a median progression-free survival (PFS) and overall survival (OS) of 8.3 and 21.1 months, respectively. At t0, *BRAF* V600E was detectable by ddPCR in 24 (62%) of 39 patients. Among 21 shedders evaluated at t1, 17 (81%) cleared the mutation. Higher baseline *BRAF* V600E allele frequency was associated with shorter PFS (hazard ratio [HR], 1.09, $P = .013$) and OS (HR, 1.10, $P = .010$), whereas clearance at t1 correlated with longer PFS (8.3 *v* 1.4 months, $P < .001$) and OS (10.5 *v* 2.2 months, $P < .001$). Biological PD anticipated radiologic/clinical PD by a median of 4.9 weeks (IQR, 1.4-9.8). Baseline *EGFR* and *MET* CNVs were associated with shorter PFS and OS. Resistance mechanisms at PD included *NRAS*, *KRAS*, *TP53* mutations and *MET*, *EGFR*, *ERBB2* CNVs.

CONCLUSION The LiBRA study supports liquid biopsy as a prognostic and monitoring tool in *BRAF* V600E–mutated NSCLC undergoing targeted therapy.

ACCOMPANYING CONTENT

Data Supplement

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INTRODUCTION

BRAF V600E–mutated non–small cell lung cancer (NSCLC) represents a distinct molecular subtype accounting for approximately 2%-4% of lung adenocarcinoma. This molecular alteration defines a subset of oncogene-addicted tumors amenable to targeted therapies.[1]

The combination of dabrafenib (a BRAF inhibitor) and trametinib (a MEK inhibitor) is currently one of the standard first-line treatments for patients with advanced *BRAF* V600E–mutated NSCLC, demonstrating durable responses and an acceptable safety.[2,3] Encorafenib plus binimetinib has also received European Medicines Agency approval as a targeted option in this setting.[4,5]

Tissue biopsy remains the gold standard for tumor diagnosis and molecular profiling through next-generation sequencing (NGS) in NSCLC. However, it is invasive and often limited by insufficient tumor material, especially due to tumor heterogeneity or challenging sampling.

Liquid biopsy, a minimally invasive technique that detects circulating tumor DNA (ctDNA) in plasma, constitutes a valid

CONTEXT

Key Objective

Could a liquid biopsy provide early insights into treatment response in patients with *BRAF* V600E–mutated non–small cell lung cancer (NSCLC) receiving first-line dabrafenib plus trametinib? This study evaluated whether liquid biopsy can predict clinical outcomes and identify emerging resistance.

Knowledge Generated

Forty patients were enrolled. Dabrafenib plus trametinib achieved a 42.5% overall response rate, with a median progression-free survival (PFS) of 8.3 months and overall survival (OS) of 21.1 months. At baseline, *BRAF* V600E was detectable in 62% of patients, and 81% of shedding patients cleared the mutation after 4 weeks of treatment. Higher baseline *BRAF* V600E allele frequency was associated with shorter PFS and OS, and biological progression anticipated radiologic/clinical progression by a median of 4.9 weeks.

Relevance

These findings suggest that baseline circulating tumor DNA (ctDNA) levels may identify patients with more aggressive disease. Monitoring ctDNA dynamics can provide early indications of progression, potentially enabling early intervention.

complementary diagnostic tool. Several studies have demonstrated a high concordance between molecular genotyping obtained from ctDNA and tissue biopsy in NSCLC, supporting the clinical utility of liquid biopsy for molecular diagnosis.[6-8] Moreover, liquid biopsy allows serial sampling over time to assess tumor dynamics, minimal residual disease, and early resistance.[9]

Although plasma monitoring of oncogenic driver mutations is well established in *EGFR*-mutated NSCLC, where it could play a role in guiding treatment and detecting resistance mechanisms to EGFR-TKIs,[9,10] its application in *BRAF* V600E–mutated NSCLC remains less defined. To address this gap, we conducted a prospective, multicenter study (LiBRA) aimed at evaluating the role of liquid biopsy in detecting and longitudinally monitoring *BRAF* V600E mutation in patients with advanced *BRAF* V600E–mutated NSCLC treated with first-line dabrafenib plus trametinib.

MATERIALS AND METHODS

Study Design

This is a prospective, multicenter study conducted in 25 Italian Centers between February 2022 and May 2025. The study included patients with advanced NSCLC carrying *BRAF* V600E mutation, detected on diagnostic tissue biopsy, eligible to receive first-line dabrafenib plus trametinib as per standard clinical practice. Patients received oral dabrafenib (150 mg twice daily) plus oral trametinib (2 mg once daily) in continuous 28-day cycles until disease progression (PD) or unacceptable toxicity.

The primary objective of the study was to describe the *BRAF* V600E blood levels in ctDNA, in terms of variant allelic frequency (VAF). Secondary objectives were (1) to study putative resistance mechanisms to dabrafenib plus trametinib on ctDNA and (2) to describe clinical outcomes of dabrafenib plus trametinib in real-life population.

The study obtained the ethical approval by local Ethics Committee (Protocol number 143/2021, September 15, 2021), and all patients provided informed consent.

