Research Article
Open Access

Frequency of BRAF V600E/E2/D and V600K/R/M mutations in patients with malignant melanoma unresectable stage III-IIIC–IV from May to August-2021 at Unigem in Medellín

Abstract

Background: Melanoma is the most aggressive skin cancer type and is responsible for 80% of deaths from this entity. It is considered one of the tumors with the highest mutational load, with approximately 50% of cases in codon600 of the BRAF gene.

Objective: To determine the frequency of BRAF V600E/E2/D and BRAF V600K/R/M mutations in patients with malignant melanoma unresectable stage III-IIIC – IV, during the period May-2018 and August-2021, at UNIGEM, in Medellín.

Methodology: Observational, retrospective study, which included 154 paraffin-embedded biopsies from patients with stage III-IIIC-IV unresectable malignant melanoma. The evaluation of the BRAF V600E/E2/D and BRAF V600K/R/M mutations was carried out through real-time PCR using the Idylla platform.

Results: 154 test results for the BRAF V600 mutation were reviewed, with a total of 60 (38.9%) mutated cases, of which 56 (93.3%) correspond to the V600E/E2/D change, predominantly in minors or equal to 63 years of age.

Conclusion: We found that in the population analyzed with metastatic melanoma, 38.9% of the patients presented alterations in the BRAF gene, more frequently in men, in the V600 E/E2/D codon and in the age group≤63 years.

Keywords: cancer, skin, melanoma, mutation, BRAF, codon V600

Volume 6 Issue 1 - 2023

Pino Erika,[1] Benítez Claudia,[2] Aristizábal Clara,[2] Orozco Laura,[2] Rincón Olga,[2] Jiménez María,[2] Aristizábal Beatriz[2]
[1]Molecular Diagnóstic-Biomoleculas group, Fundación Hospitalaria San Vicente de Paul, Colombia
[2]Molecular Diagnóstic, UNIGEM-Biomoleculas group, Colombia

Correspondence: Beatriz Aristizabal, Scientific Direction, Cl 19A 44-25, Medellín, Antioquia, Colombia, Tel+573104498719, +5730541111135, Email beatriz.aristizaba@unigem.co

Received: February 22, 2023 | **Published:** April 19, 2023

Abbreviations: DNA, deoxyribonucleic acid; MAPK, mitogen-activated protein kinase; PI3K, fosfoinositol-3-quinasa; RAF, rapidly accelerated fibrosarcoma; UNIGEM, unidad de investigación Genética molecular

Introduction

Skin cancer is a common neoplasm in humans, with 1805 (1.6%) new cases and 490 (0.89%) deaths worldwide. It is generally divided into two large groups, non-melanomas (mainly basal cell and squamous cell carcinoma) and melanomas. Basal cell carcinoma is the most frequent skin tumor, with 75% of all cases in the world, followed by squamous cell carcinoma with 20% and finally melanoma, although it represents only 4% of skin cancers, but is the most aggressive and responsible for 80% of deaths from this type of cancer due to its ability to cause metastasis.[1,2]

Melanoma arises from melanocytes, which are responsible for producing and releasing melanin (specialized pigment cells) whose function is to minimize DNA damage from ultraviolet radiation. They are found in the epidermis, meninges, inner ear, and eyes.[3] Several factors are involved in the development of melanoma, whether environmental or genetic. The first group includes sun exposure, while the second includes family history of skin cancer, the number of nevi, and the light photo type.[1,4] affects young and middle-aged people, the average age at diagnosis is 57 years.[3]

The worldwide incidence and mortality (for both sexes and all ages) in 2020 was 324,635 and 57,043 cases, respectively.[5] In Colombia, the National Institute of Cancerology reported during the period between 1996-2010, 1,853 (16.1%) cases, with an average of 124 cases per year of malignant melanoma and a mortality of 16.1 cases per year in the period 2001-2009, without differences according to sex.[6] Instead, GLOBOCAN in 2020, reported 1,805 (1.6%) new cases and 490 deaths (0.89%) for melanoma of the skin, with an estimated 5-year prevalence (for all ages) of 10.35% per 100,000 population,[5] which reflects a significant increase in the number of new cases and mortality of this entity in our setting.

