American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Review Article

BRAF and KRAS Mutations in Colorectal Cancer

Zahra Salesi[1], Fatemeh Ali Sefat Madadi[2], Maryam Eslami[2], Sina Hadilou[2], Dorsa Mohammadzadeh[3], Milad alipour[2], Nazanin Zabihi[4], Fatemeh Roshan[4] and Sayed Ali Hosseini Zavareh[2]*

[1]*Young Researcher and Elite Clube, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*

[2]*Medical student, Department of Medicine, Islamic Azad University Ardabil Medical Sciences, Iran*

[3]*Pharmacy student, Islamic Azad university pharmaceutical sciences branch, Iran*

[4]*Bachelor of Science in Nursing, Islamic Azad University Tehran Branch, Tehran, Iran*

***Corresponding author:** Sayed Ali Hosseini Zavareh, Department of Medicine, Islamic Azad University Tehran Medical Sciences, Iran.

To Cite This Article: *Zahra Salesi, Fatemeh Ali Sefat Madadi, Maryam Eslami, Sina Hadilou, Sayed Ali Hosseini Zavareh, et al., BRAF and KRAS Mutations in Colorectal Cancer. Am J Biomed Sci & Res. 2021 - 15(1). AJBSR.MS.ID.002067.* DOI: 10.34297/AJBSR.2021.15.002067.

Received: November 26, 2021; **Published:** December 09, 2021

Abstract

Activation of RAS, RAF, MEK, ERK, MAP pathways play an important role in colon cancer. The prevalence of colorectal cancer was generally in the sigmoid and rectum, but the mutations studied were more likely to cause cancer in the cecum, upper colon, and transverse colon, indicating a link between the tumor site and the type of mutation. To reduce the risk of colorectal cancer, KRAS and BRAF mutations and MSI status in precursor lesions such as HPS, SA, and adenomas can be evaluated. Mutations in the MAPK signaling genes (NRAS, KRAS, BRAF, and PIK3CA) in pairs or triplets will have a synergistic effect on CRC antitumor potency. Therefore, their identification using different methods such as PCR and NGS is important because of these mutations on treatment choice and response. Direct sequencing technique is less sensitive in detecting KRAS and BRAF mutations. Using higher sensitivity techniques such as COLD-PCR followed by HRM analysis, the sensitivity in detecting KRAS and BRAF mutations will be significantly higher than conventional PCR methods. A combination of SERS and PCR, which has high sensitivity and low Detection Limit (DL), can be used to detect these mutations in cfDNA.

The Idylla system can also be used to detect mutations in plasma. Which requires a larger volume of plasma and a suitable concentration of cfDNA. Elevated levels of cfDNA in cancer patients are primarily of tumor origin, and analysis of KRAS and BRAF mutations in plasma could be an alternative to tumor tissue analysis. Genotyping using exosomal serum mRNA can replace tissue samples in CRC patients who do not have the possibility of repeated biopsies due to their non-invasive nature and high rate and reproducibility. CTC can also be used as a non-invasive method to monitor and evaluate patients' condition. BRAF and KRAS mutations are specific mutations that occur independently. These two mutations increase FDG uptake and accumulation within tumor cells by increasing GLUT-1 expression. These mutations are associated with MSI-H type tumors and lack of hMLH1 expression. Studies show a high degree of compatibility between KRAS/BRAF mutation status and MSI status in primary CRC and corresponding peritoneal metastases. Therefore, the MSI status of cancer cells does not change as the disease progresses.

High serum levels of CA19-9 and CEA and the presence of KRAS and BRAF mutations worsen the patient prognosis in CRC. The V600E BRAF gene mutation is a negative prognostic factor in CRC and causes a recurrence of the disease in a short time and can be mentioned as an independent prognostic factor along with TNM and gender. Usually due to the mutually exclusive phenomenon, both KRAS and BRAF mutations do not occur simultaneously, but if both mutations are combined, it causes worse and more aggressive clinical manifestations and leads to a shorter OS. BRAF mutation is associated with proximal site, poor differentiation and mucinus, and tumor grade, of which tumor grade is an independent factor in the prognosis of OS. Unlike KRAS and APC mutations, MLH1 hypermethylation is significantly associated with the occurrence of BRAF mutations. Mutations in the BRAF gene can be implicated in dentate adenomas, which originate from hyperplastic polyps. BRAF mutations are also significantly associated with dMMR tumors.

Most patients with BRAF mutation have right-sided tumors, so right-sided tumors are a contributing factor to the poor prognosis of the disease. In patients with this mutation, involvement of colorectal and thoracic lymph nodes as well as renal metastasis is more common. LRRFS is also lower in BRAF mutant patients, so the overall survival of patients with KRAS mutation is higher than that of BRAF mutation. In general, it can be said that RAS family mutations are more present in the early stages of the tumor they give. Early stages of CRC with KRAS and PIK3CA double mutations, such as Stage 4 tumors, are likely to metastasize to distant sites, and this may be associated with an increased risk of liver metastasis. KRAS mutations occur following MGMT hypermethylation and their occurrence in stages is significantly associated with deeper invasion. These mutations are mostly well and moderately differentiated and mucinous adenocarcinomas and can be effective in causing dysplasia in the intestinal villi. The presence of KRAS mutations is likely to cause PDC formation in CRC.

The presence of this mutation in tumors is associated with high serum levels of CA19-9 and CEA, as well as in female patients and those who have not smoked. KRAS-mutant tumors are also significantly associated with pMMR status, so that the association of pMMR with its mutant type has a high probability of recurrence. In terms of MMR status, the risk of recurrence of dMMR is half that of pMMRs. NRAS mutations are also seen in tumors with long-term metastasis, in rectal cancer, and in tumors with poor differentiation. These mutations can affect the effectiveness of EGFR inhibitors in CRC. The risk of death and progression to wild-type tumors is lower than that of mutants, and only these individuals respond to treatments such as anti-EGFR. People who carry the Wild Type allele for all genes have better RR and DCR than patients who carry each of the mutations. Mutations in each of these three genes, BRAF, KRAS, and PIK3CA, prevent response to cetuximab therapy. KRAS, NRAS, and BRAF mutations are associated with inadequacy of treatment with stoxybim and shorter OS and lower PFS.

Keywords: BRAF and KRAS, Colorectal Cancer, BRAF Mutation, KRAS Mutation, Prognosis, Colonoscopy, Stoxybim, NRAS, Mutations, Adenomas, Morphological

Abbreviations: RAS: Rat sarcoma; RAF: Rapidly Accelerated Fibrosarcoma; MEK: Mitogen-activated protein kinase kinase; ERK: Extracellular regulated kinase; MAP: Mitogen-activated protein; KRAS: Kirsten rat sarcoma viral oncogene homolog; BRAF: V-raf murine sarcoma viral oncogene homolog; MSI: Microsatellite instability; HPS: Hyperplastic polyps; SA: Serrated adenoma; MAPK: Mitogen-activated protein kinase; NRAS: Neuroblastoma ras viral oncogene homolog; PIK3CA: Phosphatidylinositol 3-kinase catalytic a subunit; CRC: Colorectal cancer; PCR: Polymerase chain reaction; NGS: Nest generation sequencing; COLD-PCR: Co-amplification at lower denaturation temperature PCR; HRM: High-Resolution Melting; SERS: Surface enhanced roman spectroscopy; DL: Diagnosis limitation; CF DNA: Cell-free DNA; CTC: Circulation tumor cells; FDG: 18F-Fluorodeoxyglucose; GLUT-1: Glucose transporter-1; MSI-H: Microsatellite Instability-high; HMLH1: Human mutL homolog 1; CA19-9: Cancer antigen 19-9; CEA: Carcinoembryonic antigen; TNM: Tumor-node-metastasis; OS: Overall survival; APC: Adenomatous polyposis coli; MLH1: MutL homolog 1; DMMR: Deficient mismatch repair; LRRFS: Locoregional recurrence-free survival; MGMT: O6-methylguanine DNA methyltransferase; PDC: Poorly differentiated clusters; DMMR: Proficient mismatch repair; MMR: Mismatch repair; EGFR: Epidermal growth factor receptor; RR: Response rate; DCR: Disease control rate; PFS: Progression Free Survival; PET/CT scan: Positron emission tomography/computed tomography scan; SUV max: Maximum standardized uptake value; TLR: Tumor-to-liver ratio; HXK-II: Hexokinase type-ll; MCRC: Metastatic colorectal cancer; CIMP: CpG island methylator phenotype; WT: Wild type; GI: gastrointestinal; tp53: Tumor suppressor gene 53; SMAD4: Mothers against decapentaplegic of human cancer; FBXW7: A critical tumor suppressor of human cancer; FAP: Familial adenomatous polyposis; T3: Tumor grade 3; Csc: Cancer stem cell; MSS: Microsatellite stability; PTEN: Phosphatase and tensin homolog; RFS: Relapse free survival; WBC: White blood cells; DFS: Disease free survival; TCGA: The Cancer Genome Atlas; CIN: Chromosomal instability; PERK: Proline-rich extension-like receptor kinases; ctDNA: Circulating Tumor DNA; pmKRAS: Plasma mutant KRAS level; HRMA: High-Resolution Melting Analysis; CIMP: CpG island methylator phenotype; MLR: Multiple linear regression; BEAM: Beads; emulsion; amplification; magnetics. DPCR: Digital polymerase chain reaction; FFPE: Formalin-fixed and paraffin embedded; RFLP: Restriction fragment length polymorphism; LNA-clamp: Locked nucleic acid (LNA)-clamp Polymerase Chain Reaction (PCR); anti-EGFR-Moab: Anti-Epidermal Growth Factor Receptor-Monoclonal Antibody; AREG: Amphiregulin; EREG: Epiregulin; TTP: Time to progression; ORR: Overall response rate; OR: Objective response; CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy; mPFS: Median progression free survival; DOD: Died disease; NM: Nodal micro metastases; G3: Poorly differentiated clusters-grade; LVI: Lympho vascular invasion; PTNM: Pathological tumor-node-metastasis; TB: Tumor budding; HCT-116: Human colon cancer cell line; RKO: Human colon carcinoma cell line; MSP: Methylation-specific PCR; CpG: In genetics; a site where Cytosine (c) lies next to Guanine (G) in the DNA sequence. (The p indicates that c and G are connected by a phosphodiester bond). Methylation of DNA occurs at any CpG site; CDKN2A: Cyclin dependent kinase inhibitor 2A; AA: African Americans; MSH2: Muts Homolog2; SCRC: Sporadic colorectal cancer; SGC: Sporadic gastric cancer; HNPCC: Hereditary nonpolyposis colorectal cancer; hMSH2: Human muts Homolog2.