Sample Collection and ctDNA Analysis

Plasma samples were collected at baseline (t0, before starting treatment), after 4 weeks (t1), every 4 weeks during the first 16 weeks of therapy, and then subsequently every 8 weeks until PD.

ctDNA analysis was performed at the central laboratory (Department of Medical Oncology, University Hospital of Parma). Cell-free DNA (cfDNA) was extracted from 4 mL of plasma using the MagCore Circulating DNA Large Volume Kit (Diatech Labline), according to the manufacturer's instructions. Detection and quantification of the *BRAF* V600E mutation (p.V600E, c.1799T>A) were conducted using the QX200 Droplet Digital PCR (ddPCR) system (Bio-Rad, Hercules, CA). For ddPCR, VAF was defined as the ratio of mutant droplets to total droplets.

NGS analysis was performed on baseline and PD plasma samples. cfDNA was isolated from 3-4 mL of plasma, and libraries were prepared using the AVENIO ctDNA Expanded Kit (Roche, Basel, Switzerland; Data Supplement, Table S1). Sequencing was conducted on the Illumina NextSeq 550Dx (Illumina, San Diego, CA), generating approximately 30 million reads per sample. This workflow ensured high coverage and enabled detection of somatic variants with a VAF as low as 0.1%. For NGS, VAF was defined as the proportion of variant-supporting reads at a given genomic position.

The copy number variation (CNV) score summarizes amplification confidence based on log2 copy number ratio and its standard error: values >5 indicate high confidence, 0-5 borderline, and negative scores indicate absence of amplification.

Patients with *BRAF* V600E VAF ≥ 0.1% were defined as shedders. ctDNA clearance was defined as undetectable *BRAF* V600E mutation at t1 in baseline shedders.

Clinical Outcomes

A total-body CT scan for response assessment was performed every 12 weeks in accordance with clinical practice, until treatment discontinuation.

Primary resistance was intended as PD as best response or stable disease (SD) for <3 months after initial treatment.[11] The overall response rate (ORR) was defined as the proportion of patients who achieved either a complete response or partial response (PR) as their best overall response at the radiologic evaluation, based on Investigators' assessment according to standard RECIST v1.1 criteria.[12] Disease control rate (DCR) was defined as the proportion of patients who achieved a complete response, PR, or SD as their best overall response.[12] Duration of treatment (DoT) was defined as the time from treatment start until treatment discontinuation, whatever the cause. Progression-free survival (PFS) was defined as the time interval from treatment initiation to either PD or death, whichever occurred first. Overall survival (OS) was defined as the time from treatment start to death from any cause.

Statistical Analysis

Statistical analyses were performed using Jamovi (version 2.3.21) and R studio (version 2024.12.1+563). The median follow-up was calculated with the reverse Kaplan-Meier method from the start of dabrafenib and trametinib. Differences in categorical variables were examined using Fisher's exact test and the Chi-square test, as appropriate. Continuous variables were compared using the Mann-Whitney U test for unpaired data and the Wilcoxon signed-rank test for paired data, as appropriate. Median DoT, PFS, and OS were estimated using the Kaplan-Meier method and analyzed with Cox proportional hazards models. Ninety-five percent CI (95% CI) were calculated using Wald's method. Differences in survival between groups were assessed by the log-rank test. Association between VAF and PFS/OS risks was assessed using Cox proportional hazards regression models with restricted cubic splines to capture potential nonlinear effects, including VAF and sum of diameters of target lesions as continuous covariates. Multivariable Cox proportional hazards regression analyses were performed to explore the impact of *BRAF* V600E at baseline and clearance at t1 on PFS and OS, in patients evaluated for ctDNA clearance, adjusting for clinical statistically significant variables at univariable analyses ($P \leq .05$). All P values were two-sided, and $P \leq .05$ was considered statistically significant.

RESULTS

Patient Characteristics and Clinical Outcomes

A total of 41 patients with *BRAF* V600E–mutated NSCLC were enrolled from February 2022 to May 2024. One patient was excluded from the analysis due to lack of confirmation of the *BRAF* V600E mutation. Baseline patient characteristics are summarized in Table 1. The median age of the patients was 71 years (IQR, 65-77), and the majority were male (60%). Most of the patients had a history of smoking (70%). Adenocarcinoma was the predominant histology (87.5%), and many patients were diagnosed at stage IV (87.5%). PD-L1 expression had a median value of 43% (IQR, 5%-63%), with 42.5% of patients showing PD-L1 ≥50%.

Treatment with dabrafenib plus trametinib achieved an ORR of 42.5% (95% CI, 27 to 58) and a DCR of 70% (95% CI, 56 to 84; Table 2; Data Supplement, Fig S1). The median DoT was 8.1 months (95% CI, 4.6 to Not Reached [NR]). After a median follow-up of 17.8 months (95% CI, 14.0 to 21.8), the median PFS was 8.3 months (95% CI, 4.4 to NR; Data Supplement, Fig S2a) and the median OS was 21.1 months (95% CI, 8.6 to NR; Data Supplement, Fig S2b).

A total of 25 (63%) patients experienced PD, and the most frequent site of PD was the lung (n = 6, 24%), followed by the bone (n = 5, 20%). At the time of PD, 11 (44%) of 25 patients received further therapy: chemotherapy (n = 7), immunotherapy (n = 3), or radiotherapy (n = 1). Any grade adverse events occurred in 27 (68%) patients, and 11 (28%) patients experienced grade 3 to 4 adverse events (Data Supplement, Tables S2 and S3).