Melanoma is an entity that has particular characteristics such as: high metastatic potential, rapid development, poor prognosis, high mortality rate, and mutational burden. The most frequent somatic mutations refer to BRAF (50%), NRAS (15-30%), NF1 (10-15%), KIT (10%), which are genes involved in the MAPK (mitogen-activated proteinkinase) pathways and PI3K (phosphoinositol-3-kinase), these pathways are responsible for controlling cell proliferation, differentiation, or even survival.[1] These mutations lead to abnormal activation of the downstream MAPK pathway, in the absence of any extracellular stimulus, favoring cell proliferation and survival (resistance to pro-apoptotic signals), because the mutant protein is ten times more active than the wild-type protein.[7]

BRAF gene (B1 homologue of the murine sarcoma viral oncogene v-Raf) belongs to the RAF (rapidly accelerated fibrosarcoma) family of proto-oncogenes. More than 40 different BRAF mutations have been identified in human cancer, with a predominance in malignant melanomas (40-60%), followed by thyroid cancer (45%) and colorectal cancer (10%).[8] The main mutation observed in BRAF is V600E (originating in exon 15), it epresents 90% of the mutations. It is a substitution of a valine for a glutamic acid with a single nucleotide change at position 1799 from a thymine to adenine (T>A). Other reported mutations are: V600D (<5%, valine for aspartic acid, are very

rare and biochemically very similar to glutamic acid substitutions), V600K (12-15%, valine for lysine),V600R (3-5%, valine for arginine).

The main value of detecting BRAF mutations for malignant melanoma is not diagnostic, but therapeutic. The understanding of the molecular mechanisms involved in this entity has facilitated its biological understanding, and has allowed the creation of drugs that block the different pathways that are activated when this type of mutation occurs (tyrosine kinase inhibitors and inhibitors of immune control points).[9] which leads to a decrease in tumor size and increased survival in these patients.[3] Due to the increase in melanoma cases in our environment, the small number of articles published about the mutational behavior of the BRAF gene, and the impact that these mutations have on the choice of drug therapy, the objective of the study was: To determine the frequency of BRAF V600E/E2/D and BRAF V600K/R/M mutations in patients with stage III-IIIC-IV unresectable malignant melanoma between May-2018 and August-2021 at a molecular diagnostic center in Medellín (UNIGEM).

Material and methods

154 cases received at UNIGEM for molecular diagnosis were selected, from May 2018 to August 2021, previously classified with a diagnosis of malignant melanoma by pathology report. The samples were analyzed by the Idylla methodology, which detects the V600E/E2/D and V600K/R/M mutations in codon 600 of the BRAF gene. This methodology does not discriminate between V600E, E2 and D mutations, nor between V600K, R and M mutations. It also does not detect other rare mutations, such as V600G. It has an analytical specificity: 100% mutant V600E and 100% mutant V600K and an analytical sensitivity mean probability of detection of 95%, 4 copies/PCR of V600E and 10 copies/PCR of V600K. The detection limit is greater than 5% for the V600K/R/M mutation and for the V600E/E2/D mutation the detection limit is 1%. Some negative results reported as V600E/E2/D*, have additional explanations: "It is possible to miss the V600K/R/M mutation with DNA <5% in the sample tested".

Ethical considerations

The genetic tests were performed at UNIGEM, after the completion and signing of the informed consent by the patients at the different referring institutions. During the development of the work, the rights, integrity and confidentiality of the study subjects were protected.

Clinical features

Sociodemographic and clinical-histopathological data were obtained from a secondary source (Pathology Report). A classification of the type of melanoma was made, anatomical location of the tumor in 5 levels: head and neck, trunk, upper limbs, lower limbs and others; as well as, variables such as Clark, Breslow, Borders/margins and ulceration were classified (Table 1).