Introduction

KRAS and BRAF mutations can be effective in the prognosis of patients with colorectal cancer [1]. Considering the effect of these mutations on treatment choice and response, it is important to identify these mutations using various methods such as PCR and examine them with methods such as kaplen major [2]. KRAS and BRAF mutations and MSI status in precursor lesions such as HPS, SA and Adenomas can also be examined. Adenomas were the most common lesions isolated during colonoscopy. HPS lesions are mainly located in the left colon and are less than 10nm. HPSs are known as neoplastic lesions and may lead to cancer. SAs are usually larger than 10 nm and are in the right colon. In a study of 103 lesions removed by colonoscopy, none showed MSI-H phenotype. KRAS mutation was not found in any of the SAs and BRAF mutation was not found in any of the adenomas. To reduce the risk of colorectal cancer, it is better to remove precursor lesions and examine them for morphological, molecular, size and location characteristics [3]. KRAS mutations at codons 12 and 13 and BRAF mutations at codon 600 (v600e) are common mutation sequences. Mutations in the KRAS gene have been seen more as a substitution of the aspartic amino acid for glycine in exon 2 [4].

A study of 244 CRC samples found that BRAF and KRAS mutations are specific mutations that occur independently. These mutations are associated with MSI-H type tumors and lack of hMLH1 expression, and except for one case, lack of hMLH1 expression is always associated with the MSI-H phenotype. In this study, BRAF mutations all occurred in exon 15 and above in tumors of the proximal colon [5]. In another study, 51 patients underwent FDG-PET/CT scan before primary tumor resection and predicted the presence of KRAS and BRAF mutations in CRC samples based on the accumulation of FDG, SUVmax, and TLR. In general, all

primary tumors had increased FDG accumulation, but were higher in mutant variants, both BRAF, KRAS, SUVmax, and TLR, so that at 13cut off for SUV, sensitivity and specificity were predicted to predict mutation. KRAS and BRAF scores were 74% and 75%, respectively, indicating the prognostic role of FDG-PET/CT scan. Also, in terms of the expression of GLUT-1 and HXK-II receptors, in tumors with high expression of GLUT-1, SUV and TLR were higher, but the results were not the same for HXK-II. It didn't have much.

Therefore, it can be concluded that these two mutations increase the uptake and accumulation of FDG inside tumor cells by increasing the expression of GLUT-1 [6]. In a study to estimate treatment costs in 4 groups:

a) Treatment without anti-EGFR therapy

b) Treatment with anti-EGFR therapy but without examining KRAS and BRAF mutations

c) Treatment with anti-EGFR therapy only by examining KRAS mutation

d) Treatment with anti-EGFR therapy was performed along with examination of both KRAS and BRAF mutations. In general, it was found that the lowest cost is related to treatment without anti-EGFR therapy and this method is about $20,000.

Costs are reduced, but if anti-EGFR therapy is to be considered, it is best to check for both KRAS and BRAF mutations, as this will reduce costs by more than $8,000, although BRAF measurement reduces the potential for survival [7]. In a 4-year OS study of 2953 mCRC patients, the overall survival of patients with KRAS mutation was higher than BRAF mutation [8].

Investigation of the relationship between gender, race, and age with KRAS and BRAF mutations

Colorectal cancer is the fourth most common cancer in Kazakhstan. Real-time PCR showed the most common mutations in exons 2, 3 and 4 of the KRAS gene, codons 12, 13, 61 and 146 of the NRAS and V600E genes in the BRAF gene. BRAF mutations were not observed simultaneously with RAS mutations. The prevalence of mutations in men and women was equal to and greater in the G12D codon. No association was found between the type of mutation and gender, although there were cases of increased mutations in women. In examining the relationship between the type of mutation and race of individuals, the results showed the predominance of wild type in Asians, lineage and KRAS mutation in Europeans. The study of the relationship between age and mutation showed a higher incidence of wild-type mutations in individuals aged 59-55 years and RAS mutations in individuals aged 89-60 years [9]. But in Iran, this disease is more valuable for screening in middle-aged people [10].

In another study, the status of BRAF and KRAS genes was evaluated with paraffin and frozen mold samples from 581 patients. These two mutations always happened separately. The KRAS mutation was found to be unrelated to sex, age, tumor location, stage of cancer, and venous invasion, but there was a slight association between this type of mutation and lymphatic invasion. In contrast, BRAF mutations were associated with gender, age, cancer stage, and tumor location. In this population, no correlation was found between KRAS status and patient and tumor characteristics, while there was a clear association between BRAF status and patient and tumor characteristics. Thus, unlike the BRAF, the KRAS mutation status can be an independent predictor [11]. The presence of KRAS mutations in tumors is associated with high serum levels of CA19-9 and CEA, as well as in female patients and non-smokers, so KRAS mutations are less likely to occur in patients with CRC who have no history of smoking. While smoking history was associated with BRAF and CIMP mutations in CRC.

Hence, BRAF mutation can be considered as an alternative marker for a group with high CIMP and sporadic dMMR. In addition to high serum levels of CA19-9 and CEA, BRAF mutations are associated with a lower rate of overweight compared to the WT BRAF form. High serum levels of CA19-9 and CEA and the presence of KRAS and BRAF mutations worsen the patient's prognosis in CRC. Alcohol consumption, diabetes mellitus, hypertension and chronic GI status are not risk factors for KRAS and BRAF mutations [12]. In another study on CRC patients in the Arab countries of the Persian Gulf and the study of kras.braf.tp53.nras.apc gene mutations and by analyzing the status of these mutations with NGS to optimize treatment in patients with CRC, these results were obtained. In these patients, tumors on the left such as rectum, sigmoid colon and splenic flexure were about 3 times more than tumors on the right such as cecum and transverse colon.

Comparing Western and Arab CRC patients it was observed that the recurrence of KRAS, BRAF, NRAS, TP53, PIK3CA and APC mutations in the population of Arab patients is almost like the population of Western patients. However, in Arab patients, the frequency of SMAD4 mutations was lower and the frequency of FBXW7 mutations was higher than in Western patients, which could be due to differences in other samples or different ethnicity and geographical distribution. Apart from TP53, which is significantly older than 50 years, and PIK3CA and TP53 mutations, which are associated with the absence of mutations in the APC gene, other clinicopathological factors such as family history, FAP, stage and tumor location were also observed. Age was not significantly associated with mutations in KRAS, BRAF, NRAS, TP53, PIK3CA and APC genes, as well as the incidence of CRC [13]. In Indian society, CRC is more common in men than in women in the colon compared

to the rectum and in people younger than 60 years (mean age 53), but the incidence of CRC in the proximal and distal colon is equal.

In Indian patients with CRC, the tumors were mostly with stage II, more with the depth of T3 tumor invasion, and the most mutations in the genes were TP53 mutations and the least mutations were BRAF and NRAS. The results of the analysis of the relationship between mutations and clinicopathological features using NGS method are as follows: KRAS mutations were more seen in people younger than 60 years and older in the proximal colon and most KRAS mutations were in codons 12 and 13 of KRAS mutations. Was PIK3CA mutations in exons 9 and 20 were more common in men in stage I and II tumors and in the proximal colon, but their presence was not associated with lymph node involvement. NRAS mutations were more common in women and in the distal and rectal clones. APC mutations were more associated with the depth of T2-V600E tumor invasion. BRAF mutations were more common in men and the most common subtype of BRAF mutations was V600E, and eventually TP53 mutations were more common in men and the distal colon.