A CONSORT-style flow diagram summarizes patient enrollment, exclusions, and longitudinal plasma sample collection for ctDNA analyses at each time point (Data Supplement, Fig S3).

ddPCR Analysis

Baseline plasma samples were collected from 39 (98%) of 40 patients. At t0, *BRAF* V600E was detected in 24 (62%) of 39 patients (median VAF 1.1%; IQR, 0.6-5.2; Fig 1A).

At t1, 35 (89.7%) of 39 patients collected plasma samples, and *BRAF* V600E was detected in four (11%) of 35 patients (median VAF 1.1%; IQR 0.7-1.3; Fig 1A).

Among 24 baseline shedding patients, 21 (88%) were evaluated for ctDNA clearance, achieved in 17 (81%) of 21 patients. A significant variation in VAF was observed at t1 compared with t0 (median difference −1.1%, $P < .001$; Data Supplement, Fig S4).

TABLE 1. Baseline Patient Characteristics

Baseline Patient Characteristic	Value (N = 40)
Age, median years (IQR)	71 (65-77)
Gender, No. (%)	
Female	16 (40)
Male	24 (60)
Smoking status, No. (%)	
Never smokers	12 (30)
Former smokers	18 (45)
Current smokers	10 (25)
ECOG PS, No. (%)	
0	12 (30)
1	20 (50)
≥2	8 (20)
Metastatic sites, No. (%)	
Lymph nodes	20 (50)
Bone	10 (25)
Pleura	8 (20)
Liver	6 (15)
Brain	5 (13)
Adrenal gland	5 (13)
No. of metastatic sites, median (IQR)	4 (3-4)
Diagnostic sample, No. (%)	
Cytologic	10 (25)
Histologic	30 (75)
Histology, No. (%)	
Adenocarcinoma	35 (87.5)
NSCLC NOS	5 (12.5)
TNM staging 8th ed. at diagnosis, No. (%)	
IIB	2 (5)
IIIA	2 (5)
IIIB	1 (2.5)
IVA	21 (52.5)
IVB	14 (35)
PD-L1 expression, median (IQR)	43% (5%-63%)
<1%	4 (10)
1%-49%	19 (47.5)
≥50%	17 (42.5)
BRAF molecular diagnosis, No. (%)	
NGS	27 (67.5)
RT-PCR	12 (30)
Sanger sequencing	1 (2.5)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; NSCLC NOS, non–small cell lung cancer not otherwise specified; NGS, next-generation sequencing; RT-PCR, real-time polymerase chain reaction.

TABLE 2. Clinical Outcomes

Clinical Outcomes	Value (N = 40)
Best response, No. (%)	
CR	0 (0)
PR	17 (42.5)
SD	11 (27.5)
PD	12 (30)
ORR, % (95% CI)	42.5 (27 to 58)
DCR, % (95% CI)	70 (56 to 84)
mFU, months (95% CI)	17.8 (14.0 to 21.8)
Number of cycles, median (IQR)	7 (4-14)
mDoT, months (95% CI)	8.1 (4.6 to NR)
mPFS, months (95% CI)	8.3 (4.4 to NR)
mOS, months (95% CI)	21.1 (8.6 to NR)

Abbreviations: CR, complete response; DCR, disease control rate; mDoT, median duration of treatment; mFU, median follow-up; mOS, median overall survival; mPFS, median progression-free survival; NR, not reached; ORR, overall response rate; PD, progression of disease; PR, partial response; SD, stable disease.

At PD, *BRAF* V600E was detected in 11 (52%) of 21 patients (median VAF 3.2%; IQR, 0.5-18.4; Fig 1A). Among patients who experienced biological PD (n = 10), defined as a ctDNA re-positivity after clearance or increase in VAF compared with the previous sample, all showed radiologic and/or clinical PD. The median interval from biological PD to radiologic and/or clinical PD was 4.9 weeks (IQR, 1.4-9.8; Fig 2).

NGS Analysis

NGS analysis was performed at baseline in 39 patients and at PD in 21 patients with available plasma samples.

At t0, *BRAF* V600E was detected in 26 (67%) of 39 patients (median VAF 0.8%; IQR, 0.4-5.0). Of note, two patients were shedders at NGS even though ddPCR was negative (Fig 1B).

At the time of PD, *BRAF* V600E was detected in 10 (48%) of 21 patients (median VAF 2.9%; IQR, 0.8-19.8). Of note, one patient was nonshedder at NGS although ddPCR was positive (Fig 1B).

Overall concordance between ddPCR and NGS was 95% (37/39) at t0 and 95% (20/21) at PD.

At t0, co-mutations were observed in 19 (73%) of 26 shedding patients and were independent of *BRAF* V600E VAF (P = .664). The alterations involved cell cycle regulation (n = 15, 58%), the PI3K pathway (n = 7, 27%), the MAPK pathway (n = 3, 12%), metabolic differentiation (n = 3, 12%), the Hedgehog pathway (n = 2, 8%), and the EGFR/ERBB pathway (n = 1, 4%). CNVs at t0 were detected in seven (27%) of 26 shedding patients (Fig 3A) and were more likely to be found in patients with higher baseline *BRAF* V600E VAF (median *BRAF* V600E VAF in patients with *v* without CNVs: 6.6% *v* 0.7%, P = .009).

