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Original research

Cross-method comparison for *BRAF* p.V600 mutation cfDNA testing in Melanoma: BRAFI study

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ABSTRACT

Background: BRAF p.V600 mutation is the most frequent molecular driver alteration in melanoma. Detection of BRAF mutations in circulating-free DNA (cfDNA) reflects the shedding of tumor DNA and offers a potential non-invasive biomarker for disease monitoring and prognosis. However, the lack of standardized methodologies and inter-assay variability hinders its clinical implementation.

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Methods: The sensitivity, agreement and concordance of seven *BRAF* mutation detection assays were assessed across four laboratories. *BRAF* p.V600 mutation in pretreatment plasma samples was analyzed in 51 patients diagnosed with advanced stage melanoma using two digital PCR-based assays (droplet digital PCR -ddPCR- Bio-Rad and microfluidic digital PCR -Absolute Q, ThermoFisher Scientific-), three RT-PCR based assays (Idylla®, Cobas®, PNA-Q-PCR) and two NGS based assays (Oncomine™ Pan-Cancer Cell-Free Assay and Illumina Platforms).

Results: digital PCR-based assays and Cobas® exhibited the highest sensitivity (51.0 %), followed by NGS Illumina® (45.1 %), Oncomine NGS / PNA-Q-PCR (43.1 %) and Idylla® (37.2 %). Results of different techniques showed a moderate to strong agreement, except for the comparison of Cobas with Idylla that was poor (Kappa=0.57). There was near-perfect agreement on detection of *BRAF* mutation between both NGS platforms and the NGS Illumina® with PNA-Q-PCR (Kappa = 0.92). Concordance of the quantitative results in terms of mutant allele frequency was near-perfect between NGS Illumina and ddPCR Bio-Rad assays (ICC = 0.99).

Conclusions: Our study demonstrates substantial agreement among multiple cfDNA *BRAF* mutation detection assays, particularly between NGS and digital PCR assays. These findings support the potential utility of different techniques for BRAF testing in cfDNA.

1. Introduction

Mutations at codon 600 (p.V600) of the *BRAF* gene *(BRAF* V600) are found in 40–50 % of melanomas, driving tumor growth and progression. Three different combinations of targeted therapies combining BRAF and MEK inhibitors have been approved for the treatment of patients with *BRAFV600* mutation-positive advanced melanoma [1–3]. Additionally, immunotherapy based on the use of anti-PD-1 antibodies, with or without anti-CTLA-4 antibodies, has high activity in *BRAF* mutant melanoma, with most studies suggesting higher rates of long responders compared with *BRAF* non-mutant patients [4].

The detection of *BRAF* mutations in circulating-free DNA (cfDNA) has emerged as a clinically relevant biomarker as it correlates with tumor burden [5,6]. Moreover, several studies suggest that circulating tumor DNA (ctDNA) detection in cfDNA holds prognostic value [5–8] and it is an excellent tool for monitoring tumor burden evolution [9].

Various molecular strategies are available for detecting *BRAF* mutations in blood, with distinct characteristics in terms of sensitivity, breadth of analysis, technical complexity, turnaround time, and cost. While RT-PCR and digital PCR-based assays are less complex and faster, they are limited to detecting specific mutations, whereas NGS-based assays are more complex but offer a more comprehensive characterization by capturing all variants within a target region. Beyond the interpretation of the test as qualitative data, quantitative data is crucial in monitoring tumor evolution over time [10,11]. Assays may vary in mutant allele fraction (MAF) quantification, providing either absolute, relative, or semi-quantitative values.

Currently, while there is a consensus on the clinical utility of ctDNA testing for monitoring patients with advanced melanoma, its implementation as a routine clinical tool remains highly limited. Challenges include the absence of uniform recommendations in clinical guidelines [12], and technical factors such as the lack of standardized methodologies and inter-assay variability [13–15]. To address these technical challenges, the Spanish Melanoma Group (GEM) conducted a prospective multicenter study to evaluate the agreement and concordance of *BRAF* V600 mutation detection in ctDNA from metastatic melanoma patients using distinct assays employed in four Spanish laboratories. By assessing the performance of these assays, we aimed to provide technical evidence supporting the adoption of ctDNA analysis in melanoma clinical practice.

2. Methods

2.1. Study design and patients

This is a multicenter, non-interventional, prospective trial in patients with advanced melanoma. The study was conducted in 14 hospitals from the Spanish Melanoma Group (GEM) (detailed information in Supplementary methods). Eligible patients were patients with histologically confirmed melanoma, either unresectable stage III or stage IV. Documentation of melanoma with *BRAF* V600 mutation by local labs in tumor tissue was required as inclusion criteria.

2.2. Blood collection and cfDNA isolation

A single blood sample per patient (50 mL) was collected at the time of inclusion. Samples were collected in Cell-Free DNA BCT Streck collection tubes and sent to the central lab (Pangaea Oncology Laboratory, Dexeus Hospital) for DNA extraction. Isolation of cfDNA was performed from 4 mL of plasma using a custom protocol with the QIAsymphony® DSP Virus/Pathogen Midi Kit using a QIAsymphony robot (QIAGEN, Hilden, Germany) and following the manufacturer's instructions. The final elution volume was 50 μL per sample. DNA samples were stored at −80ºC and then distributed in batches of 10 samples to the rest of participating laboratories (Hospital Clinic Barcelona, Hospital General de Valencia and Complejo Hospitalario Universitario de Cartagena). The median DNA extracted was 1505 ng/ul (range 0,7–5,42 ng/ul).

