Association of KRAS, NRAS, and BRAF Mutations with Clinicopathologic Characteristics in Algerian Colorectal Cancer Patients

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Abstract

We conducted a study investigating the mutational profiles of KRAS, NRAS, and BRAF biomarkers in metastatic colorectal cancer (mCRC) patients and their correlation with clinicopathologic characteristics. Our study aimed to identify the primary cause of acquired resistance to anti-EGFR treatment in mCRC patients and to investigate the frequency and distribution of KRAS, NRAS, and BRAF mutations in mCRC patients.

We employed real-time PCR and direct sequencing for molecular biology analysis and immunohistochemistry to determine Micro Satellite Instability (MSI) status. Using XLSTAT software (version 2016.02.28451), we evaluated statistical tests. We found that KRAS, NRAS, and BRAF mutations were present in 48.7%, 5.54%, and 7.19% of the cases, respectively, with G12D, G12V, and G13D being the most common KRAS mutation subtypes identified. KRAS and BRAF mutations were more frequent in elderly patients and the left colon (p=0.004 vs. p=0.04 and p=0.001 vs. p>0.0001, respectively) and were significantly associated with a well-differentiated histological type (p=0.001 vs. p=0.02). However, we could not establish a significant relationship between NRAS mutation, MSIs, and patient-specific characteristics. Our study revealed that mutations in KRAS occurred more frequently outside of exon 2, and the left colon was the predominant location for mutations in both KRAS and BRAF.

Our findings could help oncologists identify prognostic and predictive biomarkers for selecting appropriate targeted patient therapies. Colorectal cancer is prevalent worldwide, and molecular epidemiology is increasingly crucial in personalized medicine.

Keywords: colorectal cancer, immunohistochemistry, real-time PCR, sequencing, CRC, KRAS, NRAS, BRAF, MSI.

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Introduction

Colorectal cancer (CRC) is the second deadliest and third most common malignancy worldwide. In 2020, 1.9 million new cases of CRC and 935,173 deaths were reported, representing nearly 10% of new cancer cases and deaths worldwide [1]. Algeria recorded almost 6,500 new cases of CRC, including 3,500 cases in men and 3,000 cases in women, according to the cancer registries of the National Institute of Public Health. This cancer ranks second among all types of cancer in Algeria, first in men, then lung cancer, and second in women after breast cancer [2].

Thanks to the development of primary and adjuvant therapies, the survival of patients with metastatic disease has improved significantly. Generally, complete tumor eradication remains the ideal treatment, mainly requiring surgery [3]. However, despite the emergence of many screening programs to reduce the incidence of CRC, nearly a quarter of CRC cases are diagnosed at a late stage with metastases, and 20% of cases can develop metachronous metastases, leading to difficulties in curative surgical control and subsequent cancer deaths [4]; [5]. The prognosis for patients with metastatic colorectal cancer (mCRC) remains bleak despite the impressive improvement observed in the last 20 years due to the introduction of active chemotherapy drugs [6]; [7] and targeted drugs, particularly agents that block the epidermal growth factor receptor (EGFR), such as the anti-EGFR monoclonal antibodies cetuximab or panitumumab, which present an effective therapeutic option [8]; [9].

Activation of EGFR triggers various downstream signaling pathways, RAS-RAF-BRAF-MAPK and phosphatidylinositol 3-kinase (PI3K)-AKT, which are involved in cell proliferation or metabolism and play a vital role in the initiation and progression of colorectal cancer [10]; [11]; [12]; [13]. RAS proteins are proto-oncogenes frequently mutated in human cancers. Three genes encode them expressed ubiquitously HRAS, KRAS, and NRAS. GTPases are proteins functioning as molecular switches regulating cell proliferation and survival pathways. Aberrant KRAS and NRAS function is associated with hyperproliferative developmental disorders and cancer. KRAS is the most frequently mutated isoform, detected in 22% of all cancers analyzed. NRAS and HRAS are found in 8% and 3% of cancers, respectively [14]. Mutation of the B-type proto-oncogene RAF (BRAF) is a new biomarker gaining interest due to its association with poor prognosis. However, it currently has no clear predictive role in guiding therapeutic decisions. This BRAF mutation, although rare, is detected in 5 to 10% of metastatic CRCs [15]; [16].

Distant metastases pose a significant problem in CRC patients after surgery as they are associated with high morbidity and mortality. Treatment decisions are usually based on patients' prognostic characteristics, including traditional clinicopathological characteristics such as TNM stage, degree of histopathological differentiation, invasion of surrounding tissues, and the number of lymph node metastases, as well as microsatellite instability (MSI). The DNA-MMR system comprises four MMR genes and their encoded proteins (MLH1, MSH2, MSH6, and PMS2). Deficiency in MMR leads to the production of a non-functional truncated protein or loss of a protein that causes MSI [17].

Molecular pathological epidemiology can be a powerful tool to decipher treatment outcomes based on molecular alteration patterns. Optimization of initial treatment and continuous exposure therapies have achieved maximum effectiveness. However, a paradigm shift is underway towards precision medicine and personalized therapies based on the specific molecular characteristics of the disease. In this context, improving our understanding of the biology and genetics of colorectal cancer would enable researchers to define better predictive biomarkers that would help oncologists select the most appropriate new targeted strategies and therapies for patients. This work focuses on the molecular profiles of some of the most promising biomarkers in daily practice, particularly KRAS, NRAS, BRAF, and microsatellite instability, as prognostic and predictive response factors to targeted therapies in metastatic colorectal cancer in Algerian patients.

Material and methods

Sample

Our study focused on a prospective series of 946 cases from CRC patients collected at the CHU Mustapha Department of Pathological Anatomy and Cytology (Algiers). They were evaluated between (2017-2021) after the approval of the Ethics Committee of Mustafa Bacha University

Hospital, Algiers, Algeria, and all patients provided written informed consent before enrollment. Clinicopathological characteristics recorded and analyzed included age, sex, primary tumor location, histological type, tumor size, and stage. All formalin-fixed paraffin-embedded (FFPE) tissue sections were tested in tumor areas with at least 10% tumor cells but without significant necrosis or inflammation, as determined by a pathologist onward. 946 patients were enrolled for genetic detection of KRAS, 595 NRAS, 556 BRAF, and 110 MSI due to treatment strategy and availability of specific kits.

Molecular analysis

Molecular biology research focuses on cases of metastatic colorectal cancer (mCRC). It consists of assessing the status of mutations (KRAS, NRAS, and BRAF) using real-time PCR (Idylla and Sacace platforms) and 130 cases were tested in London, UK by a semi-conductor based on next-generation high-throughput sequencing of the PGM Ion Torrent (due to missing Idylla machine cartridges at that time). The Idylla KRAS and NRAS mutation test (Biocartis, Mechelen, Belgium), carries the CE-IVD mark and allows the characterization of 23 RAS mutation hotspots in exons 2, 3, and 4, namely G12D, G12A, G12C, G13D, G12S, G12 V, and G12R, A59T/E/G, Q61H, Q61K, Q61R/L, K117N, and A146P/T/V. In addition, the Idylla™ BRAF Mutation Test uses multiple PCR reactions and allows amplification of exon 600 of the BRAF oncogene. This Idylla testing does not require prior deparaffinization, DNA quantification, or genomic DNA isolation because these steps and all PCR reactions are fully automated and performed in a single-use cartridge. FFPE tissue sections of 5-10 µm thick, selected by the pathologist, should be cut and placed directly into the Idylla cartridge. After two hours, the results were obtained.