None of the patients studied always had mutations in all 5 or 6 genes, indicating the onset of CRC through different signaling pathways. The KRAS and BRAF mutations, BRAF and NRAS, NRAS and PIK3CA were mutually incompatible. Mutation of this system in pairs or in groups will have a synergistic effect on the detoxifying power of CRC [14]. Mutations can affect the effectiveness of EGFR inhibitors in CRC, and this effect is more pronounced in women. Also, although BRAF mutation was more common in women but decreased csc in MSS tumors in men, it was not prognostic in women which could be due to a lack of hormonal factors [15,16]. In a study on 69 Chinese patients, KRAS mutation in 43.9% of patients, BRAF mutation in 25.4% and PIK3CA mutation in 8.2% of patients were identified. Also, loss of PTEN expression was observed in 47.8% of patients.

Among KRAS mutations, the most common mutation was in V14G and then G12D codons. Less common mutations such as G13D and G13G were also detected in codon 13. BRAF mutations were measured in various codons such as V600E, V600L, V600Q, V600V, and the most common mutations were in codons V600E and V600L. PIK3CA mutations were more commonly detected in exon 20 and codon H1047L, although KRAS mutations and loss of PTEN expression were more common in women and KRAS, BRAF, and PIK3CA mutations were more common in individuals with a history of smoking or alcohol use [17]. The prevalence of colorectal cancer was generally in the sigmoid and rectum, but the mutations studied further caused cancer in the cecum, upper colon, and transverse colon. Which indicates an association between tumor location and type of mutation [18].

The effect of KRAS and BRAF mutations on patient's prognosis and its relationship with clinical and pathological features of the disease

Studies have shown that mutations in the MAPK signaling genes (BRAF, NRAS, KRAS and PIK3CA) are associated with poor prognosis in CRC [19]. And the KRAS and BRAF signals in samples with CRC can be used as prognostic factors, and the BRAF gene can be used independently for prognosis [20]. Simultaneous evaluation of BRAF and KRAS mutations can also be used in prognosis, survival evaluation and other clinical evaluations [21]. The presence of these mutations is associated with a shorter OS but has no significant effect on PFS [22]. Patients with CRC with MSS tumors containing KRAS and BRAF mutations, who have undergone surgery for treatment, have a poor prognosis [23]. It is more dangerous and reduces patients' OS [24]. And the rate of recurrence and mortality is higher in BRAF gene mutations [25]. In another study of 132 patients with mCRC, BRAF mutations and PTEN deficiency were independently associated with decreased overall survival [26] and the prognostic value of BRAF in left MSS tumors [27].

In terms of MMR status, the risk of recurrence of dMMR is half that of pMMRs. dMMR tumors were more common in stage C and in right-sided tumors of mid gut embryonic origin, and since they were less common in stage Sh, it can be concluded that these tumors are less likely to metastasize. BRAF mutations were significantly associated with dMMR tumors and were more common in the midgut. Wild type or mutant types of this mutation did not differ much in recurrence. KRAS-mutant tumors are more likely to recur than wild-type tumors and are significantly associated with pMMR status, so that the association of pMMR with the wild-type KRAS type has a moderate prognosis, and with the mutant type it has a high probability of recurrence [28]. The results of a study on the MMR value of BRAF and KRAS mutations in 2010 showed that 26% of tumors were in the right colon, 3% in the left, and 1% in the anus dMMR. Also, 17% of right bowel tumors, 2% of the left colon and 2% of anal tumors related to mutations in BRAF gene and tumors resulting from mutations in KRAS gene 40% in the right side of the intestine, 28% in the left side and 37% in the anus [29]. Therefore, mutations in the KRAS gene are seen much more frequently in the right colon than in the left colon [30].

KRAS mutations are more well and moderately differentiated and mucinous adenocarcinomas [31]. RFS is considered significant and the association of this mutation in N1sage tumors with invasion of lymph nodes can affect the prognosis of OS [32]. Another study of 170 patients with a mean age of 66 years with BRAF and KRAS mutations who had stage 2 CRC cancer and underwent surgery found that in 27% of cases tumors in the right colon, 3% in the transverse colon 41% in the left colon and 29% in the rectum. The

results show that mutation in BRAF V600E gene, which is a negative prognostic factor in CRC that causes recurrence of the disease in a short time [33]. It should be noted that in rectal cancers, mutation in BRAF gene (V600E) is not involved [34]. On the other hand, considering P<0.05, it was found that KRAS mutation has little to do with factors such as age, sex, tumor site, tumor grade, MSI status, etc., but with a weaker RFS in stage 2 patients.

Also, since lymph node resection is performed carefully in patients, in multivariate studies, KRAS is considered as an independent and effective factor in the prognosis of recurrence in 2-stage patients**.** This mutation was not related to OS. BRAF mutations were associated with proximal site, poor differentiation, and mucinous and tumor grade. Although MSI-H was more common in proximal tumors, it was also seen in distal tumors with BRAF mutations. Tumor grade was also an independent factor in the prognosis of OS [35]. In 149 mCRC patients with the aim of determining the role of KRAS, BRAF, NRAS and PIK3CA mutations in the prognosis of patients, BRAF mutation with the weakest prognosis and the lowest survival rate (7.6 months) and with 3 times higher risk of death than the all-wild-type group Was associated and most patients with this mutation had right-sided tumor, so right-sided tumor is one of the factors contributing to the poor prognosis of the disease. The KRAS mutation codon12,13 was shown to be a factor in the poor prognosis of the disease with a twice as high risk of death as the all-wild-type group.

Individuals with all-wild-type populations for all these genes had better and higher prognosis and survival rates than the other groups (27.7 months). Some clinical features are also important in poor prognosis, such as no primary tumor removal, less chemotherapy and generally fewer treatment lines, WBC count>10000, and no treatment with bevacizumab. However, anti-EGFR drugs have little to do with survival [36]. It was concluded that the study of these mutations could play a role in the prognosis of patients receiving anti-EGFR antibodies [37]. A study of 600 BRAF codon mutations and KRAS codon mutations 12 and 13 in CRC patients in China found that KRAS mutations could be effective in causing dysplasia of the intestinal villi, and that BRAF gene mutations could also occur in dentate edema. It originates from hyperplastic polyps [38]. In the case report, two patients with colorectal cancer carrying both KRAS and BRAF mutations were found to have both BRAF mutations in codons other than V600E, and the association of non-V600E BRAF mutations with KRAS mutations was better than that of mutant CRCs. BRAF V600E [39].

In another case report of a 50-year-old man with advanced rectal adenocarcinoma at stage 4, T3N2M1, and hepatic metastasis, both KRAS mutations were found in codon 12 exon 2 and BRAF V600E. We usually do not expect to have both mutations in the patient at the same time, but the cause of this phenomenon can be considered a tumor consisting of different clones with different mutations and a high degree of heterogeneity, and the association of both mutations together, worse clinical manifestations. It is more aggressive [40]. Thus, BRAF V600E mutations occur in tumors with KRAS-WT and their occurrence is significantly associated with the original location of the tumor, colon tumors, and deterioration of tumor differentiation. Unlike exon 20 mutations, PIK3CA is more common in proximal tumors, such as the colon, than in distal sites such as the rectum. The occurrence of KRAS mutations in stages is significantly associated with deeper invasion [41]. The BRAF V600E mutation is associated with distant and simultaneous metastases and higher TNM, and has a weaker OS compared to WILD-TYPE variants. Therefore, it can be mentioned as an independent prognostic factor along with TNM and gender.

The KRAS mutation in codon 13 was associated with a weaker OS compared to KRAS wt/BRAF wt, and in this regard, the mutation in the G13D sequence can also be mentioned as an independent prognostic factor. But codon 12 mutations did not show such an effect [42]. Examination of the spectrum of KRAS mutations in Serbian mCRC patients showed that the most frequent mutations in KRAS in CRC were 7 different bp substitutions at codons 12 and 13, with different mutations having different effects, e.g., Increases the risk of death to T [43]. In the study of the association of KRAS and BRAF mutations with 3-year-old OS and DFS in patients with colorectal cancer at TCGA and GSE39582, considering the TNM rating scale, there was generally little association between these mutations with OS and DFS; But in TCGA database, stage C patients with BRAF mutation had shorter OS but not much difference in DFS. In GSE39582 database, stage B patients with BRAF mutation had higher DFS.

Also at this base, the KRAS mutation was associated with a shorter 3-year OS and DFS, but this did not happen at TCGA. Usually due to the mutually exclusive phenomenon, both KRAS and BRAF mutations do not occur simultaneously, but at TCGA 9 patients had these two mutations simultaneously, leading to a shorter OS but not much difference in DFS [44]. Regardless of MSI status, KRAS and BRAF mutations are associated with weaker OS and DFS. BRAF mutations are commonly seen in tumors of the proximal colon, or mucinus, and are associated with a weak OS in stage Ш patients. A patient with a male gender over the age of 65 and an advanced TNM stage who also has the KRAS mutation will have a poor prognosis. If we consider the effect of other variables and factors on prognosis, KRAS is still associated with poor prognosis [45]. KRAS mutation was associated with mucinous carcinoma pathology and tumor type histology, and positive tumor deposit. In terms of OS, two univariate and multivariate studies were performed.

