

# Clinicopathological correlations between colorectal cancer and genetic mutations

Alexandra-Alina VESA<sup>1</sup>, Octavian MAGHIAR<sup>2</sup>, Ovidiu POP<sup>1</sup>, Monica BOROS<sup>1</sup>, Andrei PASCALAU<sup>1</sup>, Adrian MAGHIAR<sup>2,3</sup>

<sup>1</sup>Morphological Sciences Department, Faculty of Medicine and Pharmacy, University of Oradea, Romania

<sup>2</sup>Surgical Sciences Department, Faculty of Medicine and Pharmacy, University of Oradea, Romania

<sup>3</sup>Surgery Department, Pelican Hospital, Oradea, Romania

## ABSTRACT

**Objective.** Colorectal cancer is an oncological pathology that, unfortunately, has increased in terms of incidence in recent years. The presence of KRAS and BRAF mutations in colorectal cancer has significant clinical implications. As a result we want to conduct research that analyzes the impact of these mutations on patients diagnosed with colorectal cancer and also to observe the clinicopathological differences between mutant and wild-type tumors.

**Material and methods.** We conducted a retrospective study in the period 2018-2022, including 118 patients diagnosed with colorectal cancer. The patients were subsequently divided into two groups equal in number of patients, depending on the presence or absence of mutations.

**Outcomes.** After analyzing the data we were able to identify several differences between the two groups, regarding the histopathological type - mucinous correlated with the mutant tumors, the degree of infiltration of the locoregional lymph nodes (more N+ cases in the mutant group), the location of the primary tumor (right colon within the mutant tumors, the rectosigmoid region in the wild-type group), the location of secondary tumors (pulmonary ones with a triple incidence in the mutant group).

**Conclusions.** The study of genetic mutations and their role in colorectal cancer has provided valuable insights into the underlying mechanisms of this complex disease. It is an ever-evolving field that promises to have a profound impact on patient care, ultimately leading us toward more effective prevention, early detection, and personalized therapies for colorectal cancer patients. By leveraging genetic information, clinicians can optimize treatment plans, minimize side effects, and increase the chances of successful outcomes for individual patients.

**Keywords:** colorectal cancer, genetic mutation

## INTRODUCTION

In terms of cancer diagnoses, colorectal cancer (CRC) is the third most common malignancy in both men and women worldwide and the second most common cause of death among malignant tumors, with approximately 9.4% of cancer-related deaths 2020 [1,2].

CRC only affects the colon or rectum, and is represented by an abnormal growth of glandular epithelial

cells. Three main subtypes of CRC exist: sporadic, inherited, and colitis-related. The risk of getting CRC is determined by both environmental and hereditary factors [3].

From a cellular and molecular perspective, colorectal cancer is a diverse illness. Kirsten rat sarcoma (KRAS) is a frequently mutated oncogene in CRC, with mutations in about 40% of all CRC cases. These mutations cause constitutive activation of the KRAS protein, which

functions as a molecular switch to persistently stimulate downstream signaling pathways, including cell proliferation and survival, and ultimately promote tumor growth [4]. Patients with CRC mutant expression of KRAS have a worse prognosis than those with wild-type KRAS CRC, particularly when the tumor has spread to other organs [5].

About 10% of CRC patients have mutations in the BRAF gene [6]. Female gender, right-sided, advanced stage, mucinous histology, deficient mismatch repair, and a serrated adenoma pathway are all related with BRAF mutation. Additionally, with a median OS of around 12 months, BRAF-mutated CRCs are characterised by a poor prognosis and resistance to conventional therapy [7].

Understanding the role of genetic mutations in colorectal cancer is essential for early detection, personalized treatment strategies and risk assessment. Advances in genetic testing and genomic research have enabled healthcare professionals to identify specific mutations associated with colorectal cancer, allowing for targeted therapies and improved patient outcomes. The presence of KRAS and BRAF mutations in colorectal cancer has significant clinical implications. Patients with these mutations often have distinct clinical features, treatment responses, and different prognosis. Importantly, these mutations can affect the responsiveness of targeted therapies, such as anti-EGFR (epidermal growth factor receptor) treatments, which are widely used in the management of colorectal cancer.

