BRAF V600 Mutations Detection in Melanoma: A Cross-Methodological Study Using cfDNA

Jantus-Lewintre, Eloisa[1]; Calabuig-Fariñas, Silvia[2]; Puig-Butille, Joan Anton[3]; Ortiz Reina, Sebastián[4]; Ana Drozdowskyj[8]; Cerezuela Fuentes, Pablo[5]; Manzano Mozo, José Luis[6]; Ayala de Miguel, Pablo[7]; Gonzalez Cao, María[8]; Mayo de las Casas, Clara[8] , on behalf of the Spanish Melanoma Group (GEM)

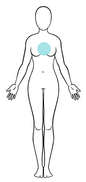
1. Universitat Politècnica de Valencia , Laboratorio Oncología Molecular, Fundación para la Investigación del Hospital General Universitario de Valencia, CIBERONC, Valencia, Spain; 2. Universidad de Valencia, Laboratorio Oncología Molecular, Fundación para la Investigación del Hospital General Universitario de Valencia, CIBERONC, Valencia, Spain; 3. CORE de Biología Molecular, Hospital Clínic de Barcelona, Barcelona, Spain; 4. Servicio de Anatomía Patológica del Complejo Hospitalario Universitario de Cartagena; 5. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain; 6. ICO Badalona, Badalona, Spain; 7. H. San Pedro de Alcántara, Cáceres, Spain; 8. Hospital Dexeus, Oncology Department, PangaeaLab, Barcelona, Spain.

INTRODUCTION

The BRAF V600 mutations are a key molecular marker for metastatic melanoma and the most common somatic point mutation in this cancer. Detecting BRAF mutations in blood has prognostic and predictive value, helping monitor responses to BRAF-targeted therapy and immunotherapy. Since BRAF mutations remain in circulation during melanoma progression and correlate with tumor burden, analyzing BRAF mutations in circulating-free DNA (cfDNA) is increasingly important.

Objective: This non-interventional study aimed to evaluate the **concordance among various methods for assessing BRAF p.V600 mutations in cfDNA** in a cohort of *BRAF*-mutated melanoma patients from 12 Spanish hospitals.

METHODOLOGY



- *Multicentric study: 14 Hospitals*
- *Recruitment: Feb 2023- Nov 2023*
- *Cut off Data Analysis June 2024*
- ***N**= 51 patients (Total=55 pts; 4 excluded: screening failure)*
- *Stage IV melanoma*

50 ml plasma samples were collected in Cell-Free DNA BCT Streck collection tubes (baseline)

Centralized isolation of cfDNA

Samples were distributed in batches to 4 laboratories

Analysis performed by seven methodologies, including 3 digital PCR-based, 2 NGS *and* 3 PCR-based platforms

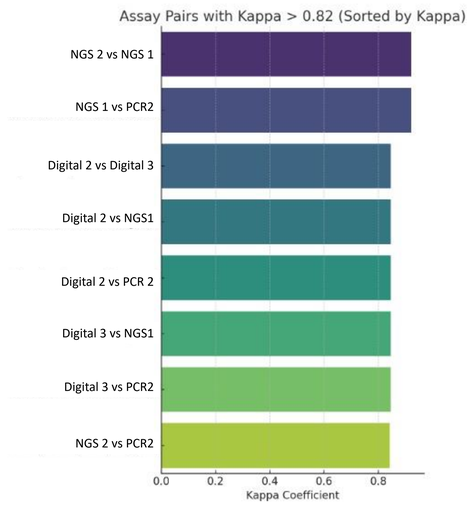
- In-house PNA-Q-PCR (Taqman®) assay for BRAF V600E/K/R mutations (PCR2) ★
- Cobas BRAFMutation Test v2 (Roche diagnostics) (PCR1) ★
- *BRAF* RT-PCR (Idylla) (PCR3) ★
- NGS Oncomine™ Pan-Cancer Cell-Free panel (Thermofisher) (NGS2)★
- NGS_QIAseq DNA Custom Panel (Illumina) (NGS1) ★
- ddPCR BRAF V600 kit (BioRad)) (Digital 2) ★
- ddPCR BRAF V600E assay (BioRad) (Digital 1) ★
- dPCR BRAF QuantStudio® Absolute Q (Applied Biosystems) (Digital3) ★

Statistical analysis

RESULTS

- Baseline clinicopathological characteristics of the cohort are shown in Table 1. Tumor tissue *BRAF* mutations were distributed as follows: 72.5% p.V600E, 11.8% p.V600K, and 15.7% other/undiscriminated p.V600 mutations.
- *BRAF* mutations were detected in cfDNA (by at least one method) in 28 (54.9%) samples.
- Concordance analysis showed near-perfect agreement (K= 0.92) among NGS platforms, strong agreement with digital PCR methods (K= 0.69-0.85), and PCR-based approaches (K= 0.77) (Figure 1).
- Variant allele frequencies (VAF%) assessed by quantitative methods were depicted in Figure 2. Comparisons among techniques were performed using intraclass correlation coefficients (ICC), with an overall ICC agreement of 0.77 and consistency of 0.79. High correlations were found among all techniques using Pearson correlation coefficients (Figure 3).
- Higher *BRAF* detection rates in plasma samples were associated with visceral metastases (p=0.0004), multiple metastatic sites (p=0.03), and high LDH levels (p=0.06).