Primary resistance was observed in 12 (30%) of 40 patients. Among these, four (33%) of 12 patients were nonshedders at both t0 and PD, two (17%) of 12 were shedders at t0 but not at

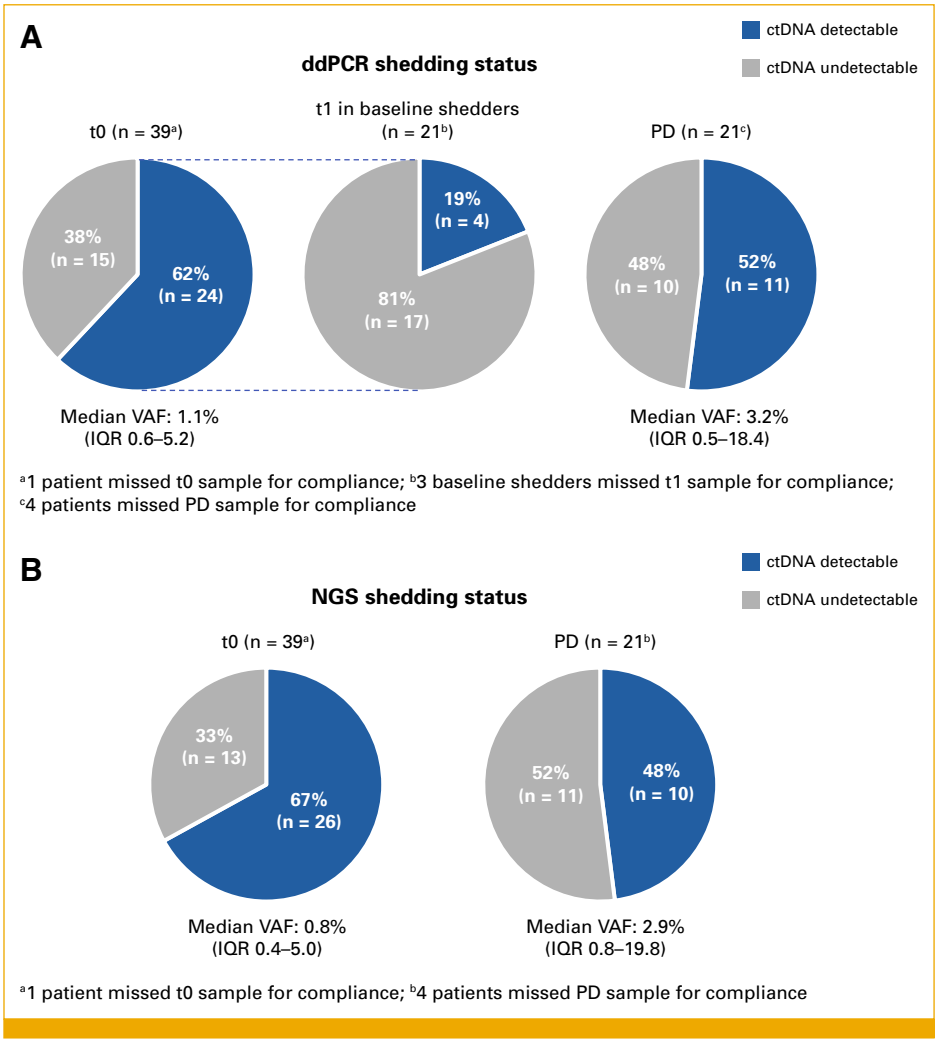

FIG 1. ddPCR and NGS shedding status. (A) ddPCR shedding status at t0, t1, and PD; (B) NGS shedding status at t0 and PD. ctDNA, circulating tumor DNA; ddPCR, digital droplet PCR; NGS, next-generation sequencing; PD, progression of disease; VAF, variant allele frequency.

PD, three (25%) of 12 were shedders at both t0 and PD, and three (25%) of 12 were shedders at t0 but missed PD for compliance.

The analysis of putative resistance mechanisms was performed in matched samples of 10 shedders at both t0 and PD. At PD, all 10 evaluated patients had co-mutations. In particular, one patient acquired the *NRAS*^{Q61K} and *MET* CNV and one patient acquired the *KRAS*^{V14I}. Other molecular alterations that occurred at PD were *NRAS*^{G13V}, *TP53*^{R213Q}, and *TP53*^{R273L} and CNVs in *EGFR*, *MET*, and *ERBB2* (Fig 3B).

Association of Clinical and Molecular Features With Clinical Outcomes

Among baseline clinical features, only the presence of liver metastases had a negative impact on PFS and OS (median PFS in patients with liver metastases *v* without: 3.9 *v* 9.0 months, $P = .008$; Data Supplement, Fig S5a; median OS: 4.0 *v* NR months, $P < .001$; Data Supplement, Fig S5b; Table S4). Baseline tumor burden, assessed as the sum of diameters of target lesions, was not associated with either PFS or OS (Cox regression for continuous variable: PFS hazard ratio [HR], 1.00 [95% CI, 0.99 to 1.01]; $P = .376$; Data Supplement, Fig S6a; OS HR, 1.00 [95% CI, 0.99 to 1.01]; $P = .763$; Data Supplement, Fig S6b). Baseline tumor burden showed no significant correlation with *BRAF* V600E VAF at t0 ($\rho = -0.062$, $P = .707$; Data Supplement, Fig S7).

Baseline ctDNA shedding status showed a trend toward worse PFS and OS (Data Supplement, Table S5). Higher *BRAF* V600E VAF at t0 was associated with shorter PFS (HR, 1.09 [95% CI, 1.02 to 1.17]; $P = .013$; Data Supplement, Fig S8a) and OS (HR, 1.10 [95% CI, 1.02 to 1.18]; $P = .010$; Data Supplement, Fig S8b).