## MATERIAL AND METHODS

We conducted a retrospective study in the period 2018-2022, including 118 patients diagnosed with colorectal cancer. The patients were selected from the database of the Oradea County Emergency Hospital Oradea, and Pelican Hospital Oradea, with the agreement of the management of the mentioned institutions. The genetic tests were carried out at the “Resident Laboratory” clinic, also with the consent of the management to access the database. The patients were subsequently divided into two groups equal in number of patients, depending on the presence or absence of mutations, namely: the group without mutations present called “group A wild-type” and the group with mutations called “group B mutant”.

Inclusion criteria for the two batches were:

- Age over 18 years;
- Histopathological diagnosis of colorectal cancer;
- Patients with genetic testing performed from 2018 to 2022 inclusive;
- Tumors with microsatellite stability (MS-S).
- Consent of the patient or relatives (in case of death) to participate in the study;

Exclusion criteria for the two lots were:

- Colorectal tumor diagnosis based only on imaging investigations, without a definite histopathological result;
- Patients from other counties, because it was not possible to follow them from the point of view of the oncological treatment and the evolution;
- Genetic testing with inconclusive result;
- Non-compliant patients or patients' refusal to participate in the study.
- Histopathological diagnosis different from adenocarcinoma;

The genetic testing was carried out in the pathology laboratory “Resident Laboratory” through an automatic real-time PCR method, according to the “cascade” algorithm, which includes 3 stages:

1. KRAS exon 2 screening - 7 mutations located at codons 12 and 13 are tested; if a mutation is detected, the testing stops, the patient not being eligible for personalized therapy. If no mutation is detected, testing continues with step 2.

2. All RAS extended testing - 28 mutations are tested at the level of exons 3 and 4 of the KRAS gene and 2, 3, and 4 of the NRAS gene;

3. BRAF mutation testing - allows the detection of 5 mutations at codon 600, including the V600E mutation.

Mutational analysis is normally performed on formaldehyde-fixed paraffin-embedded (FFPE) tissues, after removal of the paraffin and DNA extraction with standardized protocols. In the testing of KRAS mutations, PCR amplification techniques are used in the first stage. Depending on the tissue analyzed, the ratio of tumor tissue versus healthy tissue is variable and heterogeneous, resulting in a mixture of the target to be amplified in which the mutant and wild-type DNA are not present in an equimolar ratio. That is why it is important that for genotyping, the selected tissue contains enough tumor material for analysis (more than 70% invasive carcinoma cells).

## OUTCOMES

We noticed that the most frequent mutation in our research is the KRAS mutation (83.05%). In addition to this, NRAS (3.38%), BRAF (10.16%) and concurrent KRAS/BRAF mutations (3.38%) were also identified. The Anova test carried out highlights a significant difference in the standard deviation ( $p < 0.05$ ). The most frequent KRAS mutation variant was the one with a mutation present at the level of exon 2 (codon 12, codon 13). These are most often identified in colorectal cancer. In addition to these, mutations located at the level of exon 3 (codon 59, codon 61) and the level of exon 4 (codon 117, codon 146) were also noted (Figure 1).

In the cohort made up of the total number of 118 patients, it can be seen in the figure and below that the majority of patients are male (64.40%), insignificant in terms of from a statistical point of view ( $p=0.124$ -chi square test) (Figure 2).