2.3. Assays for BRAF p.V600 detection in cfDNA

The presence of *BRAF* p.V600 mutation was determined using seven assays (Supplementary methods). RT-PCR based methods included an in-house 5′-nuclease real-time PCR assay for *BRAF* p.V600E/K mutations (PNA-Q-PCR Taqman®), the Cobas BRAF Mutation Test v2 (Roche diagnostics®) and the IdyllaTM ctRAS-BRAF Mutation Assay (Idylla®). Digital PCR-based methods included microfluidic digital PCR assay for *BRAF* p.V600E (dPCR Absolute Q, ThermoFisher®) and the ddPCR *BRAF* V600 Screening Kit for *BRAF* p.V600E/K/R mutations (ddPCR BioRad®) (Supplementary Table 1). NGS-based methods included NGS Oncomine™ Pan-Cancer Cell-Free panel (Thermofisher) (NGS Oncomine) and a Custom Solid tumor panel (Qiagen ®) using the llumina platform for sequencing (NGS Illumina). The MAF value was obtained with all assays, except with Cobas and Idylla.

2.4. Statistical analysis

The sensitivity of each method was assessed by comparing cfDNA results to FFPE tissue analysis (all patients included had BRAF mutation detected in tissue). The agreement between different methodologies according to the detection of BRAF status was evaluated using the kappa coefficient values and the corresponding 95 % confidence intervals (95 % CI) [16]. The concordance of MAFs values between assays was assessed using the Lin's concordance correlation coefficient and the Passing-Bablok regression analysis [17,18]. Progression-free survival and overall survival were estimated by means of the Kaplan–Meier method and the nonparametric log-rank test was applied for comparisons of groups (Supplementary methods).

3. Results

3.1. Patient characteristics

Fifty-one patients with advanced melanoma and local confirmed *BRAF* p.V600 mutations in tumor tissue were included. The median patient age was 58 years (range, 34–92). Most patients presented with good performance status (ECOG 0–1, 90 %) and were treatment-naïve (64.7 %). The median number of metastatic sites was two (range 1–6), and LDH levels were predominantly within the normal range (82.4 %).

BRAF molecular characterization of tissue biopsies detected the p.V600E mutation in 29 patients (56.9 %), p.V600K in 3 (5.9 %), p.V600R in 1 (2.0 %), and an unknown *BRAF* p.V600X subtype in 18 (35.3 %). Subsequent ctDNA analysis enabled the identification of the specific alteration in 11 *BRAF* p.V600X patients (61.1 %). This resulted in 37 patients with p.V600E (72.5 %), 6 with p.V600K (11.8 %), 1 with p.V600R (2.0 %), and 7 classified as p.V600X subtype (13.7 %) (Table 1).

3.2. Technical inter-comparison of PCR-based and NGS-based BRAF p.V600 cfDNA assays

First, we evaluated the detection rate of *BRAF* p.V600 mutations in ctDNA as a qualitative result. Overall, *BRAF* p.V600 mutations in plasma were detected in 56.9 % of patients by at least one method. ddPCR BioRad assay, dPCR ThermoFisher assay, and Cobas® showed the highest detection rate (51.0 %), followed by NGS Illumina® (45.1 %), NGS Oncomine and PNA-Q-PCR (43.14 %), and Idylla® (37.2 %).

BRAF mutation detection was associated with the presence of visceral metastases and high tumor burden. We found a higher *BRAF* p.V600 detection rates in samples from patients with visceral metastases (81 % vs. 32 %, p = 0.0004), multiple metastatic sites (74 % vs. 43 %, p = 0.03), and elevated LDH (89 % vs. 50 %, p = 0.06) (Fig. 1).

ctDNA testing showed concordant results across assays in 38 (74.5 %) patients, with 16 testing positive and 22 negative. By contrast, 13 (25.5 %) cases showed discordant detection of the *BRAF* p.V600 mutation across assays including four (36.3 %) stage M1a patients, three (27.2 %) stage M1b patients, four (26.6 %) M1c patients and two (25 %) M1d patients (Supplementary Table 7–8). Differences in the limit of detection of the assay might cause such discrepancies, since the median MAF value of discrepant cases was lower than that of cases with concordant results. The median MAF detected in concordant cases versus discordant cases using ddPCR was 14.59 vs 0.97 (p = 0.003), with dPCR Absolute Q it was 11.59 vs 1.01 (p = 0.003), using NGS Illumina it was 11.84 vs 1.26 (p = 0.011), with NGS Oncomine it was 8.74 vs 1.07 (p = 0.034), and finally, using PNA-Q-PCR, the median MAF in concordant cases was 11.16 vs 1.06 in discordant cases (p = 0.047) (Fig. 4, Supplementary Table 8–9).

We evaluated the presence of additional alterations beyond the *BRAF* p.V600 mutations by NGS-based assays. We detected other candidate driver mutations in nine (19.6 %) patients: seven patients were treatment naïve, while in two patients, the sample was obtained after progression to anti PD-1 antibody treatment. Detected mutations involved *KRAS* gene (2 patients), *NRAS* gene (1 patient), *PIK3CA* gene (3 patients), *MAP2K1* gene (1 patient) and IDH1 gene (1 patient). (Supplementary Figure 3).

Finally, we evaluated the agreement on detection of *BRAF* p.V600 mutation across the assays. A moderate to near-perfect agreement across all the assays was observed, except for the comparison of the Idylla with Cobas (Fig. 2, Supplementary Table 4). There was a near-perfect agreement between both NGS platforms, and NGS Illumina with PNA-Q-PCR assays (K=0.92), while a strong agreement was found between results of both digital PCR assays (K=0.85), NGS Oncomine with the PNA-Q-PCR assay (K=0.84), Idylla® with both NGS platforms (K=0.80) and with PNA-Q-PCR (K=0.80), ddPCR with NGS platforms (k = 0.85) and both dPCRs with PNA-Q-PCR (K=0.85). Cobas® and Idylla® assays had a moderate agreement with the results obtained using dPCR methods or PNA-Q-PCR (K= 0.64–0.79). There was only a week agreement when comparing results obtained using Cobas® with those obtained using Idylla (K=0.57).