In both reviews in stage 4, the OS was shorter. In stage 3, a shorter univariate study was performed, but in several variables, this result was not obtained. dMMR was observed in younger patients, large tumors, poor differentiation and usually in stage1,2, and acts as a protective factor in stage3, leading to a longer OS. NRAS was associated with a shorter OS in stage 1,2 [46]. A 2019 study of non-surgical advanced mucosal adenocarcinoma patients found that the status of KRAS, BRAF, NRAS, and PIK3CA stimulus mutations was associated with the spread of metastasis. NRAS mutations and especially KRAS mutations of codons 12 and 13 in metastatic tissues were significantly more than primary tissues. In primary tumors, KRAS-stimulating mutations were more common in the sigmoid colon, whereas BRAF and PIK3CA mutations were found only in primary tumor tissue, and the PIK3CA mutation in Stages II and III CRC was slightly more than metastatic. There was little difference between the incidence of these mutations in colon and rectal cancer. However, in rectal cancer only one PIK3CA mutation was found and no NRAS gene mutation was observed [47].

Investigation of KRAS and BRAF mutations in CTC, ctDNA, cfDNA, plasma and CSC

DNA damage and alteration that occurs for a variety of reasons, including aging and environmental factors such as radiation exposure, can lead to mutations in DNA, which can cause tumors and cancer. Diagnosis Analysis of CRC-related mutations can lead to earlier diagnosis of cancer and reduction of mortality and appropriate treatment. From 35 patients with CRC, with a mean age of 60 years, before tumor resection surgery to analyze genomic DNA in tumor samples, peripheral blood samples were taken for exosomal mRNA analysis. It was observed that the repeat mutations of codons 12, 13 and 61 of KRAS gene and codon 600 of BRAF gene in serum exosome and tumor tissue are almost similar and the sensitivity, specificity, and stability rate of detection of these mutations in serum exosome are also high. Also, the most common KRAS mutations were the same in tumor tissue and serum exosome, and in both KRAS mutations, codon 12 of exon 2 doses of students t-test were significantly associated with the patient's older age, while the occurrence of BRAF mutations was correlated. Patients with no clinicopathological characteristics.

Genotyping using exosomal serum mRNA can replace tissue samples in CRC patients who do not have the possibility of repeated biopsies due to its non-invasive nature and high rate and reproducibility [48]. In the study of KRAS/BRAF mutations in serum and primary tumor among 115 patients, it was concluded that in case of similarity, serum sample was used instead of tissue sample to diagnose the mutation, but due to reasons and possibilities such as possible KRAS/metastasis BRAF occurs, or the circulating DNA is not related to primary tumors. It was concluded that mutations in serum and primary colorectal tumors do not match [49]. Analysis of circulating cell-free DNA shows that this method shows 87% for KRAS gene detection and 96% for tumor DNA matching, 7% for BRAF gene and 100% matching [50]. Another study examined mutations in the KRAS, NRAS, BRAF and PIK3CA genes in plasma and colorectal cancer tumors. 175 cases of colorectal cancer (101 men and 74 women with an average age of 59 years) were studied from 2016 to 2017. In these patients, tumor tissue is the best way to diagnose the disease, but it is difficult to obtain this tissue, which can be replaced by plasma. Used [51].

The percentage of KRAS and BRAF mutations observed in a study performed by DNA flowmetric method was 39% and 4% among 135 patients with CRC, respectively. In this study, P53 was also investigated that if it is associated with KRAS mutation, it seems that in tumors with aneuploid DNA (which is based on asymmetric chromosomal mechanisms) it can affect the mechanisms of Chromosomal Instability (CIN). [52]. In 36 cases of CRC with BRAF and KRAS mutations using Roche Cobas® and PCR kits, 13 cases were without BRAF and KRAS mutations. The association between these mutations and the activation of ERK signals was investigated. In staining with PERK antibodies, there was no difference between mutated and non-mutated cancer samples, and these mutations may not necessarily be associated with the expression of the ERK gene. There are in these mutations. In general, further studies and other methods are needed to prove this relationship [53]. The sensitivity and accuracy of real-time PCR detection for sampling liquids is estimated between 0.1-0.5%.

Using plasma DNA-binding Magnetic Beads and selective identification of mutations related to exons 2, 3 and 4 of RAS family genes and exon 15 of BRAF gene, Circulatory Tumor Status (ctDNA) of colorectal cancer patients was evaluated by qualitative real-time PCR. According to the results of liquid biopsy, the prevalence of RAS and BRAF mutations in patients was 26.5% and 10.2%, respectively. Using Liquid Biopsy to evaluate BRAF and KRAS mutations is a good clinical assessment for more information on disease prognosis. Using this method along with Tissue Biopsy can provide better and more complete information about mutations [54]. A study examined the cfDNA of KRAS in 108 patients with mCRC between April 2005 and April 2008. Quantitative measurements of cfDNA and tumor specific KRAS mutations in plasma are highly interdependent, leading to the development of the theory that elevated cfDNA levels in cancer patients are primarily of tumor origin. KRAS mutations in tumors with an incidence rate of 78% and 91% concordance were also seen in peripheral blood. For this reason, KRAS mutations that can be detected in tissue analysis can also be seen in peripheral blood.

Also, high levels of mutated Plasma KRAS (pmKRAS) are associated with deteriorating patient status [55]. Another study examined the importance of third line CFDNA therapy with Cetuximab and irinotecan in 108 colorectal cancer patients, most of whom also detected KRAS mutations in tumor plasma (78%) and 2 out of 3 mutations. BRAF was found in tumor plasma. These results suggest that analysis of KRAS and BRAF mutations in plasma could be an alternative to tumor tissue analysis, the level of which is strongly correlated with the results of the third line of mcrc treatment [56]. Biomarkers are needed to evaluate the effectiveness of EGFR inhibitors. The effect of CFDNA as a prognosis in patients with CRC with KRAS and braf mutations has been investigated [57]. To investigate the mutations of PIK3CA, KRAS NRAS, BRAF in cfDNA, Mitella patients with colorectal cancer were monitored and treated with cetaximab. From 15 patients with plasma mcRc, 8 patients were collected, and it was found that the mean level of cfDNA in these patients was significantly higher than healthy individuals. Leaving treatment with cetaximab for 2 months and resuming treatment elicited a partial response.

Continuous monitoring of PIK3CA, KRAS NRAS, and BRAF mutations in cfDNA is possible and helps in early detection of drug resistance to cetaximab [58]. After CTCs were isolated from patients' blood using the Vortex platform, mutations detected in CTC, ctDNA, and tumor tissue in mCRCs that metastasized to the liver were examined and compared. In most cases, KRAS, BRAF, and PIK3CA mutations in CTC were like tumor tissue, but in some cases, due to intratumorally heterogeneity, additional mutations were found in CTC that were not in tumor tissue. CTC as a non-invasive method can be used to monitor and evaluate patients' condition, because in this study it was found that CTC and ctDNA levels can determine the recurrence or progression of the disease, even before the imaging and CT- scan to make a change, show. KRAS, BRAF, and PIK3CA mutations were detected in some cases in CTC, in some cases in ctDNA, and in some cases in both. Therefore, it can be concluded that CTC and ctDNA are complementary and can be used together to monitor patients and use their results to select appropriate drugs [59].

KRAS and BRAF mutation detection methods in CRC samples and its common sequences

Considering the role of KRAS and BRAF mutations in colorectal cancer, identification, and familiarity with detection methods of these mutations in CRC samples is of special importance. In CRC, mutations occur with a 51% -30% probability in the KRAS gene, and in about 10% of cases, mutations in the BRAF gene occur. One of the methods for detecting the above mutations, HRMA method, is a method for fast and very sensitive screening based on the differentiation of DNA behavior when temperature increases. In a study of 116 CRC samples, variable dilution was performed on two types of mutant and non-mutant DNA, and it was found that at least 5% of mutated alleles can be detected by HRMA method [60]. In another study with a similar statistical population, KRAS, BRAF and PIK3CA mutations were identified using HRMA method and the results were evaluated by sequencing analysis using PCR. They both detected a mutation in KRAS in-frame c.30_31insGGA, which results in the addition of excess glycine to the sequence (p.10_11insG), although this mutation had not been previously reported in CRC.

Two rare KRAS mutations, including Q22K mutation and L19F, were also detected by HRMA. The BRAF V600E mutation was also detected in exon15 only. This study also showed that KRAS and BRAF mutations occur mutually exclusive, so that no BRAF mutation was observed in any of the samples containing KRAS mutation. On the other hand, the association of PIK3CA mutation with KRAS mutation was significant, so that out of 20 PIK3CA mutations in the study population, all mutations in exon20 and 54% of exon9 mutations were associated with KRAS mutation. Also, 10% of 9PIK3CA exon mutations were associated with BRAF mutation [61]. In two other studies in Taiwan that used the HRMA method to examine the above mutations, cases of PIK3CA mutations with KRAS (wild-type or mutant) mutations were reported, but no association of BRAF and KRAS mutations was observed [62,63]. PCR method can be used to identify KRAS and BRAF mutations and their role in treatment selection and response [64,65].