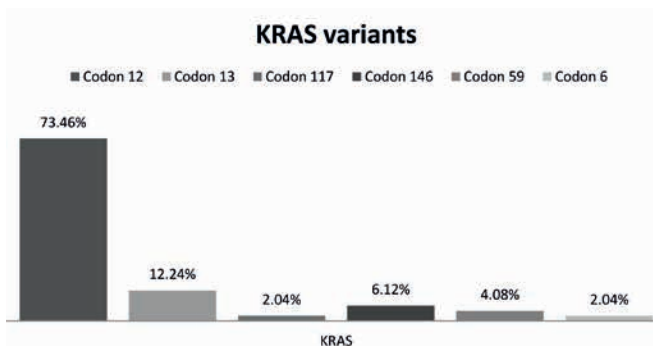


FIGURE 1. KRAS mutation variants identified in group B

**Gender \* Crosstabulation mutation**

Count		WILDE-TYPE	MUTANT	Total
Gender	Female	17	25	42
	Male	42	34	76
Total		59	59	118

**Chi-Square Test**

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.366 <sup>a</sup>	1	.124		
Continuity Correction <sup>b</sup>	1.811	1	.178		
Likelihood Ratio	2.377	1	.123		
Fisher's Exact Test				.178	.089
Linear-by-Linear Association	2.346	1	.126		
N of Valid Cases	118				

<sup>a</sup> 0 cells (0.0%) have expected count less than 5. The minimum expected count is 21.00

<sup>b</sup> Computed only for a 2x2 table

FIGURE 2. Classification of patients according to sex in relation to the presence/absence of mutations. Chi-Square Test

The next parameter examined was the age of the patients. It should be mentioned that we noted the age at which the patient was diagnosed with a malignant colorectal tumor. Thus we noticed that the youngest patient in the study cohort is in the age category between 21-30 years (27 years) and the oldest patient is in the age category of 81-90 years (86 years). The minimum age value in group A wild-type is 36, female; the minimum age value of mutant group B is 27 years, male ( $p=0.852$ ). The maximum age value in group A wild-type is 86; the maximum age value of batch B mutant 81 years, ( $p=0.972$ ). The average value of lot A wild-type is 62.14, respectively the average value of lot B mutant is 62.71, statistically insignificant ( $p=0.759$ ) (Table 1).

TABLE 1. Distribution of cases according to age

	Lot A	Lot B	p
Min	36	27	
Max	86	81	
Median	62.14	62.71	0.759*, 0.751**
DS	9.442	10.90	
ES	1.229	1.419	
N	59	59	

DS – standard deviation; ES – standard error; N – total number; \* – T test; \*\* – chi square test

For the mutant group, we further analyzed the type of mutation present, namely BRAF, KRAS, and NRAS, to try to establish a certain predominance between gender and age respectively. Thus, from the data that can be identified in the figure below, we can state that at the BRAF mutation level, with a total of 6 cases, 83.33% of them belong to the female sex, thus obtaining a ratio of 5:1 ( $p=0.0039$ ). The affected ages are between 42 and 79 years, with an average value of 62.5. At the KRAS mutation level, it is observed that the majority of affected patients are male (50.85% versus 32.20%,  $p=0.786$ ). The minimum age in this category is 27 years, and the maximum age is 81 years (both cases being male), having a mean of 63.10, with a standard error of 1.50. The NRAS mutation, observed in 2 cases (3.38%), is "distributed" equally in terms of gender and age, both cases being 68 years old.

The location of the primary tumor at the colorectal level was an important parameter monitored in this study, to identify a correlation regarding a specific "target" area in the mutant group and the wild-type group respectively. The location of the tumor at the rectosigmoid level imposes a statistically significant difference between the two groups in terms of tumor localization in this segment (group A wild type/ $n=15/59$ , 25.42% versus group B mutant/ $n=8/59$ , 13.55%,  $p=0.0016$ ). Another location with a significant statistical difference between the two groups ( $p=0.0022$ ), is the tumor location at the level of the ascending colon, in group B mutant ( $n=8/59$ , 13.55%) this location is 4 times higher compared to group A wild-type ( $n=2/59$ , 3.38%).