When the analysis was restricted to 29 patients with *BRAF* p.V600E mutation confirmed within the tissue sample, excluding other BRAFV600 mutations, all the comparisons showed a moderate to near-perfect agreement (Supplementary Table 5, Supplementary Figure 1).

The median ctDNA concentration detected by the quantitative techniques was lower for both dPCR methods, followed by both NGS methods (Supplementary Table 6, Supplementary Figure 2). The highest concordance between MAF values was found between results obtained using NGS Illumina and dPCR Absolute Q, ThermoFisher (Lins CCC 0.985; 95 % CI: 0.975, 0.995)(Passing-Bablok 1.02, 95 % CI:0.92,1.08). Overall, the remaining comparisons demonstrate excellent concordance (Supplementary Table 7), with the exception of MAF values obtained using NGS Oncomine compared with those obtained using the dPCR assays or NGS Illumina (Fig. 3).

Table 1
Patients' characteristics.

	N = 51
Age, median (range), y	58 (34−92)
Female sex, n (%)	17 (33.3)
ECOG PS 0–1, n (%)	46 (90.2)
Number of previous lines, median (range)	
0	33 (64.7)
≥ 1	18 (35.3)
Number of metastasic sites, median (range)	2 (1−6)
M1 stage, n (%)	
M1a	15 (29.4)
M1b	11 (21.6)
M1c	15 (29.4)
M1d	10 (19.6)
LDH, n (%)	
Normal	42 (82.4)
High	9 (17.6)
BRAF*, n (%)	
BRAFV600E	29 (56.9)[a]
BRAFV600K	3 (5.9)[§]
BRAFV600R	1 (2.0)
BRAFV600X	18 (35.3)[¥]
Melanoma type, n (%)	
Cutaneous	41 (80.4)
Acral	2 (3.9)
Mucosal	2 (3.9)
UK	6 (11.8)

* BRAF mutation subclassification including data from tissue (local labs). Including data results from cfDNA analysis BRAFV600 subtypes were:

[a] BRAFV600E in 37 (72.5 %) patients; §: BRAFV600K in 6 (11.8 %) patients; ¥: UK cases were 7 (13.7 %)

4. Discussion

The present collaborative study provides a comprehensive inter-comparison analysis of seven liquid biopsy assays for *BRAF* p.V600 detection in cfDNA from unresectable advanced melanoma patients. ctDNA testing might be influenced by distinct preanalytical factors such as blood collection tubes, time to plasma isolation, storage conditions [19] and the cfDNA extraction method [20]. Thus, we centralized the cfDNA extraction in a laboratory to avoid confounding factors in the ctDNA testing. Then, four distinct laboratories performed the *BRAF* p.V600 testing in ctDNA and reported the results as both quantitative and qualitative data.

The implementation of *BRAF* testing in cfDNA as a routine clinical tool in melanoma is not uniform, mainly due to reimbursement issues. ESMO Clinical Practice Guidelines in melanoma recommend analyzing ctDNA for *BRAF* p.V600 mutation testing in stage III/IV melanoma patients if no tumor tissue is available [21], but ESMO recommendations on the use of circulating tumor DNA assays for cancer patients does not