Factors such as heterozygosity of mutations affecting cancer cells, heterogeneity of clones and different mutation status in them, as well as the presence of both normal and pathological cells in the complex structure of cancers lead to limited mutation detection. In biopsies, cancers include CRC. The direct sequencing technique is less sensitive in detecting these mutations and may identify KRAS and BRAF mutant forms in low frequency sequences, such as WT BRAF and WT KRAS. By using more sensitive techniques such as COLD-PCR and enriching the ratio of mutant alleles in sequences with less frequency during PCR, more accurate mutations can be achieved and thus therapies can be more targeted. Performing COLD-PCR method followed by HRM analysis has shown that the sensitivity in detecting KRAS mutations will be 8 times and in detecting BRAF mutations will be 4 times higher than conventional PCR methods. The difference in hypersensitivity for KRAS and BRAF mutations is due to the implementation of different fast COLD-PCR protocols for KRAS mutation and full COLD-PCR for BRAF [66].

Two cases of G12V and G13D mutations for the KRAS gene and another V600E for the BRAF gene are more common than other mutations in tumors [67]. Considering the possibility of mutations in different codons and the effect of these mutations on non-response to anti-EGFR therapies, it is better not to just ignore the

common codons and do thorough research in this regard. In a study to investigate advanced cell line and CRC samples using HRM, PCR, reverse transcription-PCR, a new mutation in 117 KRAS codons in C125 was identified in the cell line for the first time. Mutations in T529A and N581Y were also detected for BRAF. All BRAF mutations were heterozygous, but there were also homozygous cases among KRAS mutations. There was also a close relationship between BRAF and CIMP+, which is the promoter methylation of 3 genes or more. Both KRAS and BRAF mutants T529A codon were also detected in one cell. In advanced CRC samples, KRAS mutation was observed in different codons on 12/13/61/146 [68].

Using a combination of SERS and PCR by adding mutation-specific probes by PCR is another way to detect KRAS, BRAF and PIK3CA mutations in the plasma of patients with CRC. This method has high sensitivity and low Detection Limit (DL), which makes the PCR-SERS method competent and efficient in detecting these mutations in cfDNA. To design the probes used in SERS, you must first identify the mutation sequence and then use the specific mutation probes that have a color tag and are attached to the target mutations. Multiple mutations in up to 3 genes are detected simultaneously without the need for separate processes such as electrophoresis, and then subsequent analyzes such as MLR determine the presence of specific mutations [69]. DNA mutations of the APC, TP53, KRAS, BRAF, and hypermethylation genes in the promoter regions of the APC and MLH1 genes can also be detected in plasma at different stages of the tumor, using the Denaturing High-Performance Liquid Chromatography platform (DHPLC) Appeared. Genetic and epigenetic studies by this method can help diagnose, determine progress, and monitor the treatment process [70]. The Idylla system can also be used to detect mutations in plasma.

This method is used to detect hotspot mutations in KRAS, NRAS and BRAF genes with low allele frequency. The Idylla system can detect these mutations at a lower cost and in less time than methods such as BEAMing dPCR and NGS, but its detection limit is higher, and its detection sensitivity is lower. In a study of 18 plasma samples from mCRC patients, both the Idylla and BEAMing dPCR systems were fully consistent in the detection of BRAF and NRAS mutations, but the Idylla system in the detection of two 146 exon 4 KRAS codon mutations by BEAMing dPCR was detected, was disabled. One of these two cases had low concentrations of cfDNA. Also, in the detection of mutations in ctDNA, several factors prior to analysis such as contact with the sample during the centrifugation process or storage conditions of the sample can affect the quality of the sample. Therefore, to detect mutations in samples with low allele frequency in the Idylla system, we need a larger volume of plasma and an appropriate concentration of cfDNA [71]. In the Idylla system, instead of using FFPE tissue sections, the extracted DNA is pipetged directly into the cartidage.

Unlike NGS, Idylla does not require good quality DNA and can be made with low quality DNA. Further investigation of tumor tissue characteristics and identification of other appropriate treatment options using NGS after Idylla is possible. Idylla also allows the analysis of hotspot mutations in much less time than NGS and when little tumor tissue samples are available, and the results are 100% consistent with NGS results, despite the advantages mentioned. In Idylla, LOD is 2.5ng for KRAS and NRAS and 5ng for BRAF DNA input. Of course, NGS also has advantages, including the need for less input DNA and DNA analysis of target points without significant limitations [72]. Point mutations in KRAS, NRAS, and BRAF are involved in the RAS/RAF/MEK/ERK carcinogenic pathways and are used in the treatment of mCRC patients as prognostic markers. KRAS/NRAS/BRAF Assays can detect 30-point mutations in FFPE-extracted DNA on a fully automated Modaplex System. The primers required for these assays to detect 13, 13, and 4 mutations in the KRAS, NRAS, and BRAF genes, respectively, were fabricated using proprietary technology. The sensitivity of the analysis of each mutation can be assessed using proprietary Ultramer Oligos or Horizon Dx Mockblock specimens.

Each assay requires 5 microliters of clinically extracted sample containing 10 to 50 ng of DNA and a preparation time of 15 to 30 minutes. The total time for data preparation and processing is 4 hours. This kit includes internal controls (to determine the mutation status) and adjustment controls (to determine the amplitude). Studies have shown that these assays have high sensitivity, accuracy, selectivity, and specificity in detecting mutations. In a study of about 110 samples, the results showed a 95% agreement between this method and the pyrosequencing method, and these assays can be used in clinical trials, although they cannot be used in clinical diagnoses [73]. Other methods for investigating KRAS and BRAF mutations include allele-specific single-base primer extension [74], reverse hybridization strip assay and RFLP [75], a combination of LNA-clamp PCR and allele specific hybridization using Among the biological microchips [76], as well as three molecular methods cobas, Thera screen assays, Digital PCR (Fluidigm). Among the last 3 methods mentioned, cobas and Digital PCR are better methods for detecting mutations [77].

KRAS and BRAF mutations and response to treatment

The risk of death and progression in wild-type tumors is lower than in mutants, and only these individuals respond to treatments such as anti-EGFR [78]. Therefore, it is better in case of this type of mutation, KRAS codon 12 & 13 mutations [79] and other RAS family mutations and BRAF mutation [80] as well as PIK3CA mutation [81] due to their poor response to anti-EGFR-moAb treatment. Be checked before starting treatment and avoid this treatment if these mutations are associated with Wild Type. Even if the tumor has the

Wild Type allele for all the above mutations, there may still be no complete response, indicating the influence of other factors such as PTEN deficiency [82] or decreased AREG and EREG expression [83]. One of the appropriate therapies could be the effect of adding Cetuximab to the second line FOLFIRI [84]. This method only improves the condition for Wild Type KRAS mutations, and the presence of other mutations such as BRAF and PIK3CA causes treatment resistance and non-response [85]. In the case of the KRAS gene, mutations on codon 13 also have the highest response to Cetuximab [86].

The same results are found in the elderly treated with first line TEGAFOX+Cetuximab, and OS and RR are higher in wild-type mutations than in mutants, and the prognosis is often determined by the RAS family of genes, although BRAF and TP53 mutations also play a small prognostic role [87]. In treating these patients, the effect of adding an anti-angiogenic to second-line chemotherapy can also be used [88]. For example, the addition of Regorafenib to FOLFIRI improves recovery in people with colorectal cancer with wild-type BRAF and KRAS mutations [89]. This treatment is not responsive in the presence of other mutations and despite improving PFS in Wild Type patients, it does not make a difference in the presence of other mutations and even worsens the OS condition in some BRAF V600E patients [88]. BRAF and KRAS mutations can be considered as two prognostic and predictive factors for the response of mCRC patients undergoing chemotherapy based on Oxaliplatin, Irinotecan and Fluoropyrimidines. Wild Type KRAS mutations are twice as good as mutant KRAS mutations and have a clinical outcome [90]. Despite the selective apoptosis of mutant cells (unlike wild type cells) caused by the addition of vitamin C (in conditions of peripheral glucose deficiency); Another prognostic role of BRAF and KRAS genes has been demonstrated [91].

This prognostic role does not exist in monotherapy with Irinotecan [92]. The type of mutation is very important in choosing the appropriate treatment [93]. The most common mutations reported in colorectal cancers are related to KRAS, BRAF and PIK3CA genes, of which KRAS mutation is the most common (about 40% of patients) [94]. In general, it can be said that RAS family mutations are more in the early stages Tumors are present, and BRAF and PIK3CA mutations occur more frequently as the tumor progresses [84]. About 60% of Wild Type patients also have mutations in the BRAF and PIK3CA genes and other genes in the RAS family [95] and do not respond to anti-EGFR therapies because of these mutations [96]. RR and DCR are better than patients carrying each of the mutations, and screening of these individuals can be effective in choosing the appropriate treatment [97]. Mutations outside this group, including KRAS, NRAS, and BRAF mutations, are associated with the inadequacy of treatment with stock oxime and shorter OS and lower PFS and require different approaches [98]. However, the status of the PIK3CA mutation does not differ between the two parameters [99]. In patients with moderate to severe skin rash, TTP and OS are significantly higher [100].