From these results, we can draw attention to the fact that tumors with mutant status are more frequent in the right colon, compared to non-mutant ones (25.42% versus 11.86%,  $p=0.059$ , CI 95% -0.005461 to 0.2766, T-test). At the opposite pole, tumors with non-mutant status more frequently affect the rectosigmoid region, compared to the same location in the case of mutant tumors.

From the point of view of the BRAF mutation, it is noted that the location of the primary tumor at the colonic level is more frequent than the location at the rectal level (4/6 at the colonic level, 2/6 at the rectal level,  $p=0.134$ ). At the level of the colonic segment, the

cecum was the portion most affected by these tumors with a positive BRAF mutation (3/6 tumors at the cecal level). The same result is obtained in the case of KRAS mutation, the colon is more often affected than the rectum (27/49 colon and 22/49 rectum respectively,  $p=0.079$ ). Mutant KRAS tumors in the right colon accumulate 20.33% of cases versus 18.64% in the case of KRAS wild-type tumors.

In both groups, the predominant histological type is the intestinal type (conventional type), without a significant difference between the two analyzed groups ( $p=0.846$ -Pearson correlation,  $r=-0.0258$ ). The Pearson correlation highlights an important relationship between mucinous adenocarcinomas, with their increased incidence in the B group mutant compared to the A wild-type group, this difference being statistically significant ( $p=0.006$ , Pearson correlation,  $r=0.876$ ) (Table 2).

**TABLE 2.** Main histological types in relation to mutant status

Histological subtype	Total n=118	Mutant status		p
		Wild type n=59	Mutant n=59	
Intestinal	100	52 (88.13%)	48 (81.35%)	
Mucinous	13	4 (6.77%)	9 (15.25%)	0.006*
Mixed	4	2 (3.385)	2 (3.38%)	
Signet ring cell	1	1 (1.69%)	0	

n = total number, \* – Pearson correlation

Well-differentiated tumors (G1) are more common in group A wild type (10/59 versus 1/59,  $p=0.025$  two-tailed,  $r=0.297$ ). At the opposite pole, although there is a small difference between them, poorly differentiated tumors (G3) are characteristic of the group B mutant (16/59 mutant and 14/59 wild-type respectively, without statistical significance,  $p=0.375$ ).

The reporting of tumor invasion by group, as seen in the table below, reveals that T3 and T4 tumors are found in a higher proportion ( $p=0.0967$ , Pearson test) in the group B mutant compared to the group A wild-type (Table 3).

**TABLE 3.** Staging of primary tumor invasion depending on the mutant status

	Total (n=118)	Wild-type n (%)	Mutant n (%)
T2	21	12 (20.33%)	9 (15.25%)
T3	56	27 (45.76%)	29 (49.15%)
T4	41	20 (33.91)	21 (35.60%)
CI 95%	-0.03999 to 0.4493		
p*	0.0967		
r	0.2183		

n = total number, p\* – test Pearson

From the point of view of the study of locoregional lymph nodes, we observed invasion in these structures

in 67.79% of cases in wild-type group A, respectively in 84.74% of cases in mutant group B ( $p$  value=0.0306, CI 95% 0.01616 to 0.3228,  $r$  squared = 0.03968). (Figure 4). We noticed a statistically significant difference in the N0 grading between the two groups (19/59 wildtype and 9/59 mutant respectively, with a  $p$ -value of 0.0286), which demonstrates the fact that mutant tumors have a greater tendency to infiltrate locoregional lymph nodes compared to non-mutant tumors.

At the time of diagnosis with colorectal malignant neoplasm, liver metastases were observed in 37.28% of the patients belonging to this study, regardless of the group, followed by lung metastases, observed in 10.16% of the patients. The difference in cases with liver metastases between group A and group B, respectively, is quite small, of only 4 cases, without any statistically significant significance (40.67% group A wild-type versus 33.89% group B mutant,  $p=0.450$ , CI 95%= -0.1096 to 0.2452, independent T-test). Statistically significant differences are observed instead when analyzing cases with secondary tumors located at the lung level. Thus, in group B mutant, their incidence is three times higher than group A wild type (15.25% group B mutant versus 5.08% group A wild type,  $p=0.047$ , CI 95%= -0.007885 to 0.2113).