Molecular analysis

Idylla™ BRAF Mutation Test kit was used for the detection of mutations, it consists of three allele-specific duplex PCR reactions, designed to specifically amplify mutations in wild-type BRAF, V600E, V600E2, V600D, V600K, V600R and V600M, each combined with an endogenous control gene that acts as a sample processing control. The kit detects 2 groups of mutations V600E/E2/D and V600K/R/M; however, it does not discriminate between V600E,E2, and D mutations, nor between V600K, R, and M mutations. The cartridge contains the necessary reagents to perform sample preparation, amplification, and real-time PCR detection, starting from the insertion of formalin- fixed paraffin-embedded tissue into the cartridge. The process steps in the test are tissue liquefaction and cell lysis, followed by real-time PCR using allele-specific primers. The presence of a mutated genotype is determined by calculating the ΔCq. (The ΔCq value is the difference between the Cq of the wild type BRAF and the Cq of V600E/E2/D or V600K/R/M.) The signal from the mutated alleles is considered valid if the ΔCq is within apredefined range (BRAF V600 positive sample). Any sample with a valid wild-type signal but with a ΔCq value outside the predefined range is considered BRAF V600 negative. The analytical specificity is 100% for V600E and V600K mutants. Analytical sensitivity, understood as the mean 95% probability of detection, is 4 copies/PCR for V600E and 10 copies/PCR for V600K.

The determination of gene mutations was evaluated by real-time PCR using the Biocartis Idylla™ platform. The interpretation of the results (analysis of the mutations) was carried out using its software.

Statistical analysis

Among the variables analyzed, there are two groups: sociodemographic variables: sex/age and clinical- histopathological variables: anatomical location, histological type, Clark, Breslow, Borders/margins, ulceration and mutational status. The qualitative variables were summarized through descriptive statistics and sector graphs and the quantitative variables (age) with measures of central tendency and their respective measure of dispersion, using the statistical program SPSS version 21.

Results

In total, 154 test results for the BRAF V600 mutation were reviewed, 2 sociodemographic variables were characterized, age and sex, we found a range between 23 and 88 years with a mean age of 62 years old and a higher frequency in men (62.3%), than in women (37.7%). According to the anatomical location, 40.9% of the cases occurred in the lower limbs. The most frequent type of melanoma was nodular melanoma (22.7%), followed by Acral lentiginous (13.6%). The clinical type was not defined in 56.5% of the pathology reports, it was classified as unspecified melanoma (malignant, amelanotic, metastatic). Most of the cases were found in a Clark IV stage 51.7% and had a lesion depth (Breslow) greater than 4 mm 52.8%. Ulceration was present in 68.6% of cases and the edges/margins were free from contact with the lesion in 70.8% of cases. (Table 1)

The cases' mutation distribution was the following: "Wild type" genotype 57.8%, (were reported 17.5% cases with additional explanations V600E/E2/D*), followed by the group of "V600E/E2/D Mutated" with 36.4%, then the "Invalid" group, 3.2% (they were executed in duplicate for confirmation) and finally, the group of V600K/R/M Mutated with 2.6% (Figure 1). The distribution of the mutations versus the sociodemographic variables, the anatomical location of the tumor and the histological type (complete data) was analyzed. Two age groups were created taking into account the median age (63 years). All mutated genotypes were more frequent in men (including the K/R/M mutation, which only occurred in this group).

The age group > 63 years, presented more frequently the "Wild Type" genotypes (62.9%), while in the group ≤ 63 years old, predominated the genotype "BRAF V600E/E2/D Mutated " (73.2%). The majority of "Wild Type" tumors were located in the lower limbs in 50.6%, and the "BRAF V600E/E2/D Mutated" cases were located in the trunk in 30.4%. There were 56.5% of classified "Without specifying the clinical type (malignant, amelanotic, metastatic)" cases, presented mayor frequently "Mutated" genotype, in 66.6%, (BRAF V600E/E2/D and V600K/R/M with 61.6%and 5.0% respectively) (Table 2).