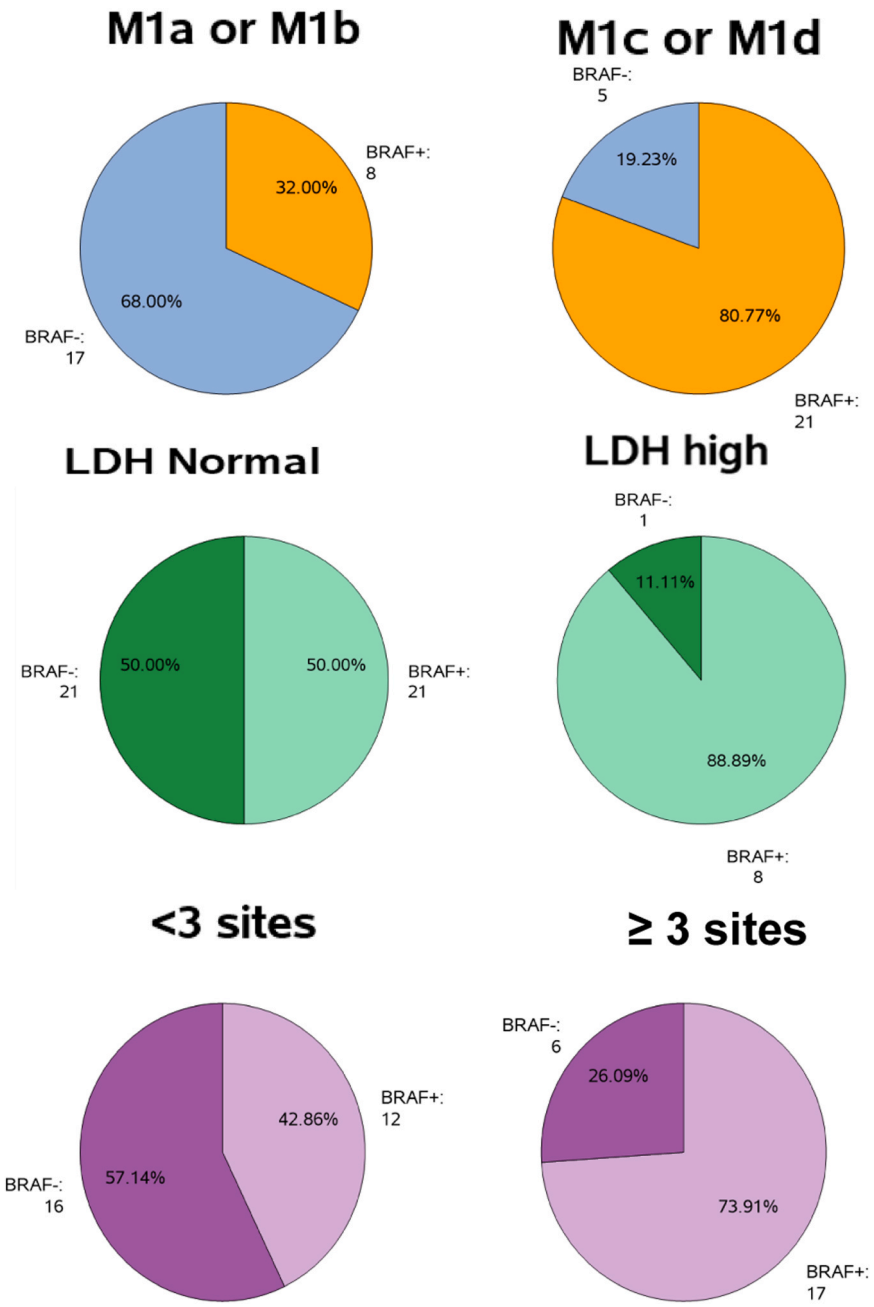

Fig. 1. Detection rate according to visceral (non-lung) metastases or no visceral (non-lung) metastases (A), LDH high versus normal values (B) and number of metastatic sites < 2 versus 3 or more (C).

included specific recommendations for melanoma [22]. In advanced melanoma the role and utility of liquid biopsy are already evident, both as prognostic markers and as a tool for monitoring tumor evolution [9]. The next step is to develop more sensitive techniques mainly for using in stage II/III melanoma to detect minimal residual disease. Thereafter, serial monitoring of ctDNA during and after treatment can flag early recurrence or emergent resistance, enabling dynamic therapy adjustments before changes appear on imaging.

In our study, a positive ctDNA test was obtained with at least one assay in 56.9 % of patients, highlighting the limitations of ctDNA testing even in advanced melanoma patients. Both PCR-based and NGS-based assays showed higher *BRAF* detection rates in plasma among patients with visceral metastases, multiple metastatic sites, and elevated LDH levels, consistent with previous studies [8,23]. *BRAF* mutant benign naevi or clinically atypical naevi do not impact in the detection of *BRAF* p.V600 in plasma [24]. However, our findings indicate that inherent biological factors to the scarcity of ctDNA in certain patients impact the sensitivity of current methods and limit its utility in a subset of advanced melanoma patients. Further development of novel methodological strategies or multimodal analytical approaches is crucial to overcome these technical challenges [25,26].

Notably, we observed minor differences in sensitivity and concordance among the assays, which have important implications for the clinical management of melanoma patients. Two Digital PCR assays (ddPCR BioRad and dPCR Absolute Q, ThermoFisher), and Cobas® demonstrated the highest detection rates (50.98 %), while Idylla® had the lowest one (37.2 %).

The detection of other mutations in addition to *BRAF* pV600 by NGS platforms in nine patients demonstrated that NGS offers the advantage of broader genomic profiling, which could provide additional insights