The appearance of new metastases during the evolution of the disease was another parameter studied in this paper. Among the 118 patients, we could observe the stationary disease status, without the appearance of new metastases or local recurrences in only 8.47%. In this category of patients, the presence of secondary tumors was not observed neither at the initial diagnosis of CRC, nor during the period of this study. Depending on the tumor genetic status, we can state that we observed an inversely proportional relationship between the two groups, regarding the incidence of liver metastases and lung metastases. If in group A wild-type, the presence of secondary liver tumors is in the first place, in group B mutant the most frequent are secondary tumors located at the lung level:

- Group A wild-type, new liver metastases found in 35.59% of patients versus group B mutant new liver metastases found in 20.33% of patients ( $p$  value=0.0618,  $rsq=0.02888$  independent T-Test); the Pearson test has a value of 0.117, there is no correlation between the seat of liver metastases and the tumor genetic status;
- Group A wild-type new lung metastases were observed at 10.16% versus group B mutant new lung metastases observed at 25.42% ( $p$  value=0.0304,  $t=2.192$ ,  $df=116$ , T-Test); the Pearson test has a value of  $p<0.0001$ , which shows a statistically significant correlation between the seat of lung tumors and the genetic status of the tumor.



## DISCUSSIONS

RAS mutation is the most common oncogenic alteration in human cancers. KRAS is the most frequently mutated, followed by NRAS. Flagship KRAS mutant cancers are pancreatic, colorectal, lung, and urogenital adenocarcinomas. It is known that approximately 30-50% of colorectal tumors have a mutated KRAS gene, and approximately 5-10% of cases have a mutated BRAF gene [8,9].

There are many studies in the specialized literature that have evaluated KRAS mutation variants in colorectal cancer, with results identical to those obtained by us in the current study. Jin Ho Baek identifies in a group of 345 patients, 40.6% patients with a mutation present in the KRAS gene. Mutation at codon 12-exon 2 was the most frequent mutation. The incidence of KRAS mutations was as follows: 90/140 (64.3%) in codon 12 exon 2, 37/140 (26.4%) in codon 13 exon 2, 1/140 (0.1%) in codon 59 exon 3, 7/140 (5.0%) in codon 61 exon 3 and 5/140 (3.6%) in codon 146 exon 4 [10].

In a cohort made up of 108 cases of CRC, performed on patients from Thailand, a KRAS mutation rate of 47.22% was identified, with the most frequent location at codon 12 (29.60%), followed by that at the level of codon 13 (8.30%) [11]. This result is also observed in the study conducted by B. Bai et al., on a group of 135 Chinese patients diagnosed with CRC, in which the mutant status of the KRAS gene is identified in 33% of the cases, the location at codon 12 being the most observed (25.19%). We add to these data the results of the current study, where we also highlight the fact that the mutation located at codon 12 and at codon 13 are the most common (36/49 and 6/49 respectively) [12].

Age is a predominant risk factor for colorectal cancer. Currently, 80% of colon cancer patients and 75% of rectal cancer patients are diagnosed over the age of 60. Colorectal cancer is traditionally a malignant tumor observed at an advanced age (average age of diagnosis = 66 years) [13]. Our study shows an average age value of 62+/- 0.71, with no significant differences between the two groups.

In a study conducted by Muhammet Azel et al. I, in 2021, on a group of over 20,000 patients, it was highlighted that patients with KRAS mutation were more likely to be older than 70 years [14]. In the case of the study carried out by us, it is shown that the incidence of colorectal cancers with the present KRAS mutation begins to increase with the age of 50 years, with a peak incidence at the age of 61-70 years. However, the incidence of this type of KRAS-mutated CRC decreases after the age of 80. The youngest patient with KRAS mutant CRC in this study is 27 years old, a rare feature at this age.