Citation: Erika P, Claudia B, Clara A, et al. Frequency of BRAF V600E/E2/D and V600K/R/M mutations in patients with malignant melanoma unresectable stage III-IIIC–IV from May to August-2021 at Unigem in Medellín. *Int J Mol Biol Open Access*. 2023;6(1):8–12. DOI: 10.15406/ijmboa.2023.06.00146

Table 1 Main characteristics of the patients studied

Variables	Female (%)	Male (%)	Total (%)
Frequency	58 (37,7)	96 (62,3)	154 (100)
Age (Media)	62,4 (DE ± 13,9)		
Anatomical localization (n=154)			
Head and neck	9 (15,5)	15 (15,6)	24 (15,6)
Trunk	10 (17,2)	20 (20,8)	30 (19,5)
Upper limbs	10 (17,2)	20 (20,8)	30 (19,5)
Lower limbs	25 (43,1)	38 (39,6)	63 (40,9)
Other*	4 (6,9)	3 (3,1)	7 (4,5)
Clinical type (n=154)			
Superficial spreading	3 (5,2)	4 (4,2)	7 (4,5)
Nodular	10 (17,2)	25 (26,0)	35 (22,7)
Acral lentiginous	9 (15,5)	12 (2,5)	21 (13,6)
Lentigo maligna	2 (3,4)	2 (2,1)	4 (2,6)
Clinical Type not defined (maligno, amelanótico, metastásico)	34 (58,6)	53 (55,2)	87 (56,5)
CLARK (n=58)a			
II	1 (4,5)	1 (2,8)	2 (3,4)
III	3 (13,6)	5 (13,9)	8 (13,8)
IV	11 (50,0)	19 (52,7)	30 (51,7)
V	7 (31,8)	11 (30,6)	18 (31,1)
Breslow (n=89)b			
≤1 mm	4 (12,9)	5 (8,6)	9 (10,1)
1,1-2,0 mm	5 (16,1)	9 (15,5)	14 (15,7)
2,1-4,0 mm	7 (22,6)	12 (20,7)	19 (21,3)
>4,0 mm	15 (48,4)	32 (55,2)	47 (52,8)
Borders/Margins (N=72)c			
Free Border-margins compromise	17 (70,8)	34 (70,8)	51 (70,8)
Compromised	7 (29,2)	14 (29,2)	21 (29,2)
Ulceration (n=86)d			
Absence	11 (33,3)	16 (30,2)	27 (31,4)
Presence	22 (66,7)	37 (69,8)	59 (68,6)

* 2 gluteus, 1 vulva, 1 vagina, 1 scrotum, 1 pubis and 1 anus cases

a No data = 96 cases, b No Data = 65 cases, c No Data = 82 cases, d No Data = 68 cases.

Table 2 Mutations distribution vs gender, age and anatomic location

Variables	Wild type (%)	Wild type BRAF V600E/E2/D* (%)	BRAF V600E/E2/D Mutated (%)	BRAF V600K/R/M Mutated (%)	Invalid (%)
Gender					
Male	39 (62,9)	17 (63,0)	33 (58,9)	4 (100)	3 (60,0)
Female	23 (37,1)	10 (37,0)	23 (41,1)	0	2 (40,0)
Age					
≤63	23 (37,1)	10 (37,0)	41 (73,2)	2 (50,0)	4 (80,0)
> 63	39 (62,9)	17 (63,0)	15 (26,8)	2 (50,0)	1 (20,0)
Anatomic location					
Head and neck	11 (17,7)	6 (22,2)	6 (10,7)	0 (0)	1 (20,0)
Trunk	8 (12,9)	3 (11,1)	17 (30,4)	2 (50,0)	0 (0)
Upper limbs	6 (9,7)	5 (18,5)	16 (28,6)	2 (50,0)	1 (20,0)
Lower limbs	33 (53,2)	12 (44,4)	15 (26,8)	0 (0)	3 (60,0)
Other*	4 (6,5)	1 (3,7)	2 (3,6)	0 (0)	0 (0)
Clinical type (n=154)					
Superficial spreading	2 (3,2)	4 (14,8)	1 (1,8)	0 (0)	0 (0)
Nodular	10 (16,1)	6 (22,2)	17 (30,4)	1 (25,0)	1 (20,0)
Acral lentiginous	15 (24,2)	5 (18,5)	0 (0)	0 (0)	1 (20,0)
Lentigo maligna	2 (3,2)	1 (3,7)	1 (1,8)	0 (0)	0 (0)
Clinical Type not defined (maligno, amelanótico, metastásico)	33 (53.2)	11 (40.7)	37 (66,0)	3 (75,0)	3 (60,0)