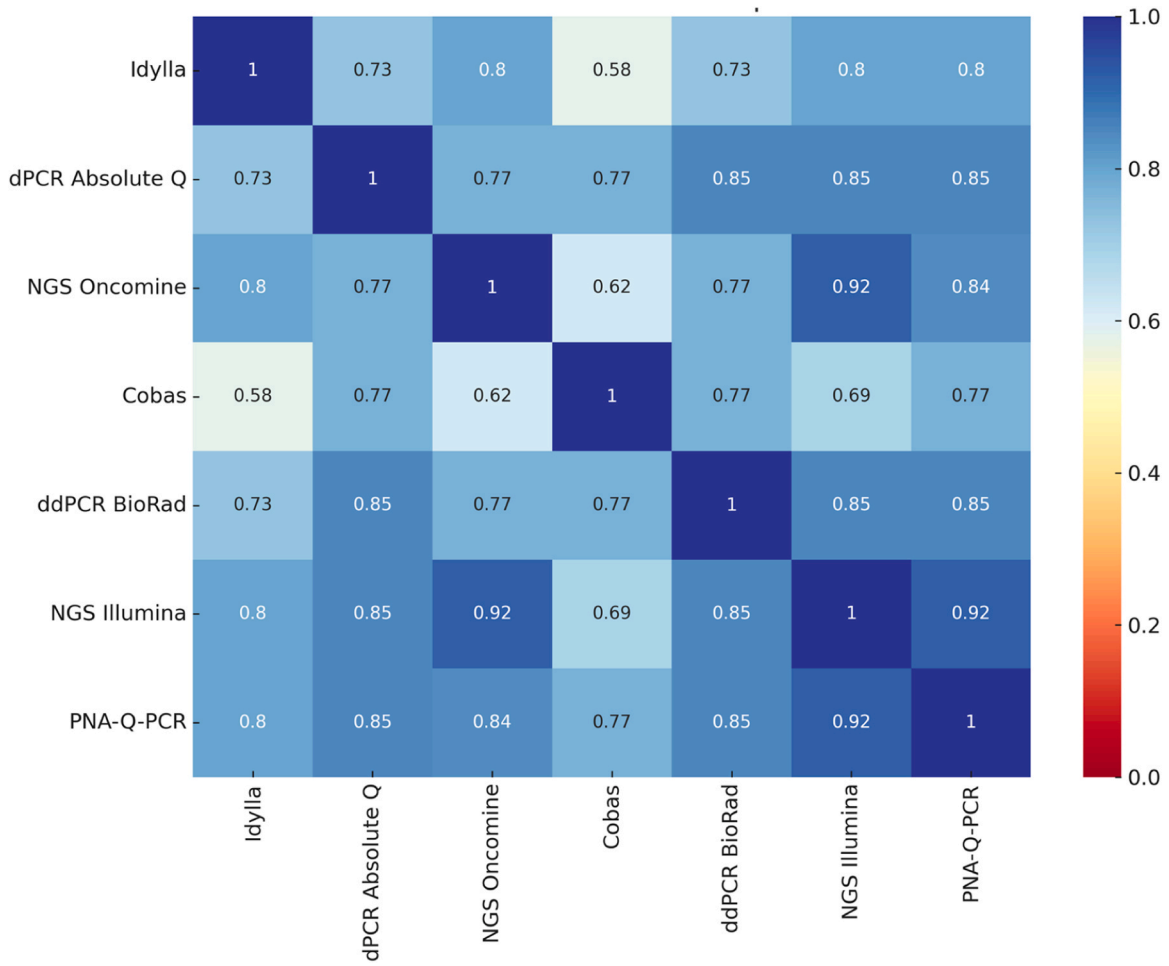

Fig. 2. Agreement analysis according to Cohen's Kappa Index, detection versus no detection of BRAFV600 in cfDNA. Correlation Matrix. The agreement between different methodologies for the assessment of BRAF status (detected vs not detected) is evaluated using the Cohen's Kappa coefficient values and the corresponding 95 % confidence intervals (95 % CI). The results of this qualitative analysis, in terms of the Cohen's Kappa Coefficient and the corresponding agreement plot are reported in this section. Agreement according to Kappa values between 0 and 0.20 no agreement, 021–0.39 minimal, 0.40 and 0.59 weak, 0.60 and 0.79 moderate, 0.80 and 0.90 strong, and almost perfect from 0.90 to 1.0.

into the molecular landscape of melanoma and potentially inform treatment decisions [27].

Overall, these assays showed a strong to near-perfect agreement indicating that most assays are highly reliable and reproducible, with minimal variability between them. Both NGS platforms and the NGS Illumina/PNA-Q-PCR showed near-perfect agreement. A strong agreement was observed between both dPCRs techniques, NGS Illumina with dPCR Absolute Q or PNA-Q-PCR. Only Cobas® and Idylla® demonstrated weaker agreement between them. Idylla® was the test that showed less concordance with the other assays. These findings are consistent with prior reports, which have noted the limitations of assays like Idylla® in detecting mutations at lower allele frequencies or in complex genomic backgrounds. However, when these analyses were restricted to *BRAF* p.V600E patients confirmed in tissue sample, all assays showed better agreement.

The Lins concordant correlation analysis and the Passing-Bablok regression analysis across these various pairings revealed that while there is generally strong agreement between the quantitative value of MAF detected by the different methodologies, there is also variability in some comparisons, mainly for those involving results of NGS Oncomine according to Passing-Bablok test. The Passing-Bablok method determines whether the quantitative results of two different techniques could be interchangeable. These findings highlight both the similarities and differences between methodologies, emphasizing the importance of cautious interpretation, especially when monitoring disease evolution, as quantitative results may not always align perfectly across different assays.

In the study, we detected discordant results between assays in 25.4 % of patients. Such differences are associated with distinct analytical sensitivity of the assays, reducing their agreement in samples with low MAFs [28].

This study is limited by its relatively low number of patients included, which may restrict the statistical power and generalizability of the results. Also, the centralized cfDNA extraction protocol, while reducing inter-laboratory variability, may not reflect the heterogeneity of pre-analytical workflows in routine clinical settings, and we cannot assess how extraction methods could affect the results.

In conclusion, this study underscores the importance of selecting appropriate methodologies for *BRAF* p.V600 detection in cfDNA. While dPCR emerges as the most reliable techniques, NGS-based assays provide information on the presence of other co-mutations. The slight variability among different assays necessitates consideration of the chosen method, especially in clinical scenarios with a low tumor burden that will require more sensitive techniques and for longitudinal MAF monitoring, which will require either the use of the same technique over time or only techniques proven to have interchangeable quantitative results. In summary, our findings highlight the potential utility of ctDNA BRAF testing as a biomarker in clinical melanoma management, providing valuable insights into its clinical relevance and the specific characteristics of each assay.