Figure 1. Concordance Analysis (Cohen's Kappa Index). Examples illustrating near-perfect and strong agreement among the various ctDNA analysis techniques utilized in the study.



Figures Legend:

- PCR 1: Cobas BRAFMutation Test v2 (Roche diagnostics)
- PCR 2: In-house PNA-Q-PCR (Taqman®) assay for BRAF V600E/K/R mutations (PCR2)
- PCR 3: *BRAF* RT-PCR (Idylla) (PCR3)
- NGS 1: QIAseq DNA Custom Panel (Illumina)
- NGS 2: Oncomine™ Pan-Cancer Cell-Free panel (Thermofisher)
- Digital 1: ddPCR BRAF V600E only assay (BioRad)
- Digital 2: ddPCR BRAF V600 kit (BioRad))
- Digital 3: dPCR BRAF QuantStudio® Absolute Q (Applied Biosystems)

Figure 2. Quantification of ctDNA (VAF%) by different methodologies

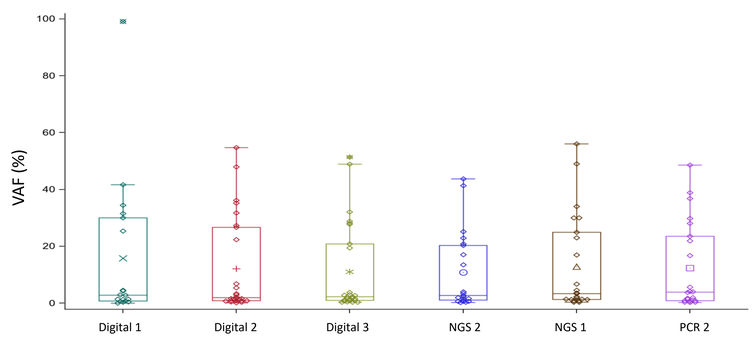


Figure 3. Pearson correlation coefficient of ctDNA VAF% comparing different techniques

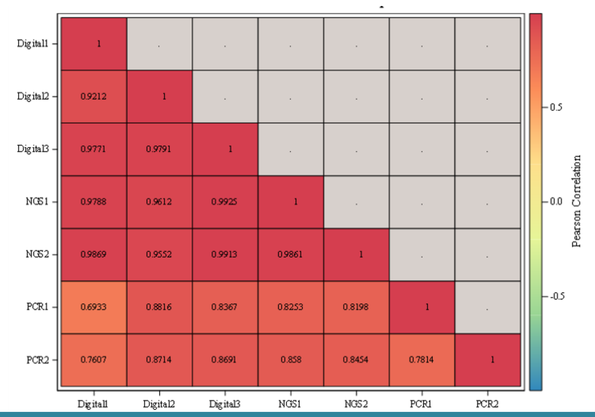


Table 1. Baseline clinicopathological characteristics

Characteristic		n=51
Age, median (range), years		58 (34-92)
Female sex, n (%)		17 (32.7)
ECOG PS 0-1, n (%)		46 (90)
Number of previous lines, median (range)		
	0	33 (63.5)
	≥1	18 (36.5)
Number of metastasic sites, median (range)		2 (1-6)
M1 stage, n (%)		
	M1a	15 (28.8)
	M1b	11 (21.1)
	M1c	16 (30.8)
	M1d	10 (19.2)
LDH, n (%)		
Normal		42 (80.8)
High		9 (19.2)
BRAF, n (%)		
	BRAFV600E	37 (72.5)
	BRAFV600K	6 (11.8)
	UK/Other BRAFV600	8 (15.7)
Melanoma type		
	Cutaneous	41 (80.4)
	Acral	2 (4)
	Mucosal	2 (4)
	UK	6 (11.8)

CONCLUSIONS

- Substantial concordance among multiple ctDNA *BRAF* mutations detection methods, particularly NGS and specific digital PCR assays.
- These findings support the potential utility of ctDNA *BRAF* testing as a biomarker in melanoma management.

Corresponding authors: cmayo@panoncology.com & ejantus@btc.upv.es