* 2 gluteus, 1 vulva, 1 vagina, 1 scrotum, 1 pubis and 1 anus cases.

Note: Some wild type results reported with V600E/E2/D*, have additional explanations: "It is possible to miss the V600K/R/M mutation with DNA <5% in the sample tested"

Citation: Erika P, Claudia B, Clara A, et al. Frequency of BRAF V600E/E2/D and V600K/R/M mutations in patients with malignant melanoma unresectable stage III-IIIC–IV from May to August-2021 at Unigem in Medellín. *Int J Mol Biol Open Access*. 2023;6(1):8–12. DOI: 10.15406/ijmboa.2023.06.00146

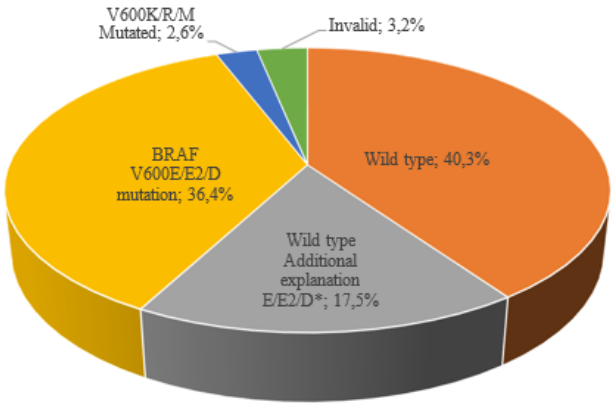

Figure 1 BRAF V600 mutational status distribution in patients with malignant melanoma.

Note: E/E2/D* Some wild type results reported with V600E/E2/D*, have additional explanations: to miss the V600K/R/M mutation with D

Discussion

Melanoma is responsible for the high mortality of skin cancer, due to this, a large number of investigations have been carried out, with the aim of clarifying the clinical-pathological and genetic mechanism of this entity. This allowed us to understand the value of mutations in the BRAF gene (exon 15, codon 600) as a marker in the choice of therapy for patients with malignant melanoma. We provide a description of the BRAF status in patients diagnosed with melanoma.

Of the total sample, 62.3% were men, and male pre-dominance was maintained in all melanoma subtypes, being more marked in nodular and acral lentiginous (26.0 and 12.5%, respectively). This differs slightly from that reported in the literature, where there is usually a slightly higher incidence in women and in Lentigo Maligna, results that do not agree with previous local studies[10,11] but do with international research[12,13] Similar to that reported globally are also observed in relation to the mean age which ranges between 57-63 years of age[14,15] however a study conducted in Japan reported an increase in cases in populations under 50 years[9] as illustrated in Table 1.

Although this study is not based on incidence, it is important to highlight that nodular melanoma was the most common histological type, followed by Acral lentiginous, with a histological representation similar to that reported in a study carried out in Bucaramanga-Colombia.[16] These results differ from studies that report a greater number of cases for superficial Spreading melanoma and Acral lentiginous melanoma, while the proportion of Lentigo maligna melanoma is below the reported values[6,17] The most frequent anatomical site was the lower limbs, as described[15,16] however several publications report the trunk as the most common site[13,15,18] followed by upper limbs; other authors have reported lower limbs as the most common site.