For the routine RT-PCR search for mutations (KRAS, NRAS, and BRAF), genomic DNA was extracted and purified from FFPE tissue sections using the "SaMag FFPE DNA Extraction, Sacace Biotechnologies, Italy" kit according to the manufacturer's instructions. The DNA was amplified using the "Sacace, Biotechnologies, COMO, Italy" Sa-Cycler 96 Real-Time PCR system. The reactions were carried out in 30µl of the "Entrogen's colorectal Cancer Mutation Detection Panel" kit, which included all of the reagents and Taq Man probes required for the amplification and specific detection of each of the mutations to be tested. The reactions were heated at 95°C for 12 minutes, then cycled 40 times for 15 seconds. Finally, the reactions were kept at 60°C for 40 seconds.

Immunohistochemical analysis

We performed immunohistochemical analysis on tissue samples collected from 110 patients with colorectal cancer. We established the tumor mismatch repair status (MSS or MSI) by using immunohistochemistry (IHC) to detect intact expression or loss of MMR proteins (MLH1, MSH2, MSH6, and PMS2). We evaluated the IHC study on 4 µm thick formalin-fixed

paraffin-embedded (FFPE) tumor tissue sections. We incubated, deparaffinized, rehydrated, and washed the slides. To unmask the antigen, we immersed the slides in a preheated TRS buffer solution at pH 7.0 in a water bath at a temperature between 97°C to 100°C, cooled them to room temperature for 20 min, and rinsed them with distilled water. We blocked non-specific antigenic sites by using hydrogen peroxide H2O2 at room temperature for 20 min, then rinsing with distilled water and PBS for 5 min (each). We sufficiently applied the primary antibodies and incubated the slides at room temperature for 55 min, then rinsed them with PBS for 5 min (twice).

We added the secondary antibodies to the slides for 20 min at room temperature, then rinsed them with PBS for 5 min (twice). We revealed the sections using the DAB detection kit for 5 to 10 min, rinsed them, immersed them in Mayer's hematoxylin, then rinsed them with running water. We used primary mouse monoclonal antibodies against MMR, including anti-MLH1 ((G168-728), Cell Marque), anti-MSH2 ((G219-1129), Cell Marque), anti-MSH6 (SP93, Cell Marque), anti-PMS2 (EPR3947, Cell Marque), and Leica, to identify the MSI status.

Statistical analysis

We performed statistical analysis using XLSTAT software (version 2016.02.28451). We compared qualitative variables using the Chi-square test or Fisher's exact test. We tested correlations between mutational status and clinicopathological characteristics using the Chi-square test. We considered all p-values less than 0.05 as statistically significant.

Results

Clinicopathologic characteristics of patients

The mean age of colorectal cancer patients is 57.9 years, with extreme values between (15 and 93) years, and a slight male predominance has been observed. The majority of tumor lesions (57.4 %) are well-differentiated adenocarcinomas. Primary tumors were found in the left colon in 47.6 % of cases (450/946) and were diagnosed as stage III in 73.4 % of cases (Table 1).

Table 1: Clinicopathological characteristics of patients

Characteristics	*N (%)*
Gender	
Men	*515 (54.4%)*
Women	*431 (45.6%)*
Age (y)	

<50	*236 (24.9%)*
≥50	*710 (75.1%)*
Tumor site	
Right Colon	*188 (19.9%)*
Left Colon	*450 (47.6%)*
Rectum	*246 (26%)*
Metastasis	*62 (6.5%)*
Histological type	
WDA	*543 (57.4%)*
MDA	*264 (27.9%)*
PDA	*70 (7.4%)*
MC	*69 (7.3%)*
Tumor size	
<5cm	*132 (49.8%)*
≥5cm	*133 (50.2%)*
NI (biopsy)	*681*
Pathological stage	
I	*3 (1%)*
II	*16 (5.3%)*
III	*224 (73.4%)*
IV	*62 (20.3%)*
NI	*641*

WDA: well differentiated adenocarcinoma; MDA: moderately differentiated adenocarcinoma; PDA: poorly differentiated adenocarcinoma; MC: mucinous carcinoma. NI: Not indicated.

Tumor genotyping and correlations of mutations with clinicopathological characteristics of patients

Among the 946 tested CRC samples, the KRAS gene was mutated in 48.7% (461) cases. The most frequent hotspots were in exon 2 (83.3%), exon 3 (6.5%), and exon 4 (10.2%). The primary mutations were G12D, found in 24.1% (101) of samples, followed by G12V, found in 19.9% (92) of mutated cases, and G13D, found in 13% (60) of mutated cases (Table 2).

The presence of KRAS mutations was not associated with sex (p=0.692). The frequency of KRAS mutations was significantly higher in older subjects (≥50 years) (p=0.004). Tumors in the mutated KRAS group were more frequently located in the left colon (p=0.001) and were of well-differentiated histological type (p=0.001) (Table 3).

Table 2: Frequency and distribution of KRAS, NRAS and BRAF mutations.

A: Gene KRAS

Exon	*Nucleotide substitution*	*Codon substitution*	*Amino acid substitution*	*Number*	*%*
2	*c.35G>A*	*GGT>AGT*	*p.G12D*	*101*	*24.1%*
	c.35G>T	*GGT>GTT*	*p.G12V*	*92*	*19.9%*
	c.34G>T	*GGT>TGT*	*p.G12C*	*35*	*7.6%*
	c.35G>C	*GGT>GCT*	*p.G12A*	*25*	*5.4%*
	c.34G>C	*GGT>CGT*	*p.G12R*	*11*	*2.4%*
	c.34 G> A	*GGT>AGC*	*p.G12S*	*10*	*2.2%*
	c.38 G>A	*GGC>GAC*	*p.G13D*	*60*	*13%*
	N.I	*N.I*	*p.G12* or G13**	*50*	*10.8%*
3	*c.175G>A/ c.176C>A/ c.176C>G*	*p.Ala59Thr/ p.Ala59Glu/ p.Ala59Gly*	*A59T/E/G*	*6*	*1.3%*
	c.183A>C	*CAA>GCT*	*p. Q61H*	*12*	*2.6%*
	c.182A>G	*CAA> CGT*	*p.Q61R*	*1*	*0.2%*
	c.182A>T/ c.183A>C	*c.182A>G/CAA>CTA/ CAA>CTA*	*p.Q61L/R/H*	*10*	*2.2%*

	N.I	N.I	p. A59*/ p.Q61*	1	0.2%
4	c.351A>T	AAA>AAT	p.K117N	5	1.8%
	c.436G>C	GCA>ACA	p.A146T	9	1.9%
	c.436G>A/ c.436G>C/ c.437C>T	GCA>ACC/ GCA>ACA/ GCA>GTA	p.A146P/T/V	33	7.2%

B: Gene NRAS

Exon	Nucleotide substitution	Codon substitution	Amino acid substitution	Number	%
2	c.34G>T	GGT>TGT	p.G12C	2	6.1%
	c.35G>A	GGT>AGT	p.G12D	1	3%
	c.35G>T	GGT>GTT	p.G12V	1	3%
	c.35G>C	GGT>GCT	p.G12A	1	3%
	c.38G>A	GGC>GAC	p.G13D	3	9%
	c.37G>C	GGC>CGC	p.G13R	1	3%
	N.I	N.I	p.G12*/G13*	1	3%
3	c.182A>G	CAA> CGA	p.Q61R	10	30.3%
	c.181C>A	CAA>AAA	p.Q61K	2	6.1%
	c.182A>T	CAA>CTA	p.Q61L	2	6.1%
	c.183A>C	CAA>GCT	p.Q61H	2	6.1%
	c.182A>T/ c.182A>G/ c.183A>C	CAA>CTA/ CAA>CGA/ CAA>CTA	p.Q61L/R/H	6	18.2%
4	c.351A>T	AAA>AAT	p.K117N	1	3%

C: Gene BRAF

Exon	Nucleotide substitution	Codon substitution	Amino acid substitution	Number	%

11	*c.1396G > A*	*GGA> GAA*	*p.G466E*	*1*	*2.5%*
	c.1405G>A	*GGA>CGA*	*p.G469R*	*1*	*2.5%*
15	*c.1799T>A*	*(GTG>GAG)*	*p.V600E*	*3*	*7.5%*
	c.1799T>A / c.1799_1800delinsAT	*GTG>GAG/ GTG>AGT*	*p.V600E /p.V600D*	*34*	*85%*
	N.I	*N.I*	*p.W604C*	*1*	*2.5%*

In 62.9% (595/946) of the samples, we analyzed the NRAS mutation status and found that only 5.54% (33/595) of patients carried NRAS mutations. The most common mutation was Q61R, present in 30.3% of mutated cases. When analyzing clinicopathological data, no significant correlation was found between NRAS-mutated status and specific patient characteristics (Table 3).