Patients who achieved molecular clearance at t1 (17/21; 81%) had a longer PFS and OS than the counterpart (median PFS,

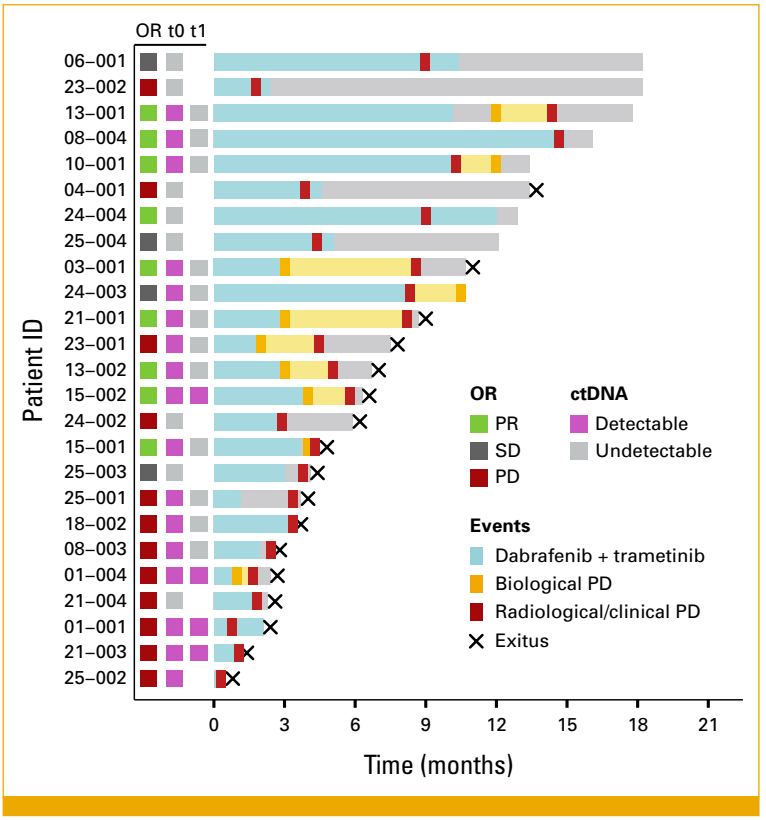

FIG 2. Timeline of key events in patients with progression of disease. CR, complete response; ctDNA, circulating tumor DNA; OR, overall response; PD, progression of disease; PR, partial response; SD, stable disease.

8.3 *v* 1.4 months, $P < .001$; Fig 4A; median OS, 10.5 *v* 2.2 months, $P < .001$; Fig 4B). The distribution of best overall response according to clearance at t1 is shown in the Data Supplement (Figure S9a). No correlation between the percentage change in ctDNA and the percentage change in tumor size at the time of best response was observed ($\rho = -0.061$; $P = .815$; Data Supplement, Fig S9b). Patients who had a t1 sample positive for *BRAF* V600E, regardless of baseline shedding status (4/35; 11%), had shorter PFS and OS than the counterpart (median PFS, 1.4 *v* 9.0 months, $P < .001$; Fig 4C; median OS, 2.2 *v* NR months, $P < .001$; Fig 4D).

Baseline *EGFR* CNV and *MET* CNV at t0 were poor prognostic factors (median PFS in patients with *EGFR* CNVs *v* without: 3.4 *v* 10.3 months, $P < .001$; Fig 4E; median PFS in patients with *MET* CNVs *v* without: 3.4 *v* 10.3 months, $P = .018$; Figure 4F; median OS in patients with *EGFR* CNVs *v* without: 3.4 *v* NR months, $P < .001$; Figure 4G; median OS in patients with *MET* CNVs *v* without: 3.7 *v* NR months, $P = .009$; Fig 4H).

Multivariable Cox analyses for PFS and OS were conducted on 21 patients who were evaluated for ctDNA clearance at t1 (Data Supplement, Tables S6 and S7). Higher baseline *BRAF* V600E VAF predicted shorter PFS (adjusted HR, 1.16 [95% CI, 1.02 to 1.32]; $P = .025$) and OS (adjusted HR, 1.15 [95% CI, 1.02 to 1.30]; $P = .027$), whereas ctDNA clearance was significantly associated with longer PFS (adjusted HR, 0.07 [95% CI, 0.01 to 0.51]; $P = .009$) and OS (adjusted HR, 0.07 [95% CI, 0.01 to 0.49]; $P = .007$).

No correlation was observed between shedding status at PD and OS (Data Supplement, Table S5). Clinical features and outcomes of shedders at PD, according to acquired genomic alterations, are reported in the Data Supplement (Table S8).