The findings regarding combination therapy are as follows: Using Cetuximab and LSN3074753 combination therapy can increase DCR, ORR and PFS in KRAS and BRAF patients [101] considering that PHA-665752 reduces cancer cell resistance The colon is resistant to treatment with Cetuximab. Concomitant use of PHA-665752 and Cetuximab for CRC treatment is more effective than any other treatment and increases the lifespan of both local tumors and metastatic tumors; [102]. Research shows the combination therapy of Panitumumab and FOLFOX increases the rate of OS in mCRC patients; [103,104]. Mutant and BRAF gene mutations do not make a difference [106]. Given that the best response to anti-EGFR therapies is seen in KRAS mutations [107]. And the mean OS and PFS in patients with PIK3CA or BRAF mutations treated with Cetuximab did not differ; [108] to save and reduce treatment costs, these mutations should be identified, and other appropriate treatment considered. Examining the status of various genes, including KRAS and BRAF before starting anti-EGFR treatment, can significantly reduce treatment costs (over $8,000) [109].

New low-cost models and methods, including the Monte Carlo Markov model [110]., have been discovered in recent years to detect these mutations. Also, studies have shown a mutation status like KRAS and BRAF genes in primary and metastatic tumor specimens, so in the absence of primary tumor specimens, metastatic specimens can be used to examine genes [111]. BRAF and KRAS mutations are often specific mutations, but a small percentage of patients have double mutations, and this group does not respond to conventional drug therapy and needs to consider new drugs to increase the life expectancy and treatment of these patients [112]. Other effective pathways in the treatment of mCRC patients besides EGFR include Insulin Growth Factor-1 Receptor (IGF-1R) and Mammalian Target of Rapamycin (mTOR) pathways.

These and other similar pathways need further study and explanation to explore newer therapies [113]. Other effective but less studied genes include the Thymidylate Synthase gene, which lacks the 3'UTR TYMS polymorphism of this gene, increasing the chances of responding to treatment [114]. Other less studied factors are environmental effects on the mutation status of cells. For example, in the case of peripheral glucose deficiency, the cells increase the expression of GLUT1, which in turn stimulates KRAS and BRAF mutations. Addition of 3-bromopyruvate to the medium can stop glycolysis and inhibit the growth of these cells [115]. All these cases need further study.

KRAS and BRAF mutations in metastatic colorectal cancer

KRAS and BRAF mutations can occur at either primary or secondary metastatic tumor sites, so they can be detected from either site; Of course, it should be borne in mind that in some cases mutations occur only at the primary site or only at the secondary site. Also in the cohort study, patients carrying the wildtype allele of the KRAS gene in the primary tumor showed a mutated form of the gene in pancreatic or adrenal metastases rather than liver metastases [116]. In mCRC patients undergoing liver resection with curative intent for liver cancer, BRAF mutation was found to be associated with a higher risk of recurrence and a more adverse outcome, whereas KRAS mutation had no association. With RFS after liver resection. The BRAF leak status can be a new warning marker for post-Liver Research status [117]. OR TTP, and OS in mCRC patients treated with first-line systemic chemotherapy in combination with monoclonal antibodies will vary according to the status of the KRAS and BRAF genes [118].

It is therefore important to determine the genetic profile to determine the response to treatment. In cetuximab-treated mCRC patients with grade 1 to 1 skin lesions carrying KRAS or BRAF mutations with reduced AREG or EREG, TTP and OS expression is extremely low [119,120]. In a study in which most patients underwent XELIRI treatment/Bevacizumab or XELOX/ Cetuximab, wild-type KRAS/wild-type BRAF patients and wild-type KRAS/ mutant BRAF patients had slight differences in OR and TTP but significant differences in OS. The results of comparing codons 12 and 13 of KRAS showed a slight difference in OR and TTP, but mutations in codon 13 showed less response and faster progression than codon 12. BRAF mutant patients have a much worse prognosis compared to wild-type BRAF patients with lower response rates and faster progression during treatment [121]. In BRAF mutant patients, colorectal and thoracic lymph node involvement as well as renal metastasis are more common. Survival without regional recurrence (LRRFS) is also lower for BRAF mutant patients [122].

A study in Norway on mCRC patients with peritoneal metastases, who underwent CRS and HIPEC between 2004 and 2015, showed that those patients with BRAF mutation with MSI-H status, after receiving these treatments, DFS and OS were significantly better, but DFS was lower for BRAF mutant patients with MSS status or KRAS mutant or double wildtype [123]. In evaluating the response to cetuximab treatment in mCRC patients who did not receive chemotherapy, patients with wild-type KRAS, RR, and mPFS had better KRAS mutants. None of the patients with KRAS mutant tumors had reduced tumor size, but toxicity profiles such as skin toxicity, diarrhea, stomatitis, and hypomagnesia did not differ between wild-type KRAS tumors and KRAS mutant tumors. Also, patients with wild-type allele had all three BRAF, KRAS and PIK3CA, RR and PFS genes better than patients with KRAS-only wild-type allele.

Mutations in each of these three genes prevent response to cetuximab treatment. However, more than half of the patients still do not respond to cetuximab treatment without any mutations in these three genes, indicating the presence of other unknown determinants [124]. The type of mutation is closely related to the two parameters OS and PFS. The various exon 2 mutations in the KRAS gene have different levels of OS and PFS. In the study of 1239 patients, the highest OS was related to G12D and G12V and the lowest OS was related to G12C, G13D and G12S. Tumors without KRAS, NRAS and BRAF mutations had higher OS levels [125]. In a study that classified mCRC samples according to Dukes' staging criteria, 4 stages were Stage A (T1-2N0M0), Stage B (T3-4N0M0), and Stage C (T1-4N1-3M0). And Stage D (T1-4N0-3M1) was defined, and it was found that the KRAS and PIK3CA mutations were closely related to Dukes' D staging, while the BRAF mutation was not significantly associated. KRAS and PIK3CA mutations can occur together, and this can be associated with an increased risk of liver metastasis [126].

Another study examining the association between the status of NRAS, BRAF, KRAS, and PIK3CA mutations with prognosis for adjuvant or surgical treatment for CRC Stage I patients at high risk of metastasis, dissemination, and progression. In general, KRAS mutation was found to be more common in DOD patients, PIK3CA mutation in patients with Nodal Micro-Metastasis (NM) and PDC G3. Multiple mutations (PIK3CA with KRAS or multiple KRAS mutations) were also more common in DOD patients, females, tumors with poor prognosis, and patients with LVI. In case of further metastasis in Stage I PDC with double or triple mutations, it was observed that this group of tumors had higher PDC G3 and more NM, which indicates the spread and progression of the disease; Therefore, these patients may need adjuvant treatment. It is also possible that early CRC stages with double mutations KRAS and PIK3CA, like Stage 4 tumors, can metastasize to distant sites. In addition, the presence of a single KRAS mutation in Stage I PTNM may not be related to PDC or TB [127].

Higher depth of invasion in the colorectal wall, tumor germination and more group metastasis, higher PTNM and PDC-G are the hallmarks of the CRC-listed mutations, and both PDC and tumor germination probably indicate the same phenomenon. Tumors with PDC-G3 were more common in CRCs with the KRAS mutant form than in CRCs with the WT KRAS form. Therefore, it is possible that the presence of KRAS mutations causes the formation of PDC in CRC. Because cases in CRCs with the mutated form of KRAS worsen the prognosis, the use of ras signaling/ras activation inhibitors is recommended to reduce cell metastasis and invasion in CRC. However, the high level of PDC in CRCs without the mutated

form of KRAS indicates other factors in the formation of PDCs. CRC is associated with the BRAF mutant form, high grade WHO and high grade PDC. However, no correlation was found between the status of the NRAS mutation and either [128]. Discovering more information about the relationship between the type of mutation and the type of treatment and the quality of the response can help to choose the appropriate treatment for each patient and improve the quality of the response [129].

KRAS and BRAF mutations and their relationship with methylation of MGMT, CDKN2A MLH-1, APC, APC2, P16, MSI, CIMP and MMR pathways

Studies showed a high degree of compatibility between KRAS/BRAF mutation status and MSI status in primary CRC and corresponding peritoneal metastases. The existence of this high correlation between primary CRC and corresponding metastases indicates that the MSI status of cancer cells does not change as the disease progresses. MSI-H in CRC consists of 3 types, in 2 of which (Lynch syndrome and MLH1 Promoter hypermethylation) there is a high compatibility between primary CRC and corresponding metastases [130]. In another study, the association between MSI and other mutations, including BRAF and KRAS, was investigated on 264 CRC tissue samples. Inhibitors of this protein are used in treatment [131]. Activation of RAS, RAF, MEK, ERK, MAP pathways also play an important role in colon cancer. In a study of more than 500 cases of colorectal cancer, 87 cases of MSI-H tumors were found in it and the relationship between these 87 cases with BRAF, KRAS gene mutations and the expression status of repair proteins was examined. BRAF gene mutations in 10 cases (11.5%) and KRAS were seen in 30 cases (34.5%).