The BRAF mutation is seen in nearly one in ten patients with advanced colorectal cancer. It represents a

state with a poor prognosis and a particular clinical phenotype, is more prevalent in women, over 70 years old, associated with a poorly differentiated histological type [15]. Similar to the data in the literature, we observed in the study carried out by us, the fact that the BRAF mutation is more common in females.

The study by Lauren C. Bylsma et al. (2019) found that KRAS mutations varied significantly by tumor location ( $p < 0.0001$ ), with 46.3% of colon tumors harboring a KRAS mutation being located at the level of the right colon, compared to 35.8% of the left tumors. Another result observed by them was the fact that the most frequent location of the tumor in patients with BRAF mutations was the cecum [16]. The same results were obtained in the studies conducted by Bleeker et al. (2000), and Loree et al. (2017), the frequency of tumors in the right colon surpassing those located in the left colon [17,18]. The results presented by us show a correlation with those described in the specialized literature, namely the increased frequency of KRAS tumors in the right colon, and the increased frequency of BRAF tumors in the cecum.

Regarding the histological type of the tumor, in our study, most of the tumors are represented by conventional adenocarcinomas (84.74%) followed by mucinous adenocarcinomas (11.01%). In 2020, Hye Seung Lee and Dae Yong Hwang analyzed 310 cases of colorectal cancer in Korea, comparing two groups (Kras mutant and Kras wild-type) according to the morphological characteristics of the tumor. Well-differentiated tumors were more frequently observed in the wild-type group [19]. In 2023, in the study conducted by Hidayati Husainy Hasbullah et al, the same result is emphasized about well-differentiated tumors, they being more often observed in tumors with mutant status wild-type [20]. Superimposing these observations with the results of our study, we obtain a statistically significant difference in terms of well-differentiated tumors, with a value of  $p=0.025$ , they being much more frequent in the wild-type group.

In the research of Xiaodong Li et al, it was shown that CRCs with mucinous component, regardless of mucin volume proportion (more than 5%), have similar clinically relevant molecular genetics (i.e., KRAS and BRAF mutations) and their genetics are different from non-mucinous CRC [21]. This result was also noted in our study, the mucinous histological type tumors being correlated to the CRC mutant group (Kras and BRAF) ( $p=0.006$ ).

In the study conducted by Tian-An Guo et al. (2019) on a cohort of 1,834 patients, one of the results is similar to our research, namely the fact that regarding the degree of tumor infiltration, T1 is more frequently observed in the wild-type group versus the mutant group [22].

The research of Xiaodong Li et al. (2020) showed that the number of cases of CRC with mutations pres-

ent in which the locoregional lymph nodes are affected is more than 25% higher than the cases where the lymph nodes are free of tumor infiltrate (99 versus 67).180 in the case of our study, we noticed that the status of N0 is more frequent in the wild-type group, their incidence being double that of the mutant group (19 versus 9), and regarding the status of locoregional lymph nodes in the mutant group, 84.7% of cases were classified N+ (84.7% versus 67.79% wild-type,  $p = 0.0306$ ) [21].

The analysis of metastases in the current study has as its first result the hepatic localization as the most frequently observed at the cohort level, this being certified in other studies in the specialized literature. For example, the study conducted by D. P. Modest et al (2016), on a group of 1239 patients, reveals that the majority of secondary colorectal tumors, regardless of genetic status, are located in the liver (558 wild-type, 366 KRAS mutant, 57 BRAF mutant) [23]. Secondary tumors with pulmonary localization, similar to our study, are more often observed in KRAS/BRAF mutant patients (201 mutant versus 194 wild-type). A. L Pereira et al (2014) and collaborators describe in a group of 494 patients that pulmonary metastases that appeared during the disease were more common in the mutant group compared to hepatic ones, a result that coincides with the one described by us [24].

#### Author's contributions

All authors reviewed the manuscript.