DISCUSSION

In this prospective, multicenter study, we confirmed the clinical activity of the dabrafenib plus trametinib combination in patients with *BRAF* V600E–mutated NSCLC, reporting an ORR of 42.5%, a median PFS of 8.3 months, and a median OS of 21.1 months. These findings are in line with those reported in the pivotal phase II trial by Planchard et al, which documented an ORR of 63.9%, median PFS of 10.8 months, and median OS of 17.3 months.[2,3] The slightly lower ORR and PFS in our cohort may reflect the clinical heterogeneity of a real-world population, including older patients and those with ECOG PS 2-3 and brain metastases.

ctDNA analysis revealed *BRAF* V600E in 62% of patients at baseline using ddPCR and 67% using NGS, with a median VAF of 1.1% and 0.8%, respectively, consistent with literature data of 71%-74% *BRAF*-mutated shedding patients.[13,14]

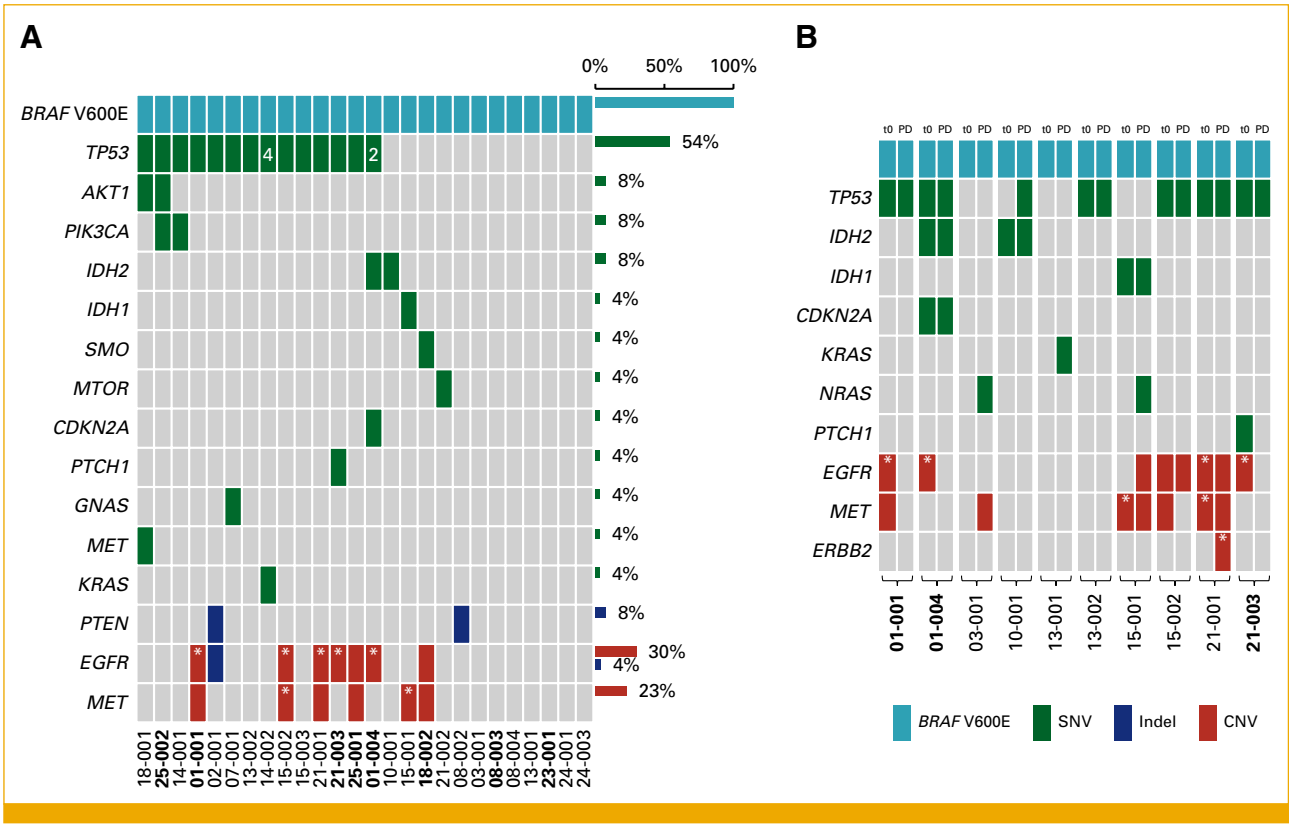

FIG 3. NGS study at baseline and at progression of disease. (A) NGS study at baseline and (B) matched NGS at PD versus baseline. *The value has a score between 0 and 5; Patient IDs shown in bold indicate primary resistance. CNV, copy number variation; NGS, next-generation sequencing; PD, progression of disease; SNV, single nucleotide variant.

Notably, we observed high concordance between NGS and ddPCR, in agreement with previous reports.[15,16] Baseline ctDNA burden showed clear prognostic relevance, as higher *BRAF* V600E VAF at t0 was significantly associated with shorter PFS and OS (PFS HR, 1.09 [95% CI, 1.02 to 1.17]; P = .013; OS HR, 1.10 [95% CI, 1.02 to 1.18]; P = .010) and remained significant in multivariable analyses. This finding supports the notion that ctDNA burden at baseline may reflect underlying tumor biology.[17,18] By contrast, baseline radiologic burden did not correlate with survival outcomes, suggesting that ctDNA may be a more sensitive marker of aggressiveness. Of note, baseline tumor burden did not correlate with *BRAF* V600E VAF at t0, nor did ctDNA changes with radiologic tumor size at best response. These findings should be interpreted with caution, as the very low baseline VAF values and frequent complete molecular clearance limit the ability to detect meaningful correlations.

ctDNA clearance at t1 was significantly associated with prolonged PFS and OS, remaining significant at multivariable analyses and highlighting its role as an early biomarker. However, without a control arm treated with chemotherapy and/or immunotherapy, the association between early ctDNA clearance and improved outcomes should be interpreted as prognostic rather than predictive, and its value as a treatment-specific biomarker cannot be formally established.