Out of 10 cases of BRAF gene, 9 cases were related to V599E. BRAF mutation was more common in cancers with loss of hMLH1 protein and rarely in Cancers with loss of hMLH6 and hMLH2 are seen, but these relationships are not true for KRAS mutant cancers [132]. BRAF mutation is seen in CIMP, MLH1 and MSI tumors. 17 blockade of colorectal polyps and FFPE 103 out of 103 patients with CRC were examined. These three mutations are seen in 71% of colorectal cancers. Distinct types of colon polyps indicate precancerous lesions of the gastrointestinal tract. BRAF mutants have a mild oncogenic effect compared to KRAS and indicate that BRAF mutant colorectal cells need to accumulate additional epigenetic changes to achieve complete transformation. The results show that these three mutations are associated with precancerous lesions in early colorectal cancers [133]. In another study, 26% of MSI-high patients had higher rates of colorectal tumors. Also, in patients with BRAF, a higher proportion of MSI was seen compared to WT type of BRAF gene. [134].

Mutations in the KRAS, BRAF, and PIK3CA genes are more likely to occur when the tumor is in the large intestine (98%) opposite the rectum (2%). Mutations in the BRAF gene were also recurrent in patients with stage III CRC (n=7) compared with patients in stage III with recurrence (n=2). Patients with mutations in the BRAF and stage III genes had higher survival than patients with stage III without the BRAF mutation. In tumors with high MSI, the frequency of BRAF mutations was 63% and KRAS was 1% [135]. KRAS mutations cause changes in CSC cells and thus affect tumor progression. Various markers have been found for CSC, including CD44 and CD133, where CD44 is strongly influenced by the KRAS gene in HCT-116 cells. Also, reducing the expression of integrin a6 in SW480, HCT-116 and RKO cells influences inhibiting PIK3CA, BRAF and KRAS genes. In general, integrin a6 acts as a mediator of CRC cells induced by PIK3CA, BRAF, and KRAS [136]. In a prospective cohort study, 734 Dutch patients with CRC (279 males and 573 females aged 55-69 years) who were initially disease-free were studied for 7.3 years, and MSP analysis was performed for 693 patients.

Were performed for MGMT promoter and for 686 of them for MLH1 promoter. But regardless of whether mutations occur in CpG or nonCpG dinucleoids, correlations with BRAF or A: T<G: C mutations in APC are independent and probably occur before MGMT hypermethylation. While the KRAS mutation occurs following MGMT hypermethylation. On the other hand, MLH1 hypermethylation, unlike KRAS and APC mutations, has a significant relationship with the occurrence of BRAF mutations, so in CRCs without mutation in KRAS and APC10, MLH1 hypermethylation and BRAF mutations occur more. Thus, MLH1 hypermethylation develops from the MGMT methylator pathway. Unlike MLH1 hypermethylation, which occurs more frequently in proximal colon, rectosigmoid, and rectal tumors and is more common in women, the occurrence of MGMT hypermethylation is independent of tumor location and sex. EXOGEN factors play an important role in the development of CRCs that develop through the hypermethylation pathway [137]. Another study examines mutations in KRAS, APC, BRAF genes and analyzes of CDKN2A, MLH1, and MGMT expression in colorectal cancer patients.

Examination of patients' DNA showed mutations in the codon 1365 of the APC gene in 3 patients and mutations in the codon of 600 BRAF genes in one patient and mutations in the codon 12 of the KRAS gene in one sample. In 80% of cases the expression of MLH1 and MGMT was undetectable and in the remaining 20% MLH1 was decreased. CDKN2A was also undetectable in 100% of cases [138]. A study examines the BRAF, KRAS (V600E) mutations and colorectal cancer in African Americans (AA). The rate of CRC and mortality in the AA lineage is higher than the general population. A total of 222

CRC tumors from AA individuals were examined for the expression of repair genes MLH1 and MSH2. BRAF mutations were found only in MSI-H tumors, while 64% of KRAS mutations were found in the non-MSI-H group. 41% of MLH1 and 33% of MSH2 were found to be inactive. Also, analysis with MSP showed high incidence of MLH1 (66%), APC (53%) and ACP2 (90%) [139].

To evaluate APC, APC2, MLH-1, P16 markers as well as BRAF V600E and KRAS codon12,13 mutations and their role in CRC in patients, to evaluate various genetic and epigenetic factors in 222 African American patients with CRC Who had their tumor removed has been treated. BRAF mutations were all associated with MSI-H status, and BRAF was associated with MLH-1 unmethylation, suggesting that the MLH-1 methylation pathway and its effect on CRC may be independent of the BRAF signaling pathway. In general, the methylation of APC, APC2, MLH-1, P16 promoters had no significant relationship with MSI-H status, but the association of APC, APC2 methylation with MSI-H could be one of the causes of worsening of the disease in African Americans. MSI-H tumors were also associated with reduced or no expression of MLH-1 and MSH2 proteins. In terms of the association of promoter methylation with the tumor site, P16 was significantly higher in the right colon but other methylations were similarly distributed at different sites [140].

Phenotype

CpG Island Methylator (CIMP-high or CIMP1)

A distinct phenotype is associated with Microsatellite Instability and BRAF mutations in colorectal cancers. Recent studies indicate another phenotype

CpG Island Methylator (CIMP-low or CIMP2)

Is associated with the KRAS mutation. Tumors were divided into four groups: the first group with wild-type KRAS/wild-type BRAF (n=440), the second group with mutant KRAS/wild-type BRAF (n=308), the third group with wild-type KRAS/mutant BRAF (n=107), the fourth group with KRAS/mutant BRAF mutants (n=6). In this analysis, the correlation structures between CIMP, Locus-Specific CpG Island Methylation and MSI were determined according to KRAS and BRAF status. The mutation status of KRAS and BRAF affects the correlation structures between Locus-Specific CpG Island Methylation and CIMP status in colorectal cancer [141]. To evaluate RASSF1A methylation, 76 primary gastrointestinal tumors with MSI, which included 31 MSI SCRC, 20 MSI CRC and 25 MSI SGC, were examined for repetition of RASSF1A methylation stimulus.

Conclusion

RASSF1A methylation is associated with poor differentiation of gastric and colon tumors and occurs in all three types of MSI including SCRC, HNPCC and SGC, but its recurrence in MSI SCRC is more than HNPCC CRC and NSI SGC. RASSF1A methylation is independent of dMMR and the simultaneous occurrence of RASSF1A methylation with KRAS and BRAF mutations in many MSI SCRC cases indicates their synergistic effect at the beginning of SCRC with dMMR. Several HNPCCs with hMSH2 germline mutations have a combination of RASSF1A methylation and KRAS mutations, whereas HNPCCs with hMLH1 germline mutations lack this combination and only half of HNPCCs have KRAS mutations. However, the frequency of RASSF1A methylation is similar in hMLH1 and hMSH2. Also, more than half of MSI SGC tumors had RASSF1A methylation [142].

Conflict of Interest

No conflict of interest

Acknowledgement

None

References

1. Edita Baltruškevičienė, Ugnius Mickeys, Tadas Žvirblis, Rokas Stulpinas, Teresė Pipirienė Ž, et al. (2016) Significance of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer patients receiving Bevacizumab: a single institution experience. Acta Med Litu 23(1): 24-34.
2. Passardi A, Ulivi P, Valgiusti M, Scarpi E, Moscati R, et al. (2011) The role of KRAS, BRAF, and PI3K mutations as markers of resistance to cetuximab in metastatic colorectal cancer. J Clin Oncol 29(15): 3603-3603.
3. Yamane LS, Scapulatempo NC, Alvarenga L, Oliveira CZ, Berardinelli GN, et.al. (2014) KRAS and BRAF mutations and MSI status in precursor lesions of colorectal cancer detected by colonoscopy. Oncol Rep 32(4): 1419-1426.
4. Marchoudi N, Amrani Hassani JH, Jouali F, Fekkak J, Rhaissi H, et al. (2013) Distribution of KRAS and BRAF mutations in Moroccan patients with advanced colorectal cancer. Pathol Biol 61(6): 273-276.
5. Colomer, Anna (2004) BRAF Mutations in Colorectal Cancer are Linked to Loss of hMLH1 Expression and Proximal Location, while Independent of KRAS Activation. J Molec Diagnost 4(9650): 20814-3993.
6. Kenji Kawada, Yuji Nakamoto, Mayumi Kawada, Koya Hida, Takuya Matsumoto, et al. (2012) Relationship between 18F-fluorodeoxyglucose accumulation and KRAS/BRAF mutations in colorectal cancer. Clin Cancer Res 18(6): 1696-1703.
7. Behl Ajay S, Katrina A B, Goddard, Thomas J Flottemesch, David Veenstra, et al. (2012) Cost-effectiveness analysis of screening for KRAS and BRAF mutations in metastatic colorectal cancer. J Natl Cancer Inst 104(23): 1785-1795.
8. Yucai Wang, Jonathan ML, Cecilia Yu, Marcella Tschautscher, Andrew MB, et al. (2018) Distinct impacts of KRAS, NRAS and BRAF mutations on survival of patients with metastatic colorectal cancer. J Clin Oncol 36(15): 3513-3513.
9. Smagulova K, Ukolova, Y Kurmankulova (2020) The study of mutations of the KRAS, NRAS, and BRAF genes in patients with colorectal cancer depending on gender, age, race in the population of the Republic of Kazakhstan. Annals of Oncology 3: 176.
10. Roya Dolatkhah, Md Hossein Somi, Mortaza Jabbarpour B, Iraj Asvadi K, Faris Farassati, et al. (2015) KRAS and BRAF mutations in colorectal cancer patients in northwest of Iran. J Cancer Epidemiol pp.14508.