The Breslow and Clark are considered the most important parameters for the prognosis of melanoma, because the survival of patients is closely related to the invasion of the tumor, therefore an early diagnosis is essential to reduce the presentation of metastases. Median survival time for melanoma patients with metastatic disease is 8 to 9 months,and the 3-year overall survival rate is less than 15%.[19]

Among the cases reported as unspecified malignant melanoma, three cases diagnosed as amelanotic melanoma were found, two of which had a mutated state for BRAF E/E2/D (2 in the lower limbs and 1 in the arm). This type of melanoma is one of the most difficult to identify due to the atypical presentation characteristics, for this reason the cases are diagnosed in advanced stages (the three cases were in the advanced Breslow and Clark stage). This type of lesion is located more frequently in the trunk, head and neck, and in the lower part of the extremities and has a worse prognosis compared to patients with pigmented metastases.[20] The frequency of mutations in codon 600 of BRAF in patients with melanoma ranges from 40-60%. In general, BRAF V600 mutations were detected in 39%, a value below the range reported by other Latin American countries[21,22] the United States[13] and Europe.[12] 93.3% (56/60) of the mutated cases corresponded to the V600E/E2/D mutation, a value consistent with that reported by other studies.[9,23]

The literature reports that there is a non-concordance between the presence of the BRAF V600E mutation and age, while some studies have shown that this mutation is frequent in younger patients, others show the opposite. In our case, we observed a higher frequency of BRAF V600E/E2/D in patients under 63 years of age (73.2%)[14] while the BRAF V600K/R/M mutation had the same number of cases in both groups of ages. Most of the pathological anatomy results do not describe the Breslow, Clark, Edges/Margins and Ulceration variables, including some of the reports, only documenting the site where the tumor is located and the diagnosis of "malignant melanoma"; this was a great limitation for the research, preventing the exploration of the association of these variables with mutational status.

Conclusion

Exploring the genomic panorama has allowed us to know the mutational profile in patients with Type III-IIIC-IV metastatic melanoma in our population and its high impact for timely establishment with the choice of therapy for the signaling pathways affected in patients with this disease. According to studies published in the country, our research contains one of the largest populations studied. We found that in the population analyzed with metastatic melanoma, 38.9% of the patients presented alterations in the BRAF gene, more frequently in men, in the V600 E/E2/D codon and in the age group ≤63 years old, mainly in lesions originating in the trunk and upper extremities; values consistent with the international literature found.

Acknowledgments

We thank the patients for having authorized the use of the information for this study, providing the opportunity to learn about the behavior of cancer that may be useful for the benefit of the scientific community and public health.

Conflicts of interest

The authors declare that there is no conflicts of interest that may affect the validity of the information.

References

1. Longvert C, P Saiag. News in cutaneous melanomaMelanoma update. *La Revue de medecine interne*. 2019;40(3):178–183.
2. Arias VC, Häsbleidy VRA, Juan David RC, et al. Epidemiological aspects of skin cancer diagnosed in 2012 in 4 reference centers in Popayán, Colombia: an observational cross-sectional study. *Revista Colombiana de Cirugía Plástica y Reconstructiva*. 2016;22(1):36–43.
3. Leonardi GF, Luca F, Rossella S, et al. Cutaneous melanoma: from pathogenesis to therapy. *Int J Onco*. 2018;52(4):1071–1080.