Although ctDNA clearance did not uniformly predict radiologic response, absence at t1 was consistently associated with PD. Interestingly, among evaluated patients, all four individuals with radiologic SD achieved ctDNA clearance. In this subset, PD occurred in only one patient after 8.3 months, and all patients were alive at the data cutoff. These findings indicate that radiologic SD may underestimate the clinical benefit of therapy, whereas ctDNA clearance may more accurately discriminate responders and identify patients deriving meaningful benefit from dabrafenib plus trametinib.

An increase in *BRAF* V600E VAF during treatment anticipated radiologic and/or clinical progression with a median of approximately 5 weeks, suggesting that serial ctDNA monitoring could enable early detection of progression and potentially guide treatment decisions. This is particularly relevant given the role of immunotherapy-based strategies in *BRAF* V600E–mutated disease,[19] where an early therapy switch could meaningfully affect patient prognosis.[20] Indeed, one patient in our cohort who had primary resistance to dabrafenib plus trametinib achieved a prolonged benefit from subsequent immunotherapy, indicating that alternative therapeutic strategies may remain effective despite early resistance to targeted therapy and highlighting the potential clinical utility of ctDNA monitoring in patient management. It should also be noted that biological PD was observed

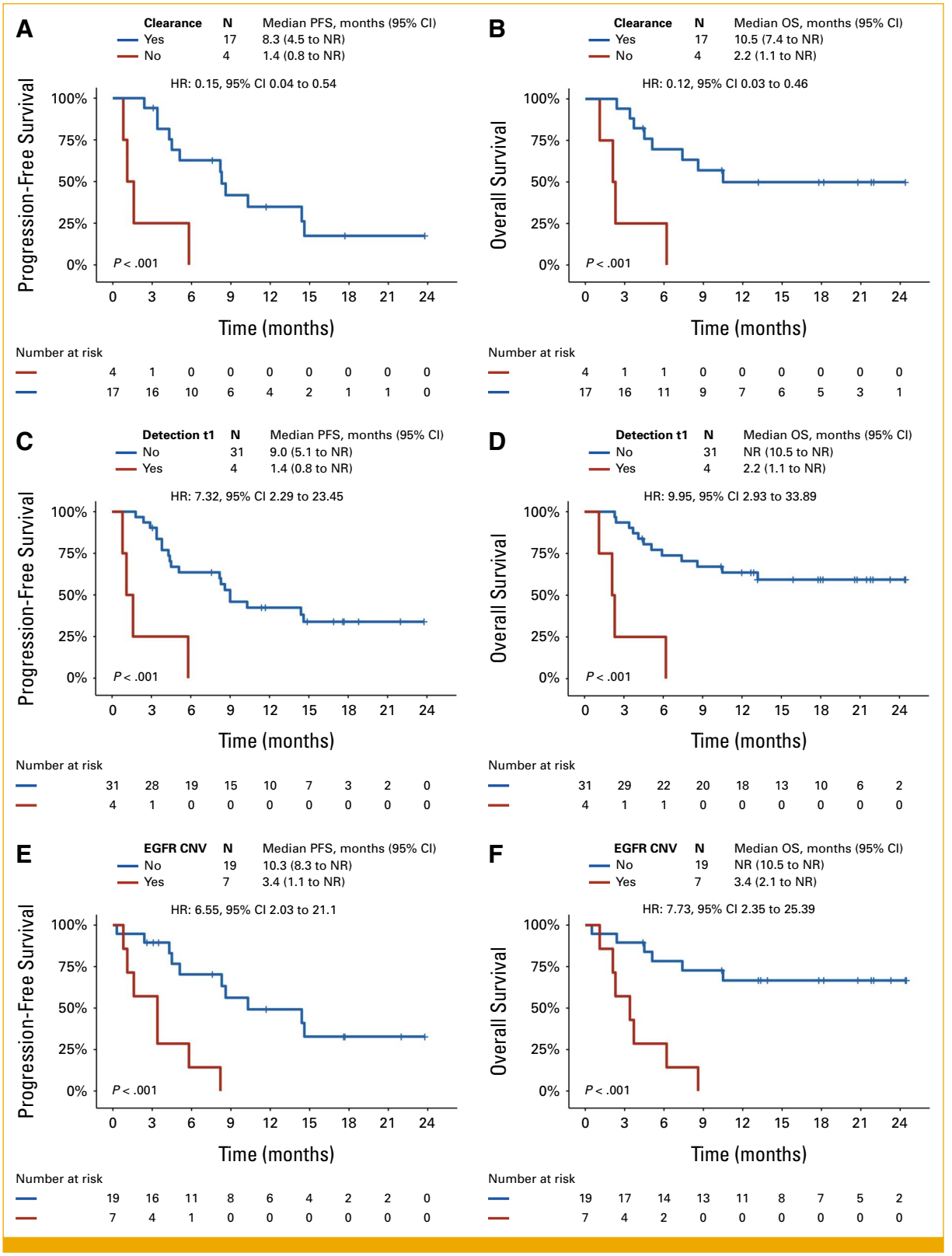

FIG 4. Kaplan-Meier curves for PFS and OS according to ctDNA clearance at t1, *BRAF* V600E detection at t1, and *EGFR/MET* CNVs. (A) PFS by ctDNA clearance at t1; (B) OS by ctDNA clearance at t1; (C) PFS by *BRAF* V600E detection at t1; (D) OS by *BRAF* V600E detection at t1; (E) PFS by *EGFR* CNVs; (F) OS by *EGFR* CNVs; (G) PFS by *MET* CNVs; (H) OS by *MET* CNVs. CNV, copy number variation; ctDNA, circulating tumor DNA; HR, hazard ratio; N, number; NR, not reached; OS, overall survival; PFS, progression-free survival. (continued on following page)