We detected the BRAF mutation status in 58.7% (556/946) of cases and found that 7.19% (40/556) of patients had BRAF mutations, with 92.5% (37/40) of cases occurring in exon 15 and 7.5% (3/40) of cases in exon 11. The most frequent BRAF mutation subtypes were V600E/p.V600D, found in 85% of mutated cases. The researchers found that BRAF mutation was frequently observed in older subjects (≥50 years) (15% vs. 85%, p=0.04) and in the left colon (p>0.0001). Tumors with BRAF mutations were statistically associated with the well-differentiated adenocarcinoma histological subtype (p=0.02). However, no significant associations existed with other clinicopathological characteristics, such as sex and tumor size (Table 3).

Table 3: Correlation of RAS and BRAF mutations with clinicopathological characteristics of patients.

Features	*KRAS Wild-Type(WT)*	*KRAS Mutant*	*NRAS WT*	*NRAS M*	*BRAF WT*	*BRAF M*
Gender	*P = 0.6*		*P = 0.9*		*P = 0.1*	
Men	*261*	*254*	*310*	*18*	*291*	*18*
	-53.80%	*-55.10%*	*-55.20%*	*-54.50%*	*-56.40%*	*-45%*
Women	*224*	*207*	*252*	*15*	*225*	*22*
	-46.20%	*-44.90%*	*-44.80%*	*-45.50%*	*-43.40%*	*-55%*
Total	*485*	*461*	*562*	*33*	*516*	*40*

Age (y)	***P = 0.004***		*P = 0.9*		***P = 0.04***	
<50	*140* *-28.90%*	*96* *-20.80%*	*157* *-27.90%*	*9* *-27.30%*	*153* *-29.70%*	*6* *-15%*
≥50	*345* *-71.10%*	*365* *-79.20%*	*405* *-72.10%*	*24* *-72.70%*	*363* *-70.30%*	*34* *-85%*
Total	*485*	*461*	*562*	*33*	*516*	*40*
Tumor site	***P = 0.018***		*P = 0.2*		***P < 0.001***	
Right Colon	*83* *-17.10%*	*105* *-22.80%*	*107* *-19%*	*4* *-1.10%*	*84* *-16.30%*	*20* *-50%*
Left Colon	*224* *-46.20%*	*226* *-49.00%*	*256* *-45.60%*	*18* *-54.60%*	*236* *-75.70%*	*15* *(37.5%*
Rectum	*139* *-28.60%*	*107* *-23.20%*	*155* *-27.60%*	*11* *-33.30%*	*157* *-30.40%*	*3* *-3.50%*
Metastasis	*39* *(8,0%)*	*23* *-5%*	*44* *-7.80%*	*0*	*39* *-7.60%*	*2* *-5%*
Total	*485*	*461*	*562*	*33*	*516*	*40*
Histologic-al Type	***P = 0.001***		*P = 0.6*		*P = 0.1*	
WDA	*266* *-54.80%*	*277* *-60.10%*	*312* *-55.50%*	*17* *-51.50%*	*292* *-56.60%*	*16* *-40%*
MDA	*131* *-27%*	*133* *-28.80%*	*154* *-27.40%*	*12* *-36.40%*	*139* *-26.90%*	*13* *-32.50%*
PDA	*52* *-10.70%*	*18* *-3.90%*	*56* *-10%*	*3* *-9.10%*	*52* *-10.10%*	*6* *-15%*
MC	*36* *-7.40%*	*33* *-7.20%*	*40* *-7.10%*	*1* *-3%*	*33* *-6.40%*	*5* *-12.50%*

Total	*485*	*461*	*562*	*33*	*516*	*40*
Tumor Size	*P = 0.8*		*P = 0.1*		*P = 0.4*	
>5cm	*65* *-50.80%*	*67* *-48.90%*	*81* *-52.9*	*2* *-25%*	*70* *-51.10%*	*10* *-62.50%*
≤5cm	*63* *-49.20%*	*70* *-51.10%*	*72* *-47.10%*	*6* *-75%*	*67* *-48.90%*	*6* *-37.50%*
NI (biopsy)	*357* *0%*	*324* *0%*	*409* *0%*	*25* *0%*	*379* *0%*	*24* *0%*
Total	*485*	*461*	*562*	*33*	*516*	*40*
Pathologic -al stage	*P = 0.9*		*P = 0.8*		***P = 0.02***	
I	*2* *-1.30%*	*1* *-0.70%*	*2* *-1.10%*	*0*	*0*	*1* *-6.30%*
II	*8* *-5.20%*	*8* *-5.30%*	*8* *-4.40%*	*0*	*4* *-2.50%*	*0*
III	*115* *-74.20%*	*109* *-72.70%*	*136* *-76.40%*	*9* *-81.80%*	*126* *-78.70%*	*11* *-68.7*
IV	*30* *-19.30%*	*32* *-21.30%*	*34* *-19.1*	*2* *-18.20%*	*30* *-18.80%*	*4* *-25%*
NI	*330* *0%*	*311* *0%*	*384* *0%*	*20* *0%*	*356* *0%*	*24* *0%*
Total	*485*	*461*	*562*	*33*	*516*	*40*

WT: wild-type; M:mutant.

Correlations were tested by the Chi-square test (Chi²). P values less than 0.05 were considered statistically significant.

We analyzed 110 tumors and found that 8.18% (9/110) of patients had microsatellite instability, with 6.36% (7/110) of patients having an MSI-H phenotype and 1.81% (2/110) of patients

having an MSI-L phenotype (see Figure 2). Most cases were MSS, accounting for 91.81% (101/110) (see Figure 1). We did not observe any significant correlations between the clinicopathological characteristics of patients and the different KRAS, NRAS, and BRAF mutations and MSI-H status (Table 4).