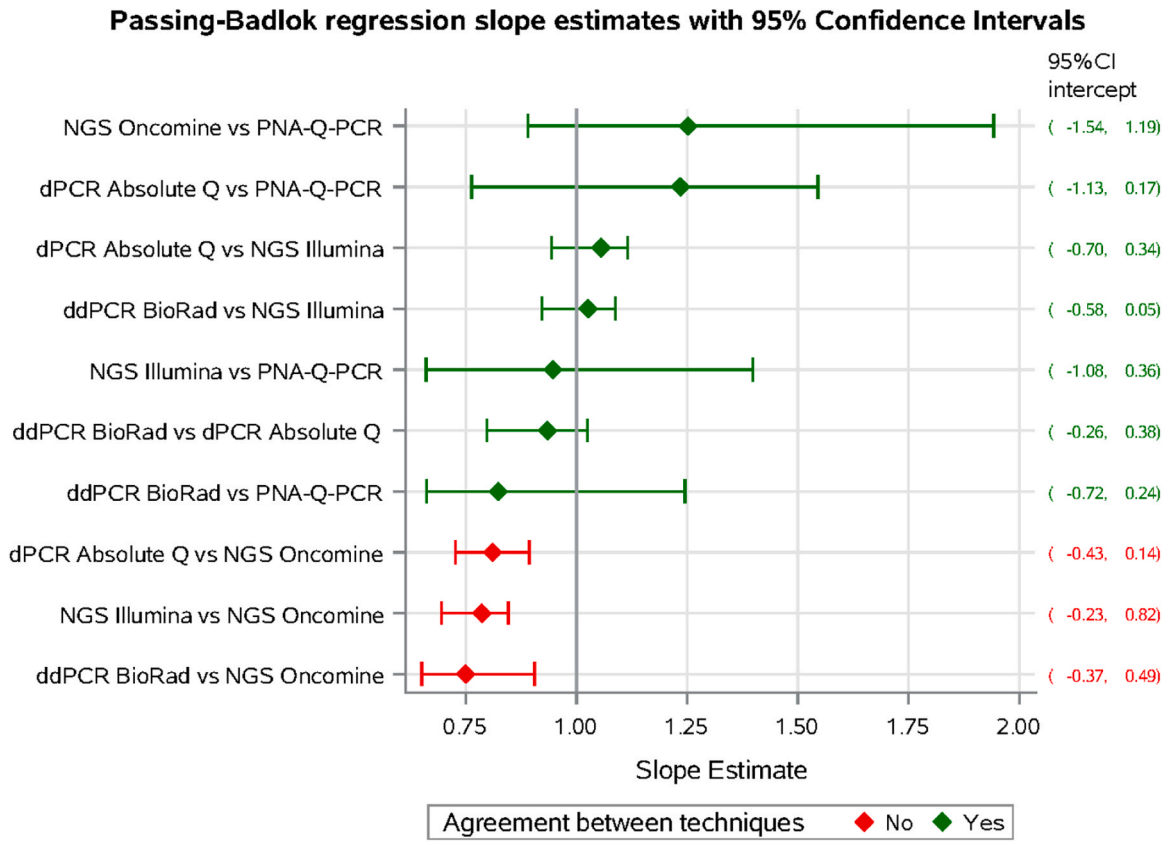

Fig. 3. Passing-Bablok regression analysis: agreement of MAF values between different methodologies. In terms of the regression estimates, the CI for the intercept (a) must contain 0 and the CI for the slope (b) must contain 1, for defining a good agreement. Green lines represent techniques with a good agreement for MAF results, and in red those techniques with no comparable MAF results.

CRediT authorship contribution statement

Silvia Calabuig-Fariñas: Writing – review & editing, Visualization, Validation, Formal analysis. **Miguel Angel Berciano Guerrero:** Investigation, Visualization, Validation, Writing – review & editing. **Susana Muñoz:** Investigation, Visualization, Validation, Writing – review & editing. **Ana Arance Fernandez:** Writing – review & editing, Validation, Investigation, Data curation, Visualization, Supervision. **Joselyn Valarezo:** Validation, Visualization, Investigation, Writing – review & editing. **Pablo Ayala de Miguel:** Visualization, Investigation, Writing – review & editing, Validation, Data curation. **Enrique Espinosa:** Writing – review & editing, Visualization, Validation, Investigation. **Jose Luis Manzano:** Investigation, Writing – review & editing, Visualization, Validation, Data curation. **Eva Muñoz-Couselo:** Investigation, Visualization, Validation, Writing – review & editing. **Pablo Cerezuela-Fuentes:** Validation, Investigation, Writing – review & editing, Data curation, Visualization. **Alfonso Berrocal:** Writing – review & editing, Validation, Funding acquisition, Visualization, Investigation. **Ana Drozdowskyj:** Investigation, Resources, Project administration, Data curation, Writing – review & editing, Supervision, Methodology, Software, Formal analysis, Validation. **Sebastian Ortiz Reina:** Formal analysis, Project administration, Methodology, Investigation, Writing – review & editing, Data curation. **Juan Francisco Rodriguez-Moreno:** Validation, Writing – review & editing, Visualization. **Maria Quindos-Varela:** Investigation, Visualization, Validation, Writing – review & editing. **Eloisa Jantus-Lewintre:** Methodology, Supervision, Resources, Formal analysis, Conceptualization, Writing – review & editing, Project administration, Validation, Investigation, Data curation. **Joan Anton Puig-Butille:** Visualization, Validation, Investigation, Data curation, Writing – review & editing, Supervision, Conceptualization, Writing – original draft, Methodology, Formal analysis, Resources. **Guillermo Crespo:** Writing – review & editing, Validation, Visualization, Investigation. **Clara Mayo de las Casas:** Supervision, Formal analysis, Data curation, Writing – review & editing, Resources, Methodology, Conceptualization, Validation, Investigation, Software, Project administration. **Maria Gonzalez-Cao:** Funding acquisition, Resources, Project administration, Data curation, Visualization, Investigation, Supervision, Formal analysis, Conceptualization, Writing – original draft. **Rafael Rosell:** Project administration, Investigation, Writing – review & editing, Validation, Methodology, Visualization. **Teresa Puertolas:** Resources, Writing – review & editing, Visualization, Data curation, Validation, Investigation. **Miguel Angel Molina:** Writing – review & editing, Investigation, Visualization, Methodology, Formal analysis, Validation. **Almudena Garcia Castano:** Data curation, Visualization, Validation, Investigation, Writing – review & editing.

Author contributions

Dr M. Gonzalez-Cao had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Concept and design:* Drs M. Gonzalez-Cao, C. Mayo. *Acquisition, analysis, or interpretation of data*: Drs M. Gonzalez-Cao, C. Mayo de las Casas, A. Drozdowskyj, J.A. Puig-Butille, S. Ortiz-Reina, E. Jantus-Lewintre. *Drafting of the manuscript*: Dr M. Gonzalez-Cao, C. Mayo de las Casas, A. Drozdowskyj, J.A. Puig-Butille, E. Jantus-Lewintre. *Critical revision of the manuscript for important intellectual content*: Drs M. Gonzalez-Cao, C. Mayo de las Casas, P. Cerezuela, E. Espinosa, T. Puertolas, A. Drozdowskyi, M.A. Molina, A. Berrocal, J. A. Puig-Butille, S. Ortiz Reina, E. Jantus-Lewintre, J. L. Manzano, P. Ayala de Miguel, A. Arance Fernandez, M. A. Berciano Guerrero, A. Garcia Castano, T. Puertolas, G. Crespo, M. Quindos-Varela, J. F. Rodriguez-Moreno, M. A. Molina, E. Muñoz-Couselo, S. Calabuig-Fariñas, R. Rosell, J. Valarezo, S.