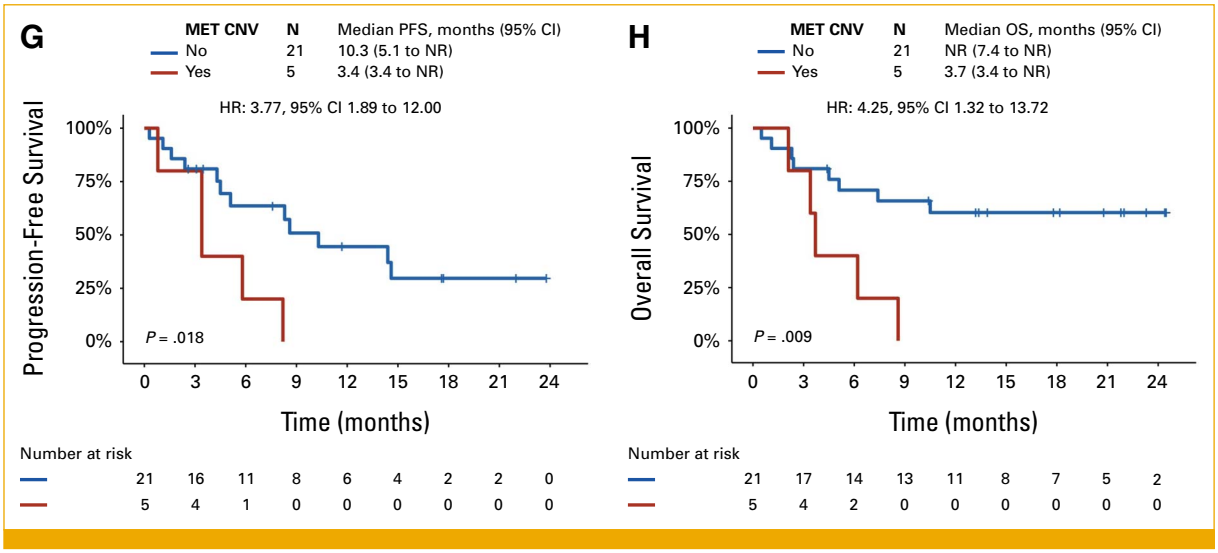

FIG 4. (Continued).

exclusively in baseline shedders, and molecular monitoring in nonshedders was not effective in anticipating PD. Moreover, among patients experiencing biological PD (n = 10), two showed ctDNA positivity only after clinical and/or radiologic PD. Despite strong association with outcomes, current evidence does not support treatment modification based on ctDNA dynamics in *BRAF* V600E–mutated NSCLC. No study has demonstrated an overall survival benefit from switching therapy at the time of molecular progression, and, from a pragmatic perspective, ctDNA clearance should therefore be considered a prognostic and monitoring biomarker rather than a guide for treatment decisions. The feasibility of interventional strategies based on ctDNA remains challenging in the context of a rare disease and requires collaborative, multicenter efforts.

NGS analysis revealed a high rate (73%) of co-occurring genomic alterations in shedding patients. The most frequent alterations involved *TP53*, *PI3K/AKT/mTOR* pathway genes, and cell cycle regulators, similarly to previous reports.[13,14,21,22] CNVs were observed in 27% of patients, with *EGFR* and *MET* CNVs enriched in primary resistant patients, and were significantly associated with higher *BRAF* V600E VAF, suggesting a possible correlation between tumor burden and genomic instability. This correlation should be interpreted with caution, as CNV detection by ctDNA is limited in low tumor fraction, contributing to uncertainty in CNV calling. Several CNVs scored 0-5, considered borderline, and were not confirmed in tissue by fluorescence in situ hybridization, representing a limitation of the study.

Mechanisms of acquired resistance were explored in 10 shedding patients with paired NGS at baseline and PD. Although no recurrent alterations emerged across all cases, several candidate resistance mechanisms were identified, including mutation in the *MAPK* pathway, such as *NRAS*^{G13V}, *NRAS*^{Q61K}, *KRAS*^{V14I}, and *TP53* mutations or CNVs involving *EGFR*, *MET*, and *ERBB2*. These findings are consistent with known resistance mechanisms to BRAF inhibition described in both NSCLC and melanoma, where MAPK pathway reactivation represents key adaptive pathway.[13,14,21,23]

Limitations of this study include the relatively small sample size, lack of matched tissue at PD, and possible false negatives in low-shedding patients. Despite these constraints, the real-world setting and prospective nature provide valuable insight into the molecular landscape and therapeutic outcomes in *BRAF* V600E–mutated NSCLC.

In conclusion, the LiBRA study highlighted the potential role of ctDNA analysis as a dynamic biomarker of treatment response and resistance in advanced *BRAF* V600E–mutated NSCLC and confirmed the clinical benefit of dabrafenib plus trametinib in this setting.

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