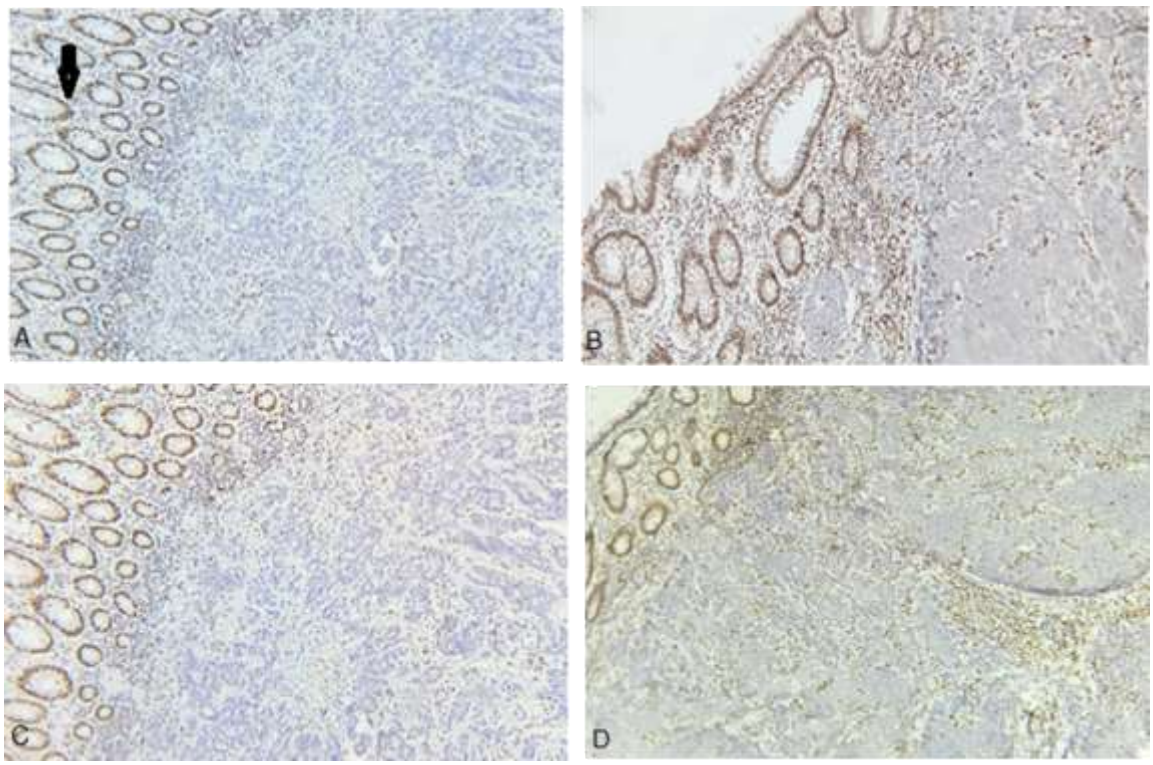

Figure 01: (X10) The immunohistochemical profile shows that proteins MLH1(A), MSH2(B), MSH6(C) and PMS2 have lost their nuclear expression (D). The arrow represents a positive internal control.

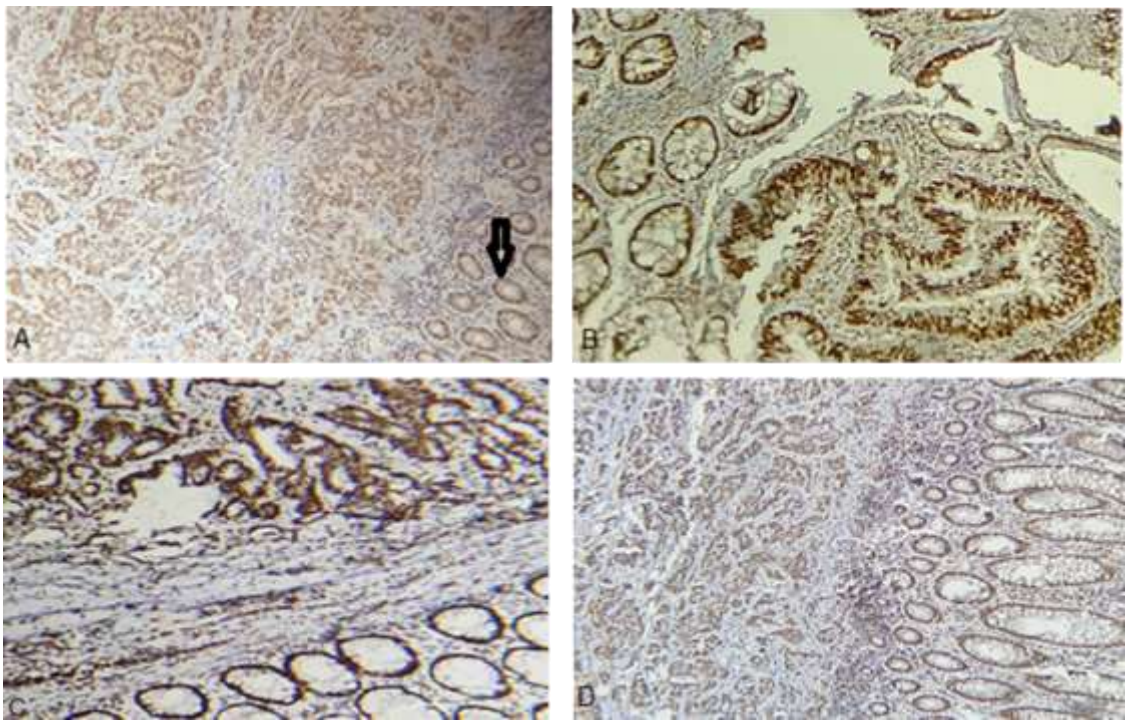

Figure 02: (x10) The immunohistochemical profile shows that the proteins MLH1(A), MSH2(B), MSH6(C), and PMS2 (D) are highly expressed in the nucleus. The arrow represents positive internal control.

Table 4: Correlation of MSI with clinicopathological characteristics of patients and different mutations.

Features	*MSS/MSI-L*	*MSI-H*	*Total*	*P-value*
Gender				*0.9*
Men	*62 (60.2%)*	*4 (57.1%)*	*66*	
Women	*41 (39.8%)*	*3(42.9%)*	*44*	
Total	*103*	*7*	*110*	
Age (y)				*0.1*
< 50	*29 (28.2%)*	*4 (57.1%)*	*33*	
≥ 50	*74 (71.8%)*	*3 (42.9%)*	*77*	
Total	*103*	*7*	*110*	
Tumor site				*0.2*
RC	*33 (32%)*	*5 (71.4%)*	*38*	

LC	*50 (48.6%)*	*2 (28.6%)*	*52*	
R	*16 (15.5%)*	*0*	*16*	
M	*4 (3.9%)*	*0*	*4*	
Total	*103*	*7*	*110*	
Histological type				*0.8*
WDA	*52 (50.5%)*	*4 (57.1%)*	*56*	
MDA	*34 (33%)*	*2 (28.6%)*	*36*	
PDA	*9 (8.7%)*	*0*	*9*	
MC	*8 (7.8%)*	*1 (14.3%)*	*9*	
Total	*103*	*7*	*110*	
Tumor size				*0.3*
< 5cm	*11 (21.6%)*	*2 (40%)*	*13*	
≥ 5cm	*40 (78.4%)*	*3 (60%)*	*43*	
NI	*52 (0%)*	*2 (0%)*	*54*	
Total	*103*	*7*	*110*	
Pathological stage				*0.9*
I	*1 (1.8%)*	*0*	*1*	
II	*5 (8,.%)*	*0*	*5*	
III	*40 (71.4%)*	*4 (80%)*	*44*	
IV	*10 (17.9%)*	*1 (20%)*	*11*	
NI	*47 (0%)*	*2 (0%)*	*49*	
Total	*103*	*7*	*110*	
KRAS				*0.9*
KRAS WT	*59 (57.3%)*	*3 (42.9%)*	*62*	
KRAS M	*44 (42.7%)*	*4 (57.1%)*	*48*	

Total	*103*	*7*	*110*	
NRAS				*0.4*
NRAS WT	*74 (90.2%)*	*6 (100%)*	*80*	
NRAS M	*8 (9.8%)*	*0*	*8*	
Total	*82*	*6*	*88*	
BRAF				*0.3*
BRAF WT	*58*	*6*	*64*	
BRAF M	*10*	*0*	*10*	
Total	*68*	*6*	*74*	

RC: right Colon; LR: left Colon; R: rectum; M: metastasis.

Discussion

Colorectal cancer accounts for a significant proportion of cancer cases globally and is a leading cause of cancer-related deaths. While the incidence of CRC in the Western world is low before age 50, it increases with age. In our study, patients had a mean age of 57.9 years (range: 15-93). This age is younger than that reported in a European study [18] but similar to Arab and African studies, where the mean age was 55.56 years in Morocco [19], 40 years in Egypt [20], and 46.7 years in Togo [21]. The literature, both nationally and internationally, usually reports a male predominance [18]; [22]; [23]; [24].