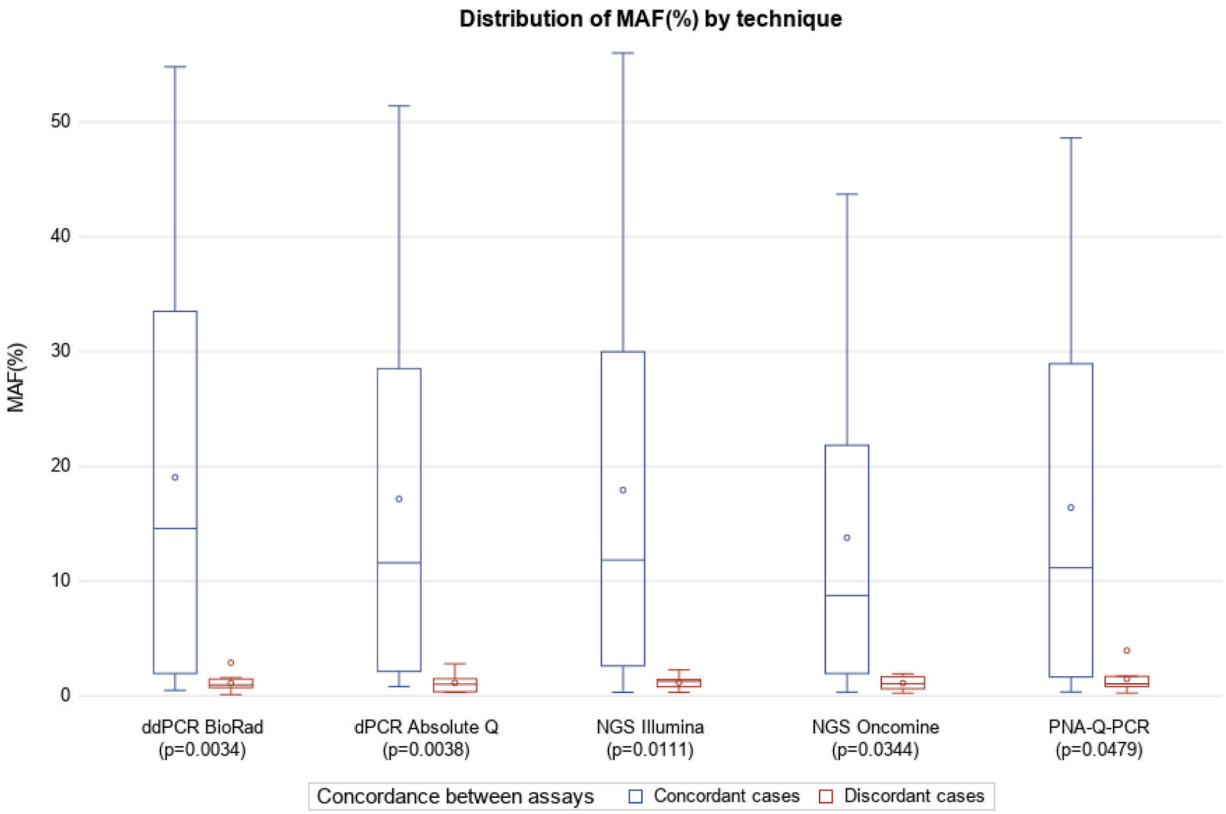

Fig. 4. Distribution of MAF by technique comparing concordant versus discordant cases. Cases with discordant results between techniques (n 13) had a lower MAF detectable mutations.

Muñoz. *Statistical analysis*: Drs A. Drozdowskyj and M. Gonzalez-Cao. *Obtained funding:* Dr M. Gonzalez-Cao, Dr A. Berrocal *Administrative, technical, or material support*: Drs M. Gonzalez-Cao, A. Drozdowskyj, C. Mayo de las Casas, R. Rosell, *Supervision*: Dr M. Gonzalez-Cao, C. Mayo de las Casas.

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Role of the funder

The funder had no role in the design and conduct of the study, collection, management, analysis and interpretation of the data, preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

Additional contributions

Sara Charro works for CRO company and has carried out the administrative and technical duties for conducting the clinical trial. We confirm that we have obtained written permission to include their name in the Acknowledgment section.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejcskn.2025.100738.

References

[1] P.A. Ascierto, G.A. McArthur, B. Dreno, et al., Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial, Lancet Oncol. 17 (9) (2016) 1248–1260, https://doi.org/10.1016/S1470-2045(16)30122-X.

[2] G.V. Long, D. Stroyakovskiy, H. Gogas, et al., Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial, Lancet 386 (9992) (2015) 444–451, https://doi.org/10.1016/S0140-6736(15)60898-4.

[3] R. Dummer, P.A. Ascierto, H.J. Gogas, et al., Overall survival in patients with BRAF-mutant melanoma receiving encorafenib plus binimetinib versus vemurafenib or encorafenib (COLUMBUS): a multicentre, open-label, randomised, phase 3 trial, Lancet Oncol. 19 (10) (2018) 1315–1327, https://doi.org/10.1016/S1470-2045(18)30497-2.

[4] J.D. Wolchok, V. Chiarion-Sileni, R. Gonzalez, et al., Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma, J. Clin. Oncol. 40 (2) (2022) 127–137, https://doi.org/10.1200/jco.21.02229.

[5] M. Gonzalez-Cao, C. Mayo-de-Las-Casas, M.A. Molina-Vila, et al., BRAF mutation analysis in circulating free tumor DNA of melanoma patients treated with BRAF inhibitors, Melanoma Res. 25 (6) (2015) 486–495, https://doi.org/10.1097/CMR.0000000000000187.

[6] N. Karachaliou, C. Mayo-de las Casas, C. Queralt, et al., Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC trial, JAMA Oncol. 1 (2) (2015) 149–157, https://doi.org/10.1001/jamaoncol.2014.257.