Patients with KRAS-mutant colorectal cancer develop lung and brain metastases more frequently than the KRAS wild-type counterpart, (Michele Ghidini et al, 2016), possibly explaining why data from studies of CRC with lung metastases occasionally have higher RAS mutation rates (Igarashi T et al, 2020) [25, 26].

## CONCLUSIONS

In conclusion, our study on colorectal cancer and genetic mutations has shed valuable light on the intricate relationship between genetics and the evolution of this devastating disease. Through a comprehensive analysis of genetic data and clinical information, we have uncovered compelling evidence supporting the pivotal role of genetic mutations in colorectal cancer susceptibility and progression.

First and foremost, we have established that KRAS and BRAF mutations are prevalent in colorectal cancer, with distinct clinical implications. While KRAS mutations are associated with increased aggressiveness and resistance to certain therapies, BRAF mutations are indicative of a poorer prognosis and limited treatment options. Understanding the prevalence and implications of these mutations is crucial for tailoring individualized treatment strategies and improving patient outcomes.

*Conflict of interest:* none declared

*Financial support:* none declared

## REFERENCES

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021 May;71(3):209–49. doi: 10.3322/caac.21660
- Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M et al. Cancer statistics for the year 2020: An overview. *Int J Cancer.* 2021 Apr 5. doi: 10.1002/ijc.33588
- Hossain MS, Karuniawati H, Jairoun AA, Ooi DJ, Urbi Z, John A et al. Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. *Cancers (Basel).* 2022 Apr; 14(7): 1732. doi: 10.3390/cancers14071732
- Zhu G, Pei L, Xia H, Tang Q, Bi F. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. *Mol Cancer.* 2021 Nov 6;20(1):143. doi: 10.1186/s12943-021-01441-4
- Dienstmann R, Mason MJ, Sinicrope FA, Phipps AI, Tejpar S, Nesbakken A et al. Prediction of overall survival in stage II and III colon cancer beyond TNM system: a retrospective, pooled biomarker study. *Ann Oncol.* 2017 May 1;28:1023–31. doi: 10.1093/annonc/mdx052
- Tejpar S, Bertagnoli M, Bosman F, Lenz HJ, Garraway L, Waldman F et al. Prognostic and predictive biomarkers in resected colon cancer: Current status and future perspectives for integrating genomics into biomarker discovery. *Oncologist.* 2010;15:390–404. doi: 10.1634/theoncologist.2009-0233
- Sinicrope F, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J et al. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology.* 2015;148:88–99. doi: 10.1053/j.gastro.2014.09.041
- Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. *Cancer and Metastasis Reviews.* 2020 Dec;39(4):1029–38. doi: 10.1007/s10555-020-09915-5
- Maurie Markman. Colorectal Cancer and KRAS/BRAF, Genomic Medicine, Last update January 2022, available at <https://emedicine.medscape.com/article/1690010-overview>
- BAEK JH, KIM J, BAEK DW, Clinical Implication of KRAS Mutation Variants in Patients With Resected Colon Cancer. *Cancer Diagn Progn.* 2022 Jan-Feb; 2(1): 78–83. doi: 10.21873/cdp.10079
- Korphaissarn K, Pongpaibul A, Roothumngong E. High frequency of KRAS codon 146 and FBXW7 mutations in Thai patients with stage II-III colon cancer. *Asian Pac J Cancer Prev.* 2019 Aug 1;20(8):2319–26. doi: 10.31557/APJCP.2019.20.8.2319
- Bai B, Shan L, Xie B, Huang X, Mao W, Wang X et al. Mutations in KRAS codon 12 predict poor survival in Chinese patients with metastatic colorectal cancer. *Oncol Lett.* 2018 Mar; 15(3): 3161–6. doi: 10.3892/ol.2017.7709
- Rogers JE, Johnson B. The reality of early-onset colorectal cancer: highlighting the needs in a unique but emerging population. *Dig Med Res.* 2021 Dec;4:63. doi: 10.21037/dmr-21-77
- Ozer M, Goksu SY, Sanford NN, Ahn C, Beg MS, Kazmi SMA. Age-dependent prognostic value of KRAS mutation in metastatic colorectal cancer. *Future Oncol.* 2021 Dec;17(35):4883–93. doi: 10.2217/fo-2021-0650
- Luu LJ, Price TJ. BRAF Mutation and Its Importance in Colorectal Cancer. "Advances in the Molecular Understanding of Colorectal