Our study found that well-differentiated adenocarcinomas were the most common histological type, accounting for 57.39% of cases. The proportions of moderately and poorly differentiated (35.90%) and mucinous (7.29%) adenocarcinomas were similar to those reported in the literature, with 27.5% and 7.8%, respectively [25]. Most of our patients had histological features associated with a good prognosis. However, the diagnosis of CRC was made late in most cases, with 73.44% at stage III and 20.33% at stage IV. Similarly, in Joachim et al.'s study, 62.4% of patients were diagnosed at stage III-IV, of whom 36.6% had metastases at diagnosis (stage IV) [26].

Nowadays, selecting patients with metastatic colorectal cancer for anti-EGFR targeted therapy commonly involves genetically analyzing hotspots of somatic mutations in the KRAS, NRAS, and BRAF genes [27]. KRAS gene mutations occur in 33-48% of tumors in colorectal cancer [28]. We found that the frequency of KRAS mutations in our series was 48.7% (461/946), which is similar to frequencies identified in Arab, European, Asian, and Latin American studies at 43% [29], (48.42%) [30], 42.9% [31], 48.9% [32], 43.4% [33] and

48.1% [34] respectively. KRAS mutations in exon 2 (codons 12 and 13) accounted for 83.3% of mutated cases, which is comparable to those reported in other studies from Jordan, Denmark, Morocco, and China at 84%, 86.1%, 88.3%, and 90%, respectively [30]; [19]; [35]; [36]. 16.7% of patients had KRAS mutations outside exon 2, with 30 (6.51%) having mutations in KRAS exon 3 and 47 (10.2%) in KRAS exon 4. Likely explained by the increased sensitivity of the tests used to determine KRAS mutational status. The rate of KRAS mutations in exon 4 (10.2%) is higher than those found in other preliminary studies at 1.9% [37], ~0.1% [38], 4.1% [39] . Guo et al. [39] recently found that patients with KRAS mutations in exon 3 predict a poor prognosis, while patients with mutations in exon 4 predict a better prognosis.

E21.9% of cases had the most frequent KRAS mutations, which were c.35>A (p.G12D), 19.95% of cases had c.35G>T (p.G12V), 7.59% of cases had c.34G>T (p.G12C), and 13% of cases had C.38G>A (p.G13D), which accounted for 62.44% of all mutated cases. In a recent Moroccan series [19], they identified these mutations in 75.4% of mutated cases.

Yuan (2021) [40] and Hayama (2019) [41] reported that the most common mutation subtypes in Europe and Asia are G12D, G12V, and G13D, which is consistent with our findings of G12D (32.19%), G12V (17.96%), and G13D (17.59%). Additionally, according to Agy (2021) [19] and Araujo (2021) [34] , the most frequent mutation subtypes at codon 12 were G12D (14.9%) and G12V (10.7%), followed by G12C (3.4%) in 167 samples.

The objective of somatic genetic analysis and epidemiology of KRAS alleles is to determine how the choice of KRAS allele affects the clinicopathological aspects of a given cancer. Researchers have extensively studied the prognostic value of KRAS mutations in various cancer contexts. For example, in colorectal cancer, KRAS-G12D, the most common allele, and KRAS-G12V mutations have been linked to poor overall survival compared to wild-type (WT) KRAS cancers patients, while codon 13 mutations are not [42]; [41]; [43]; [44]. In our series, we found that the prevalence of the KRAS-G12D point mutation was 23.9% of mutated cases.

ASP2453, a new KRAS G12C inhibitor, powerfully and selectively inhibited KRAS-G12C-induced tumor growth, making KRAS-G12C a promising target for new targeted therapies in solid tumors [45]. Researchers first reported the development of a selective KRAS-G12C inhibitor in 2013. Initially designed to bind to the Cys 12 residue in the switch II pocket, selective KRAS-G12C inhibitors block KRAS-G12C in an inactive GDP-bound state since KRAS is a GTPase. AMG510 and MRTX849 act similarly by binding in the switch II pocket [46] [47]. The prevalence of the KRAS-G12C mutation was 7.6% of KRAS-mutated cases in this study, suggesting that many patients could benefit from these targeted therapies. Although the frequency of the KRAS-G12C mutation in our series was lower than that reported in an Italian publication (12%) [47], it remained slightly higher than the frequency reported in a Taiwanese study (5.7%) [48]. Differences in mutation rates may be due to sample size variations.

Clinicians can better select patients for a given treatment by studying prognostic factors. Our study observed a higher prevalence of KRAS mutations in older subjects (≥50, p=0.004) [49]. However, an American study reported that KRAS mutations occur more frequently in younger subjects (<40) [50]. Agy et al. [19] identified that the left colon harbors more KRAS mutations, also observed in our series (p=0.001). Nevertheless, other studies have shown that KRAS mutations are frequent in the right colon [51]; [52]. Well-differentiated tumors were significantly associated with KRAS mutations, which was also confirmed in other studies [19]; [37]. Although no significant association was found between KRAS and TNM stage in our analysis [36]], other studies have shown a correlation between KRAS mutations and advanced TNM stage [19]; [53].

A distinct subgroup of mCRC patients with clinical and molecular differences are those with NRAS mutations, with 3 to 5% of CRCs presenting mutations in NRAS exon 2, 3, or 4. NRAS gene mutations were detected in 5.54% of cases in our analysis, consistent with studies published in the literature [30]; [48] but relatively high compared to that reported in another Chinese study (3.4%) [36]. However, an Iranian research group found a higher rate of NRAS mutations (14%) [54]. Our study found no significant correlation between NRAS mutation and different clinicopathological parameters, similar to results in French patients reported by Rimbert and colleagues [55].

The BRAF mutation plays a significant role in colorectal cancer (CRC). Recently, BRAF inhibitors, such as vemurafenib and dabrafenib, have revolutionized the treatment of metastatic melanoma. Researchers are currently studying their potential efficacy in treating metastatic CRC (mCRC). The scientific community is still debating the predictive value of BRAF mutation regarding chemoresistance. Identifying BRAF mutation as a predictive marker is difficult due to its low prevalence. BRAF mutations appear in 8% of all tumors and 5-12% of mCRCs [56]; [37]; [57]. We found that 7.19% of tested mCRC cases showed BRAF mutation status, consistent with Hernández-Sandoval et al. rate [58] in Latin American and Caribbean populations and lower than that reported in a Chinese population of 14.9% [59]. Interestingly, we discovered three BRAF mutations outside of exon 15.

Our study identified the clinicopathological characteristics of BRAF-mutated mCRC. Recent studies suggest that patients with BRAF mutation are more likely to be older [60]; [59]. Right-sided colon cancer has more BRAF mutations than left-sided colon cancer, which could lead to resistance to anti-EGFR targeted therapy [61] and worsen the prognosis [62]. We observed that BRAF mutations mainly localize in the left colon, contrary to several studies [54]; [60]; [63] . These BRAF-mutated tumors are also more frequently observed at an advanced stage [64].

It is recommended to analyze the MSI status of all resected colorectal cancers to identify MSI patients, including those with Lynch syndrome. High MSI (MSI-H) status is associated with a better prognosis in early-stage CRC and a lack of benefit from adjuvant 5-fluorouracil treatment

in stage II disease. MSI status has become a predictive biomarker for sensitivity to immunotherapy-based treatments, and checkpoint inhibitors have shown success in metastatic MMR-D CRC [65]. MSI-H CRCs have unique molecular and clinicopathological features, and research efforts are increasing [17]. The MSI phenotype was identified in 8.18% of patients, of whom 6.36% had MSI-H phenotype. This rate is lower than that reported in previous studies [66]. MSI tumors develop through a different mutational pathway than MSS tumors and exhibit distinctive pathological characteristics. However, no correlation was found between MSI status, clinicopathological characteristics, or other mutations.