4. de Vries EA, Julio R Amador, Carlos Javier Rincon, et al. Cutaneous melanoma attributable to solar radiation in Cali, Colombia. *Int J cancer.* 2017;140(9):2070–2074.
5. Ferlay JE. Global cancer observatory. *Cancer today*. 2019.
6. Pozzobon FC, Alvaro EA, Juan SC. Skin cancer in Colombia: figures from the National Institute of Cancerology. *Journal of the Colombian Association of Dermatology and Dermatological Surgery.* 2018;26(1):12–17.
7. Piamo Morales AJ. Diagnostic, therapeutic and prognostic implications of the BRAF V600E mutation in cutaneous melanomas. *Revista Información Científica*. 2019;98(3):413–424.
8. Cantwell-Dorris ER, John O, Orla M Sheils. BRAFV600E: implicaciones para la carcinogénesis y la terapia molecular. *Mol Cancer Ther*. 2011;10(3):385–394.
9. Sakaizawa KA, Atsuko A, Aya Uchiyama, et al. Clinical characteristics associated with BRAF, NRAS and KIT mutations in Japanese melanoma patients. *J Dermatol Sc*. 2015;80(1):33–37.
10. Puentes C, Estrada A, Bohórquez M, et al. Melanoma: clinical-pathological and molecular analysis in patients of Ibague city, Colombia. *Duazary*. 2020;17(1):5–18.
11. Gutiérrez-Castañeda LD, Mauricio G, John AN, et al. Mutations in the BRAF, NRAS, and C-KIT genes of patients diagnosed with melanoma in Colombia population. *BioMed Res Int*. 2020:2046947.
12. Gaiser MR, Alexander S, Diana G, et al. Variables that influence BRAF mutation probability: A next-generation sequencing, non-interventional investigation of BRAFV600 mutation status in melanoma. *PLoS One*. 2017;12(11):e0188602.
13. Broekaert SM, Ritu Roy, Ichiro Okamoto, et al. Genetic and morphologic features for melanoma classification. *Pigment cell & melanoma res*. 2010;23(6):763–770.
14. Kang XZ, Ying Zeng, Junqin Liang, et al. Aberrations and clinical significance of BRAF in malignant melanoma: A series of 60 cases in Chinese Uyghur. *Medicine (Baltimore)*. 2018;97(1):e9509.
15. Adler NR, Rory Wolfe, John W Kelly, et al. Tumour mutation status and sites of metastasis in patients with cutaneous melanoma. *Br J Cancer*. 2017;117(7):1026–1035.
16. Reyes E, Claudia U, Esther de V. Population□based incidence and melanoma□specific survival of cutaneous malignant melanoma in a Colombian population 2000–2009. *Int J Dermatol*. 2018;57(1):21–27.
17. Yamazaki NT, Ryota T, Arata T, et al. BRAF V600 mutations and pathological features in Japanese melanoma patients. *Melanoma Res*. 2015;25(1):9–14.
18. García AL, Constanza L, Boris E, et al. BRAF mutational status and histopathological parameters in cutaneous malignant melanoma. *Medicina (B Aires)*. 2019;79(4):265–270.
19. Choi DY, Sang PL, Sanghui Park. Prevalence of BRAF and NRAS mutations and a comparative analysis in primary and metastatic melanoma of Korean patients. *Ewha Med J.* 2014;37(1):30–35.
20. Cabrera R, Francisca R. Unusual clinical presentations of malignant melanoma: a review of clinical and histologic features with special emphasis on dermatoscopic findings. *Am J Clin Dermatol*. 2018;19(Suppl 1):15–23.
21. Fajardo-Ramírez ÓR, Julio C Salas-Alanis, Guzmán-Huerta E. et al. BRAF mutations among patients from the Northeast of México with malignant melanoma. Rev Invest Clin. 2014;66(3):288–290.
22. Mazzei ME, Jimena H, Gonzalo M, et al. Determinación de la mutación BRAF V600E en melanomas de pacientes uruguayos. *Rev. Méd. Urug*. 2013;29(2):97–102.
23. Lovly CM, Kimberly BD, Laurel E F, et al. Routine multiplex mutational profiling of melanomas enables enrollment in genotype-driven therapeutic trials. *PloS One*. 2012;7(4):e35309.

Citation: Erika P, Claudia B, Clara A, et al. Frequency of BRAF V600E/E2/D and V600K/R/M mutations in patients with malignant melanoma unresectable stage III-IIIC–IV from May to August-2021 at Unigem in Medellín. *Int J Mol Biol Open Access*. 2023;6(1):8–12. DOI: 10.15406/ijmboa.2023.06.00146