- Cancer". 2018 Sept.6. doi: 10.5772/intechopen.82571
16. Bylsma LC, Gillezeau C, Garawin TA, Kelsh MA, Fryzek JP, Sangaré L et al. Prevalence of RAS and BRAF mutations in metastatic colorectal cancer patients by tumor sidedness: A systematic review and meta-analysis. *Cancer Med.* 2020 Feb;9(3):1044-1057. doi: 10.1002/cam4.2747
  17. Bleeker WA, Hayes VM, Karrenbeld A. Impact of KRAS and TP53 mutations on survival in patients with left- and right-sided Dukes' C colon cancer. *Am J Gastroenterol.* 2000 Oct;95(10):2953-7. doi: 10.1111/j.1572-0241.2000.02327.x
  18. Loree JM, Pereira A, Lam M. Classifying colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation profiles and consensus molecular subtypes. *Clin Cancer Res.* 2018 Mar 1;24(5):1062-1072. doi: 10.1158/1078-0432.CCR-17-2484
  19. Lee HS, Hwang DY, Han HS. Histology and its prognostic effect on KRAS-mutated colorectal carcinomas in Korea. *Oncology letters.* 2020 May 13. <https://doi.org/10.3892/ol.2020.11606>
  20. Hasbullah HH, Sulong S, Jalil NAC, Aziz AAA, Musa N, Muas M. KRAS Mutational Profiles among Colorectal Cancer Patients in the East Coast of Peninsular Malaysia. *Diagnostics.* 2023,13(5),822; <https://doi.org/10.3390/diagnostics13050822>
  21. Li X, Sun K, Liao X, Gao H, Zhu H, Xu R. Colorectal carcinomas with mucinous differentiation are associated with high frequent mutation of KRAS or BRAF mutations, irrespective of quantity of mucinous component. *BMC Cancer.* 2020 May 08. doi: 10.1186/s12885-020-06913-2
  22. Guo TA, Wu YC, Tan C, Jin YT, Sheng WQ, Cai SJ et al. Clinicopathologic features and prognostic value of KRAS, NRAS and BRAF mutations and DNA mismatch repair status: A single-center retrospective study of 1,834 Chinese patients with Stage I-IV colorectal cancer. *Int J Cancer.* 2019 Sep 15;145(6):1625-1634. doi: 10.1002/ijc.32489
  23. Modest DP, Ricard I, Heinemann V, Hegewisch-Becker S, Schmiegel W, Porschen R et al. Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group. *Ann Oncol.* 2016 Sep;27(9):1746-53. doi: 10.1093/annonc/mdw261
  24. Pereira AAL, Rego JFM, Morris V, Overman MJ, ENG C, Garrett CR et al. Association between KRAS mutation and lung metastasis in advanced colorectal cancer. *Br J Cancer.* 2015 Feb 3;112(3):424-8. doi: 10.1038/bjc.2014.619
  25. Ghidini M, Personeni N, Bozzarelli S, Baretti M, Basso G, Bianchi P et al. KRAS mutation in lung metastases from colorectal cancer: prognostic implications. *Cancer Med.* 2016 Feb; 5(2): 256-64. doi: 10.1002/cam4.592
  26. Igarashi T, Shimizu K, Usui K, Yokobori T, Ohtaki Y, Nakazawa S et al. Significance of RAS mutations in pulmonary metastases of patients with colorectal cancer. *Int J Clin Oncol.* 2020;25:641-50. doi:10.1007/s10147-019-01582-z