Conclusion

We collected a series of samples at CHUMA's Department of Pathological Anatomy to provide an overview of the molecular profile of KRAS, NRAS, and BRAF mutations and their clinicopathological features in Algerian patients with metastatic colorectal cancer. Our findings indicate a higher rate of KRAS mutations outside of exon 2 than reported in other studies. Furthermore, we identified G12D, G12V, G13D, and G12C as the most common KRAS mutation subtypes. KRAS and BRAF mutations were more prevalent in elderly patients; in contrast to several previous studies, we found that they mainly occur in the left colon.

Our study is the first to analyze the frequency of KRAS, NRAS, and BRAF gene mutation subtypes in Algeria. By better understanding the genetic analysis of colorectal cancer, we can identify prognostic and predictive biomarkers, which would aid oncologists in selecting the most appropriate targeted therapies for their patients. Our findings suggest that the presence of mutations in KRAS (exons 2, 3, and 4), NRAS (exons 2, 3, and 4), and BRAF (exons 11 and 15) are associated with resistance to monoclonal antibodies against the epidermal growth factor receptor. Additionally, KRAS-G12C has emerged as a promising target for new targeted treatments in solid tumors. Furthermore, patients with advanced colorectal cancers carrying MSI-H may benefit from immunotherapy.

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References

1. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, **71** (3), 209–249.
2. Hamdi-Cherif, M. (2020) *Cancer Registries of the National Institute of Public Health (INSP)*.
3. Kuipers, E.J., Grady, W.M., Lieberman, D., Seufferlein, T., Sung, J.J., Boelens, P.G., Velde, C.J.H. van de, and Watanabe, T. (2015) Colorectal cancer. *Nature Reviews Disease Primers*, **1** (1).
4. Stok, E.P. van der, Spaander, M.C.W., Grünhagen, D.J., Verhoef, C., and Kuipers, E.J. (2016) Surveillance after curative treatment for colorectal cancer. *Nature Reviews Clinical Oncology*, **14** (5), 297–315.
5. Xie, Y.-H., Chen, Y.-X., and Fang, J.-Y. (2020) Comprehensive review of targeted therapy for colorectal cancer.*Signal Transduction and Targeted Therapy*, **5** (1).
6. Cutsem, E.V., Cervantes, A., Nordlinger, B., and Arnold, D. (2014) Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, **25**, iii1–iii9.
7. Sforza, V., Martinelli, E., Ciardiello, F., Gambardella, V., Napolitano, S., Martini, G., Corte, C. della, Cardone, C., Ferrara, M.L., Reginelli, A., Liguori, G., Belli, G., and Troiani, T. (2016) Mechanisms of resistance to anti-epidermal growth factor receptor inhibitors in metastatic colorectal cancer. *World Journal of Gastroenterology*, **22** (28), 6345.
8. Douillard, J.-Y., Oliner, K.S., Siena, S., Tabernero, J., Burkes, R., Barugel, M., Humblet, Y., Bodoky, G., Cunningham, D., Jassem, J., Rivera, F., Kocákova, I., Ruff, P., Błasińska-Morawiec, M., Šmakal, M., Canon, J.L., Rother, M., Williams, R., Rong, A., Wiezorek, J., Sidhu, R., and Patterson, S.D. (2013) Panitumumab–FOLFOX4 Treatment and RAS Mutations in Colorectal Cancer. *New England Journal of Medicine*, **369** (11), 1023–1034.
9. Cutsem, E.V., Lenz, H.-J., Köhne, C.-H., Heinemann, V., Tejpar, S., Melezínek, I., Beier, F., Stroh, C., Rougier, P., Krieken, J.H. van, and Ciardiello, F. (2015) Fluorouracil, Leucovorin, and Irinotecan Plus Cetuximab Treatment and RAS Mutations in Colorectal Cancer. *Journal of Clinical Oncology*, **33** (7), 692–700.
10. Arteaga, C.L., and Engelman, J.A. (2014) ERBB Receptors: From Oncogene Discovery to Basic Science to Mechanism-Based Cancer Therapeutics. *Cancer Cell*, **25** (3), 282–303.
11. Mizukami, T., Izawa, N., Nakajima, T.E., and Sunakawa, Y. (2019) Targeting EGFR and RAS/RAF Signaling in the Treatment of Metastatic Colorectal Cancer: From Current Treatment Strategies to Future Perspectives. *Drugs*, **79** (6), 633–645.
12. Siena, S., Sartore-Bianchi, A., Nicolantonio, F.D., Balfour, J., and Bardelli, A. (2009) Biomarkers Predicting Clinical Outcome of Epidermal Growth Factor ReceptorTargeted

Therapy in Metastatic Colorectal Cancer. *JNCI: Journal of the National Cancer Institute*, **101** (19), 1308–1324.

13. Zenonos, K. (2013) RAS signaling pathways, mutations and their role in colorectal cancer. *World Journal of Gastrointestinal Oncology*, **5** (5), 97.
14. Prior, I.A., Lewis, P.D., and Mattos, C. (2012) A Comprehensive Survey of Ras Mutations in Cancer. *Cancer Research*, **72** (10), 2457–2467.
15. Loupakis, F., Cremolini, C., Masi, G., Lonardi, S., Zagonel, V., Salvatore, L., Cortesi, E., Tomasello, G., Ronzoni, M., Spadi, R., Zaniboni, A., Tonini, G., Buonadonna, A., Amoroso, D., Chiara, S., Carlomagno, C., Boni, C., Allegrini, G., Boni, L., and Falcone, A. (2014) Initial Therapy with FOLFOXIRI and Bevacizumab for Metastatic Colorectal Cancer. *New England Journal of Medicine*, **371** (17), 1609–1618.
16. Taieb, J., Malicot, K.L., Shi, Q., Lorca, F.P., Bouché, O., Tabernero, J., Mini, E., Goldberg, R.M., Folprecht, G., Laethem, J.L.V., Sargent, D.J., Alberts, S.R., Emile, J.F., Puig, P.L., and Sinicrope, F.A. (2016) Prognostic Value of BRAF and KRAS Mutations in MSI and MSS Stage III Colon Cancer. *Journal of the National Cancer Institute*, **109** (5), djw272.
17. Popescu, R.C., Tocia, C., Brînzan, C., Cozaru, G.C., Deacu, M., Dumitru, A., Leopa, N., Mitroi, A.F., Nicolau, A., and Dumitru, E. (2021) Molecular profiling of the colon cancer in South-Eastern Romania. *Medicine*, **100** (1), e24062.
18. Turpin, A., Genin, M., Hebbar, M., Occelli, F., Lanier, C., Vasseur, F., Descarpentries, C., Pannier, D., and Ploquin, A. (2019) Spatial heterogeneity of KRAS mutations in colorectal cancers in northern France. *Cancer Management and Research*, **Volume 11**, 8337–8344.
19. Agy, F.E., Bardai, S. el, Otmani, I.E., Benbrahim, Z., Karim, I.M.H., Mazaz, K., Benjelloun, E.B., Ousadden, A., Abkari, M.E., Ibrahimi, S.A., and Chbani, L. (2021) Mutation status and prognostic value of KRAS and NRAS mutations in Moroccan colon cancer patients: A first report. *PLOS ONE*, **16** (3), e0248522.
20. Kassem, N.M., Emera, G., Kassem, H.A., Medhat, N., Nagdy, B., Tareq, M., Moneim, R.A., Abdulla, M., and Metenawy, W.H.E. (2019) Clinicopathological features of Egyptian colorectal cancer patients regarding somatic genetic mutations especially in KRAS gene and microsatellite instability status: a pilot study. *Egyptian Journal of Medical Human Genetics*, **20** (1).
21. Darré, T., Sonhaye, L., Tchaou, M., Kanassoua, K., Amadou, A., Bagny, A., N'Guiessan, A.A., Amegbor, K., and Napo-Koura, G. (2015) Histo-Epidemiological Profile of Digestive Cancers in Togo. *Journal of Cancer Research Updates*, **4** (3).
22. Amira, A.T., Mouna, T., Ahlem, B., Raoudha, A., Majid, B.H., Amel, H., Rachida, Z., and Nadia, K. (2014) Immunohistochemical expression pattern of MMR protein can specifically identify patients with colorectal cancer microsatellite instability. *TumorBiology*, **35** (7), 6283–6291.

