BRAF testing techniques in the management of patients with advanced malignant melanoma: a real-world comparative study.

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Objective

Malignant melanoma (MM) is one of the deadliest skin cancers. BRAF mutation testing plays a predominant role in the management of MM patients, because modern targeted therapies essentially consist of inhibitors of BRAF. BRAF V600 mutation must be detected using a FDA-approved (USA) or CE-IVD certified (Europe) test. The aim of this study was to compare BRAF mutational testing performed by conventional nucleotide sequencing approaches with either real-time PCR (rtPCR) or next-generation sequencing (NGS) assays in a real-life, hospital-based series of advanced MM patients.

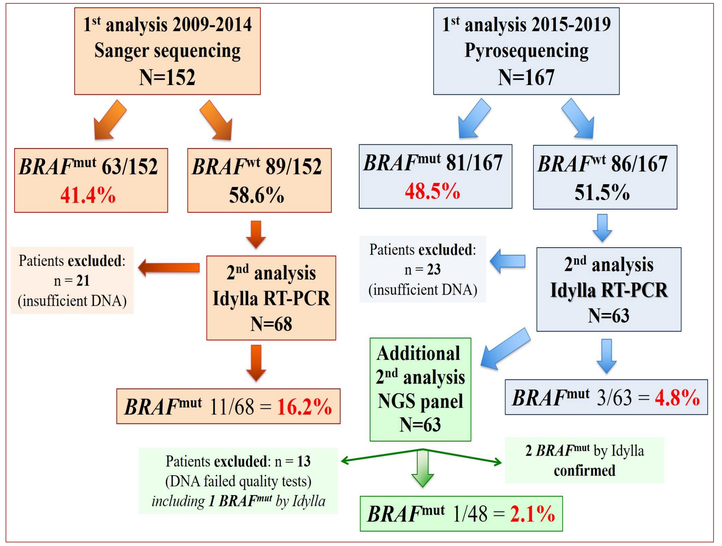
Methods and Materials

Formalin-fixed and paraffin embedded tissues from consecutive patients with AJCC stage IIIC and IV MM from Sardinia, Italy, who were referred for molecular testing, were enrolled into the study. Initial screening was performed to assess the mutational status of the BRAF and NRAS genes, using the conventional techniques recognized by the nationwide guidelines at the time of the molecular testing: at beginning, Sanger-based sequencing (SS) and, after, pyrosequencing. The present study subsequently focused on BRAF mutation detecting approaches only. BRAF wild-type cases with available tissue and adequate DNA were further tested with rtPCR (Idylla™) and NGS assays. The study was approved by the Committee for the Ethics of the Research and Bioethics of the National Research Council.

CHARACTERISTICS	Mutated	Wild-type	p
BRAF (n=319)			
Mutated, n (%)	144 (45.1)	175 (54.9)	
Gender, n (%)			
Male, n (%)	79 (54.9)	104 (59.4)	**0.479**
Female, n (%)	65 (45.1)	71 (40.6)	
Age at diagnosis, n (%)			
≤55 years, n (%)	58 (40.3)	37 (21.1)	**0.003**
>55 years, n (%)	86 (59.7)	138 (78.9)	
NRAS (n=272)			
Mutated, n (%)	40 (14.7)	232 (85.3)	
Gender, n (%)			
Male, n (%)	25 (62.5)	129 (55.6)	**0.522**
Female, n (%)	15 (37.5)	103 (44.4)	
Age at diagnosis, n (%)			
≤55 years, n (%)	4 (10)	73 (31.5)	**0.004**
>55 years, n (%)	36 (90)	159 (68.5)	

Results

Globally, 319 patients were included in the study; pathogenic BRAF mutations were found in 144 (45.1%) cases examined with initial screening; BRAF mutations were significantly more frequent in individuals older than 55. The V600E variant was the most common BRAF mutation found (83.4%). The rtPCR detected 11 (16.2%) and 3 (4.8%) additional BRAF mutations after SS and pyrosequencing, respectively. NGS detected one additional BRAF-mutated case (2.1%) among 48 wild-type cases, previously tested with pyrosequencing and rtPCR.



Exon	Mutation	Base Change	Amino Acid Change	Mutated samples	%
BRAF					
15	V600D	1799-1800 TG>AT	Val to Asp	3	**2.1**
15	V600E	1799 T>A	Val to Glu	117	**81.2**
15	V600E	1799_1800TG>AA	Val to Glu	3	**2.1**
15	V600K	1798-99 GT>AA	Val to Lys	19	**13.2**
15	V600R	1798-99 GT>AG	Val to Arg	1	**0.7**
15	K601E	1790 T>G	Leu to Arg	1	**0.7**

Exon	Mutation	Base Change	Amino Acid Change	Mutated samples	%
NRAS					
2	G12A	35 G>C	Gly to Ala	1	**2.5**
2	G13D	38 G>A	Gly to Asp	2	**5.0**
2	G13R	37 G>C	Gly to Arg	2	**5.0**
3	Q61H	183 A>T	Gln to His	1	**2.5**
3	Q61K	181 C>A	Gln to Lys	12	**30.0**
3	Q61L	182 A>T	Gln to Leu	3	**7.5**
3	Q61R	182 A>G	Gln to Arg	19	**47.5**

Conclusion

Our data evidenced that rtPCR and NGS are able to detect additional BRAF mutant cases in comparison with conventional sequencing methods; therefore, we argue for the preferential utilization of the former assays (NGS, rtPCR) in clinical practice to reduce false negative cases and improve the global accuracy of BRAF detection.