[7] M. Gonzalez-Cao, C. Mayo de Las Casas, J. Oramas, et al., Intermittent BRAF inhibition in advanced BRAF mutated melanoma results of a phase II randomized trial, Nat. Commun. 12 (1) (2021) 7008, https://doi.org/10.1038/s41467-021-26572-6.

[8] M. Gonzalez-Cao, C. Mayo de Las Casas, N. Jordana Ariza, et al., Early evolution of BRAFV600 status in the blood of melanoma patients correlates with clinical outcome and identifies patients refractory to therapy, Melanoma Res. 28 (3) (2018) 195–203, https://doi.org/10.1097/CMR.0000000000000432.

[9] A. Bartolomucci, M. Nobrega, T. Ferrier, et al., Circulating tumor DNA to monitor treatment response in solid tumors and advance precision oncology, npj Precis. Oncol. 9 (1) (2025) 84, https://doi.org/10.1038/s41698-025-00876-y.
[10] O.A. Zill, K.C. Banks, S.R. Fairclough, et al., The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients, Clin. Cancer Res. 24 (15) (2018) 3528–3538, https://doi.org/10.1158/1078-0432.CCR-17-3837.
[11] F. Darbeheshti, F. Yu, G.M. Makrigiorgos, Pre-PCR mutation-enrichment methods for liquid biopsy applications, Cancers 14 (13) (2022), https://doi.org/10.3390/cancers14133143.
[12] R.A. Fischer, I. Ryan, K. De La Torre, et al., US physician perspective on the use of biomarker and ctDNA testing in patients with melanoma, Crit. Rev. Oncol. Hematol. 196 (2024) 104289, https://doi.org/10.1016/j.critrevonc.2024.104289.
[13] T. Koessler, V. Paradiso, S. Piscuoglio, et al., Reliability of liquid biopsy analysis: an inter-laboratory comparison of circulating tumor DNA extraction and sequencing with different platforms, Lab Invest. 100 (11) (2020) 1475–1484, https://doi.org/10.1038/s41374-020-0459-7.
[14] L. Dong, X. Wang, S. Wang, et al., Interlaboratory assessment of droplet digital PCR for quantification of BRAF V600E mutation using a novel DNA reference material, Talanta 207 (2020) 120293, https://doi.org/10.1016/j.talanta.2019.120293.
[15] J. Xu, S. Qu, N. Sun, et al., Construction of a reference material panel for detecting KRAS/NRAS/EGFR/BRAF/MET mutations in plasma ctDNA, J. Clin. Pathol. 74 (5) (2021) 314–320, https://doi.org/10.1136/jclinpath-2020-206745.
[16] J.R. Landis, G.G. Koch, The measurement of observer agreement for categorical data, Biometrics 33 (1) (1977) 159–174.
[17] L.I. Lin, A concordance correlation coefficient to evaluate reproducibility, Biometrics 45 (1) (1989) 255–268.
[18] H. Passing, Bablok, A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I, J. Clin. Chem. Clin. Biochem. 21 (11) (1983) 709–720, https://doi.org/10.1515/cclm.1983.21.11.709.
[19] S. Nikolaev, L. Lemmens, T. Koessler, J.L. Blouin, T. Nouspikel, Circulating tumoral DNA: preanalytical validation and quality control in a diagnostic laboratory, Anal. Biochem 542 (2018) 34–39, https://doi.org/10.1016/j.ab.2017.11.004.
[20] M. Wang, X. Huang, X. Li, et al., Performance comparison of commercial kits for isolating and detecting circulating tumor DNA, Scand. J. Clin. Lab Invest 81 (4) (2021) 276–281, https://doi.org/10.1080/00365513.2020.1821394.
[21] T. Amaral, M. Ottaviano, A. Arance, et al., Cutaneous melanoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up, Ann. Oncol. 36 (1) (2025) 10–30, https://doi.org/10.1016/j.annonc.2024.11.006.
[22] J. Pascual, G. Attard, F.C. Bidard, et al., ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group, Ann. Oncol. 33 (8) (2022) 750–768, https://doi.org/10.1016/j.annonc.2022.05.520.
[23] K. Kozak, A. Kowalik, A. Gos, et al., Cell-free DNA BRAF V600E measurements during BRAF inhibitor therapy of metastatic melanoma: long-term analysis, Tumori (2020), https://doi.org/10.1177/0300891619900928, 300891619900928.
[24] N. Calbet-Llopart, M. Potrony, G. Tell-Martí, et al., Detection of cell-free circulating BRAF(V) (600E) by droplet digital polymerase chain reaction in patients with and without melanoma under dermatological surveillance, Br. J. Dermatol. 182 (2) (2020) 382–389, https://doi.org/10.1111/bjd.18147.
[25] J.M. Burgener, J. Zou, Z. Zhao, et al., Tumor-naïve multimodal profiling of circulating tumor DNA in head and neck squamous cell carcinoma, Clin. Cancer Res. 27 (15) (2021) 4230–4244, https://doi.org/10.1158/1078-0432.ccr-21-0110.
[26] E.S. Kuligina, G.A. Yanus, E.N. Imyanitov, Diversity of the circulating tumor markers: perspectives of a multimodal liquid biopsy, Biochemistry (Mosc) 89 (11) (2024) 1985–1997, https://doi.org/10.1134/s0006297924110129.
[27] L. De Mattos-Arruda, G. Siravegna, How to use liquid biopsies to treat patients with cancer, ESMO Open 6 (2) (2021) 100060, https://doi.org/10.1016/j.esmoop.2021.100060.
[28] A. Romero, E. Jantus-Lewintre, B. García-Peláez, et al., Comprehensive cross-platform comparison of methods for non-invasive EGFR mutation testing: results of the RING observational trial, Mol. Oncol. 15 (1) (2021) 43–56, https://doi.org/10.1002/1878-0261.12832.