23. Meddah, D., Meddah, B., Touil, A.T., Ghalek, M., and Sahraoui, T. (2009) Étude épidémiologique du cancer du côlon chez des patients de l'Ouest algérien. *Journal africain du cancer / African Journal of Cancer*, **1** (1), 31–35.
24. Richter, M., Sonnow, L., Mehdizadeh-Shrifi, A., Richter, A., Koch, R., and Zipprich, A. (2021) German oncology certification system for colorectal cancer relative survival rates of a single certified centre vs. national and international registry data. *Innovative Surgical Sciences*, **6** (2), 67–73.
25. Aissi, S. (2013) Somatic molecular changes and histo-pathological features of colorectal cancer in Tunisia. *World Journal of Gastroenterology*, **19** (32), 5286.
26. Joachim, C., Macni, J., Drame, M., Pomier, A., Escarmant, P., Veronique-Baudin, J., and Vinh-Hung, V. (2019) Overall survival of colorectal cancer by stage at diagnosis. *Medicine*, **98** (35), e16941.
27. Nagakubo, Y., Hirotsu, Y., Amemiya, K., Oyama, T., Mochizuki, H., and Omata, M. (2019) Accurate detection of KRAS, NRAS and BRAF mutations in metastatic colorectal cancers by bridged nucleic acid-clamp real-time PCR. *BMC Medical Genomics*, **12** (1).
28. Janowski, E., Timofeeva, O., Chasovskikh, S., Goldberg, M., Kim, A., Banovac, F., Pang, D., Dritschilo, A., and Unger, K. (2016) Yttrium-90 radioembolization for colorectal cancer liver metastases in KRAS wild-type and mutant patients: Clinical and ccfDNA studies. *Oncology Reports*, **37** (1), 57–65.
29. Zahrani, A., Kandil, M., Badar, T., Abdelsalam, M., Al-Faiar, A., and Ismail, A. (2014) Clinico-pathological study of K-ras mutations in colorectal tumors in Saudi Arabia. *Tumori*, (2014January-February).
30. Awidi, M., Ababneh, N., Shomaf, M., Fararjeh, F.A., Owaidi, L., AlKhatib, M., Tarawneh, B.A., and Awidi, A. (2019) KRAS and NRAS mutational gene profile of metastatic colorectal cancer patients in Jordan. *PLOS ONE*, **14** (12), e0226473.
31. Jones, R.P., Sutton, P.A., Evans, J.P., Clifford, R., McAvoy, A., Lewis, J., Rousseau, A., Mountford, R., McWhirter, D., and Malik, H.Z. (2017) Specific mutations in KRAS codon 12 are associated with worse overall survival in patients with advanced and recurrent colorectal cancer. *British Journal of Cancer*, **116** (7), 923–929.
32. Loong, H.H.-F., Du, N., Cheng, C., Lin, H., Guo, J., Lin, G., Li, M., Jiang, T., Shi, Z., Cui, Y., Jin, X., Yao, J., Xing, Y., Yao, M., Wang, K., Mok, T.S.K., and Liu, L. (2020) KRAS G12C mutations in Asia: a landscape analysis of 11, 951 Chinese tumor samples. *Translational Lung Cancer Research*, **9** (5), 1759–1769.
33. ZANATTO, R.M., SANTOS, G., OLIVEIRA, J.C., PRACUCHO, E.M., NUNES, A.J.F., LOPES-FILHO, G.J., and SAAD, S.S. (2020) IMPACT OF KRAS MUTATIONS IN CLINICAL FEATURES IN COLORECTAL CANCER. *ABCD.ArquivosBrasileiros de CirurgiaDigestiva (São Paulo)*, **33** (3).

34. Araujo, L.H., Souza, B.M., Leite, L.R., Parma, S.A.F., Lopes, N.P., Malta, F.S.V., and Freire, M.C.M. (2021) Molecular profile of KRAS G12C-mutant colorectal and non-small-cell lung cancer. *BMC Cancer*, **21** (1).
35. Therkildsen, C., Bergmann, T.K., Henrichsen-Schnack, T., Ladelund, S., and Nilbert, M. (2014) The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. *ActaOncologica*, **53** (7), 852–864.
36. Guo, F., Gong, H., Zhao, H., Chen, J., Zhang, Y., Zhang, L., Shi, X., Zhang, A., Jin, H., Zhang, J., and He, Y. (2018) Mutation status and prognostic values of KRAS, NRAS, BRAF and PIK3CA in 353 Chinese colorectal cancer patients. *Scientific Reports*, **8** (1).
37. Rimbert, J., Tachon, G., Junca, A., Villalva, C., Karayan-Tapon, L., and Tougeron, D. (2017) Association between clinicopathological characteristics and RAS mutation in colorectal cancer. *Modern Pathology*, **31** (3), 517–526.
38. Sánchez-Ibarra, H.E., Reyes-Cortes, L.M., López-Tavera, E., Luna-Aguirre, C.M., and Barrera-Saldaña, H.A. (2021) Complete Screening of Exons 2, 3, and 4 of KRAS and NRAS Genes Reveals a Higher Number of Clinically Relevant Mutations than Food and Drug Administration Quantitative Polymerase Chain Reaction-Based Commercial Kits. *Revista de investigacionClinica*, **72** (6).
39. Guo, T., Wu, Y., Huang, D., Jin, Y., Sheng, W., Cai, S., Zhou, X., Zhu, X., Liu, F., and Xu, Y. (2021) Prognostic Value of KRAS Exon 3 and Exon 4 Mutations in Colorectal Cancer Patients. *Journal of Cancer*, **12** (17), 5331–5337.
40. Yuan, Y., Liu, Y., Wu, Y., Zhang, J., Shen, C., Zhang, F., Wu, C., and Hu, W. (2021) Clinical characteristics and prognostic value of the KRAS mutation in Chinese colorectal cancer patients. *The International Journal of Biological Markers*, **36** (2), 33–39.
41. Hayama, T., Hashiguchi, Y., Okamoto, K., Okada, Y., Ono, K., Shimada, R., Ozawa, T., Toyoda, T., Tsuchiya, T., Iinuma, H., Nozawa, K., and Matsuda, K. (2019) G12V and G12C mutations in the gene KRAS are associated with a poorer prognosis in primary colorectal cancer. *International Journal of Colorectal Disease*, **34** (8), 1491–1496.
42. Fiala, O., Buchler, T., Mohelnikova-Duchonova, B., Melichar, B., Matejka, V.M., Holubec, L., Kulhankova, J., Bortlicek, Z., Bartouskova, M., Liska, V., Topolcan, O., Sedivcova, M., and Finek, J. (2015) G12V and G12A KRAS mutations are associated with poor outcome in patients with metastatic colorectal cancer treated with bevacizumab. *Tumor Biology*, **37** (5), 6823–6830.
43. Bai, B., Shan, L., Xie, B., Huang, X., Mao, W., Wang, X., Wang, D., and Zhu, H. (2017) Mutations in KRAS codon 12 predict poor survival in Chinese patients with metastatic colorectal cancer. *Oncology Letters*.
44. Li, W., Liu, Y., Cai, S., Yang, C., Lin, Z., Zhou, L., Liu, L., Cheng, X., and Zeng, W. (2019) Not all mutations of KRAS predict poor prognosis in patients with colorectal cancer. *Int. J. Clin. Exp. Pathol.*, **12** (3), 957–967.

45. Nakayama, A., Nagashima, T., Nishizono, Y., Kuramoto, K., Mori, K., Homboh, K., Yuri, M., and Shimazaki, M. (2021) Characterisation of a novel KRAS G12C inhibitor ASP2453 that shows potent anti-tumour activity in KRAS G12C-mutated preclinical models. *British Journal of Cancer*, **126** (5), 744–753.
46. Seton-Rogers, S. (2019) KRAS-G12C in the crosshairs. *Nature Reviews Cancer*, **20** (1), 3–3.
47. Giampieri, R., Lupi, A., Ziranu, P., Bittoni, A., Pretta, A., Pecci, F., Persano, M., Giglio, E., Copparoni, C., Crocetti, S., Mandolesi, A., Faa, G., Coni, P., Scartozzi, M., and Berardi, R. (2021) Retrospective Comparative Analysis of KRAS G12C vs. Other KRAS Mutations in mCRC Patients Treated With First-Line Chemotherapy Doublet + Bevacizumab. *Frontiers in Oncology*, **11**.
48. Chuang, S.C., Huang, C.W., Chen, Y.T., Ma, C.J., Tsai, H.L., Chang, T.K., Su, W.C., Hsu, W.H., Kuo, C.H., and Wang, J.Y. (2020) Effect of KRAS and NRAS mutations on the prognosis of patients with synchronous metastatic colorectal cancer presenting with liver-only and lung-only metastases. *Oncology Letters*, **20** (3), 2119–2130.
49. Yari, A., Samoudi, A., Afzali, A., Karam, Z.M., Karimaldini, N.K., Abadi, M.F.S., Ziasistani, M., Zangouey, M.R., and Dabiri, S. (2020) Mutation Status and Prognostic Value of KRAS and BRAF in Southeast Iranian Colorectal Cancer Patients: First Report from Southeast of Iran. *Journal of Gastrointestinal Cancer*, **52** (2), 557–568.
50. Serebriiskii, I.G., Connelly, C., Frampton, G., Newberg, J., Cooke, M., Miller, V., Ali, S., Ross, J.S., Handorf, E., Arora, S., Lieu, C., Golemis, E.A., and Meyer, J.E. (2019) Comprehensive characterization of RAS mutations in colon and rectal cancers in old and young patients. *Nature Communications*, **10** (1).
51. Dai, D., Wang, Y., Zhu, L., Jin, H., and Wang, X. (2020) Prognostic value of KRAS mutation status in colorectal cancer patients: a population-based competing risk analysis. *PeerJ*, **8**, e9149.
52. Bylsma, L.C., Gillezeau, C., Garawin, T.A., Kelsh, M.A., Fryzek, J.P., Sangaré, L., and Lowe, K.A. (2019) Prevalence of RAS and BRAF mutations in metastatic colorectal cancer patients by tumor sidedness: A systematic review and meta-analysis. *Cancer Medicine*, **9** (3), 1044–1057.
53. Pang, X.-L., Li, Q.-X., Ma, Z.-P., Shi, Y., Ma, Y.-Q., Li, X.-X., Cui, W.-L., and Zhang, W. (2017) Association between clinicopathological features and survival in patients with primary and paired metastatic colorectal cancer and KRAS mutation. *OncoTargets and Therapy*, **Volume 10**, 2645–2654.
54. Eshraghi, A., Esfandbod, M., SafaeiNodehi, S.R., Shahi, F., and Eshraghi, A. (2020) The predictive value of KRAS and NRAS mutations in metastatic colorectal cancer. *Med. J. Islam. Repub. Iran*, **34**, 39.
55. Rimbert, J., Tachon, G., Junca, A., Villalva, C., Karayan-Tapon, L., and Tougeron, D. (2018) Association between clinicopathological characteristics and RAS mutation in colorectal cancer.. *Mod Pathol*, **31**, 517–526.

56. Holderfield, M., Deuker, M.M., McCormick, F., and McMahon, M. (2014) Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. *Nature Reviews Cancer*, **14** (7), 455–467.
57. Djanani, A., Eller, S., Öfner, D., Troppmair, J., and Maglione, M. (2020) The Role of BRAF in Metastatic Colorectal CarcinomaPast, Present, and Future. *International Journal of Molecular Sciences*, **21** (23), 9001.
58. Hernández-Sandoval, J.A., Gutiérrez-Angulo, M., Magaña-Torres, M.T., Alvizo-Rodríguez, C.R., Ramírez-Plascencia, H.H.F., Flores-López, B.A., Valenzuela-Pérez, J.A., Peregrina-Sandoval, J., Moreno-Ortiz, J.M., Domínguez-Valentín, M., and Luz Ayala-Madrigal, M. de la (2020) Prevalence of the BRAF p.v600e variant in patients with colorectal cancer from Mexico and its estimated frequency in Latin American and Caribbean populations. *Journal of Investigative Medicine*, **68** (5), 985–991.
59. Cen, S., Liu, K., Zheng, Y., Shan, J., Jing, C., Gao, J., Pan, H., Bai, Z., and Liu, Z. (2021) BRAF Mutation as a Potential Therapeutic Target for Checkpoint Inhibitors: A Comprehensive Analysis of Immune Microenvironment in BRAF Mutated Colon Cancer. *Frontiers in Cell and Developmental Biology*, **9**.
60. Eurboonyanun, K., Lahoud, R.M., Kordbacheh, H., Pourvaziri, A., Promsorn, J., Chadbunchachai, P., O'Shea, A., Atre, I.D., and Harisinghani, M. (2020) Imaging predictors of BRAF mutation in colorectal cancer. *Abdominal Radiology*, **45** (8), 2336–2344.
61. Brummelen, E.M.J. van, Boer, A. de, Beijnen, J.H., and Schellens, J.H.M. (2017) BRAF Mutations as Predictive Biomarker for Response to Anti-EGFR Monoclonal Antibodies. *The Oncologist*, **22** (7), 864–872.
62. Salem, M.E., Weinberg, B.A., Xiu, J., El-Deiry, W.S., Hwang, J.J., Gatalica, Z., Philip, P.A., Shields, A.F., Lenz, H.-J., and Marshall, J.L. (2017) Comparative molecular analyses of left-sided colon, right-sided colon, and rectal cancers. *Oncotarget*, **8** (49), 86356–86368.
63. Li, Y., and Li, W. (2017) BRAF mutation is associated with poor clinicopathological outcomes in colorectal cancer: A meta-analysis. *Saudi J. Gastroenterol.*, **23** (3), 144–149.
64. Wang, J., Shen, J., Huang, C., Cao, M., and Shen, L. (2019) Clinicopathological Significance of BRAFV600E Mutation in Colorectal Cancer: An Updated Meta-Analysis. *Journal of Cancer*, **10** (10), 2332–2341.
65. Battaglin, F., Naseem, M., Lenz, H.-J., and Salem, M.E. (2018) Microsatellite instability in colorectal cancer: overview of its clinical significance and novel perspectives. *Clin. Adv. Hematol. Oncol.*,**16** (11), 735–745.
66. Gelsomino, F., Barbolini, M., Spallanzani, A., Pugliese, G., and Cascinu, S. (2016) The evolving role of microsatellite instability in colorectal cancer: A review. *Cancer Treatment Reviews*, **51**, 19–26.