11. Pechanska P (2010) KRAS-and BRAF-Mutations and their clinical correlates in a prospective, multi-center, diagnostic clinical trial, Molecular Signatures in Colorectal Cancer (MSKK). Zeitschrift für Gastroenterology 48(08): P178.

12. Wenbin Li, Tian Qiu, Yun Ling, Lei Guo, Lin Li, et al. (2015) Molecular pathological epidemiology of colorectal cancer in Chinese patients with KRAS and BRAF mutations. Oncotarget 6(37): 39607-39613.

13. Al Shamsi HO, Jones J, Fahmawi Y, Dahbour I, Tabash A, et al. (2016) Molecular spectrum of KRAS, NRAS, BRAF, PIK3CA, TP53, and APC somatic gene mutations in Arab patients with colorectal cancer: determination of frequency and distribution pattern. J Gastrointest Oncol 7(6): 882-902.

14. Jauhri M, Bhatnagar A, Gupta S, Minhas S, Shokeen Y, et al. (2017) Prevalence and coexistence of KRAS, BRAF, PIK3CA, NRAS, TP53, and APC mutations in Indian colorectal cancer patients: next-generation sequencing-based cohort study. Tumor Biol 39(2): 1010428317692265.

15. Sakarias Wangefjord, Magnus Sundström, Nooreldin Zendehrokh, Kajsa Ericson L, Björn Nodin, et al. (2013) Sex differences in the prognostic significance of KRAS codons 12 and 13, and BRAF mutations in colorectal cancer: a cohort study. Biol Sex Differ 4(1): 1-17.

16. Warsinggih, Liliyanto, Marhamah, M Ihwan Kusuma, Julianus Aboyaman Uwuratuw, et al. (2020) Relationship between BRAF V600E and KRAS mutations in stool for identifying colorectal cancer: A cross-sectional study. Ann Med Surg 23(60): 121-125.

17. Chen Mao, Junhua Zhou, Zuyao Yang, Yafang Huang, Xinyin Wu, et al. (2012) KRAS, BRAF and PIK3CA mutations and the loss of PTEN expression in Chinese patients with colorectal cancer. PloS One 7(5): 36653-36653.

18. Bożyk A, Krawczyk P, Reszka K, Krukowska K, Kolak A (2021) Correlation between KRAS, NRAS and BRAF mutations and tumor localizations in patients with primary and metastatic colorectal cancer. AMS.

19. Poulsen TS, De Oliveira DV, Espersen ML, Klarskov LL, Skovrider RW, et al. (2021) Frequency and coexistence of KRAS, NRAS, BRAF and PIK3CA mutations and occurrence of MMR deficiency in Danish colorectal cancer patients. APMIS 129(2): 61- 69.

20. Seth R, Crook S, Ibrahem S, Jackson M, Ilyas M, et al. (2009) Concomitant mutations and splice variants in KRAS and BRAF demonstrate complex perturbation of the Ras/Raf signalling pathway in Colorectal Cancer. Gut 58(9): 1234-1241.

21. Amar D, Georgios AM, Nikolaos A, Carlotta B, Jaeyun W, et al. (2018) Double KRAS and BRAF mutations in surgically treated colorectal cancer liver metastases: an international, multi-institutional case series. Anticancer Res 38(5): 2891-2895.

22. Susan DR, Matthew TS, Philip C, Faye E, Catherine L, et al. (2009) KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol 27(35): 5931-5937.

23. Smeby J, Sveen A, Bergsland CH, Bruun J, Merok M, Danielsen S, et al. (2017) Prognostic impact of BRAF and KRAS mutations according to the consensus molecular subtypes of colorectal cancer. Ann Oncol 28(5): 186.

24. Chenghao Yi, Yanqing Huang, Xing Yu, Xiaofen Li, Shu Zheng, et al. (2016) Clinicopathologic distribution of KRAS and BRAF mutations in a Chinese population with colorectal cancer precursor lesions. Oncotarget 7(13): 17265-17274.

25. Sun P, Wan J, Lianghua WU, Xiao Y, Lyu H, et al. (2016) Relationship between KRAS/NRAS/BRAF gene mutations and clinical pathological characteristics in colorectal cancer. Chinese J General Surg (12): 50-54.

26. Di Nicolantonio, Sartore B, Molinari F, Martini M, Saletti P, et al. (2009) Abstract# LB-93: BRAF, PIK3CA, and KRAS mutations and loss of PTEN expression impair response to EGFR-targeted therapies in metastatic colorectal cancer. Clin Rese 69(9):

27. Popovici, Vlad C, Arnaud R, Fred B, Sabine T, et al. (2013) BRAF and KRAS mutations as additional risk factors in the context of clinical parameters of patients with colorectal cancer. 31(15): 3522-3522.

28. Gordon Hutchins, Katie Southward, Kelly Handley, Laura Magill, Claire Beaumont, et al. (2011) Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol 29(10): 1261-1270.

29. Hutchins G, Southward K, Handley K, Magill L, Beaumont C, et al. (2011) Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol 29(10): 1261-1270.

30. Hou Y, LI SB, Shi XQ, FU GH, Jun WU, et al. (2018) Retrospective analysis of KRAS, NRAS and BRAF gene mutations and clinicopathological features in patients with colorectal cancer. J Shanghai Jiaotong Univ (12): 4-9.

31. Jianming Zheng, Yinghong Yang, Junliang Lu, Jie Gao, Tao Lu, et al. (2015) Molecular spectrum of KRAS, NRAS, BRAF and PIK3CA mutations in Chinese colorectal cancer patients: analysis of 1,110 cases. Sci Rep 5(1): 1.

32. Popovici Vlad, Eva Budinska, Fred TB, Sabine Tejpar, Arnaud DR, et al. (2013) Context-dependent interpretation of the prognostic value of BRAF and KRAS mutations in colorectal cancer. BMC Cancer 13(1): 439.

33. Murata EP, Stradella A, Páez D, Puyal MT, Garcia AS, et al. (2012) Prognostic value of BRAF and KRAS mutations in colorectal cancer patients. Ann Oncol 23: 216.

34. Stover DG, Cushman Vokoun AM, Vnencak Jones CL, Berlin J (2011) Analysis of BRAF and KRAS mutations in colorectal cancer and rectal carcinogenesis via fluorescent allele-specific PCR. J Clin Oncol 29(4): 436.

35. Nakanishi Ryota, Jun Harada, Munkhbold Tuul, Yan Zhao, Koji Ando, et al. (2013) Prognostic relevance of KRAS and BRAF mutations in Japanese patients with colorectal cancer. Int J Clin oncol 18(6): 1042-1048.

36. Foltran, Luisa, Nicoletta Pella, Paola Ermacora, Giuseppe Aprile, et al. (2015) Prognostic role of KRAS, NRAS, BRAF and PIK3CA mutations in advanced colorectal cancer. Future Oncol 11(4): 629-640.

37. Takahashi N, Yasuhide Y, Hirokazu T, Kohei A, Yoshitaka H, et al. (2012) Mutations in NRAS codon 61, KRAS codon 146, and BRAF V600E as prognostic factors in patients who received anti-EGFR antibody for metastatic colorectal cancer. J Clin Oncol 30(15): e14126.

38. Yi Chenghao, Yanqing Huang, Xing Yu, Xiaofen Li, Shu Zheng, et al. (2016) Clinicopathologic distribution of KRAS and BRAF mutations in a Chinese population with colorectal cancer precursor lesions. Oncotarget 7(13): 17265-17274.

39. Midthun Lauren, Shagufta Shaheen, Jeremy Deisch, Maheswari Senthil, James Tsai, et al. (2019) Concomitant KRAS and BRAF mutations in colorectal cancer. J Gastrointest Oncol 10(3): 577-581.

40. Larki P, Ehsan G, Md Yaghoob T, Fatemeh K, Ehsan NM, et al. (2017) Coexistence of KRAS and BRAF mutations in colorectal cancer: a case report supporting the concept of tumoral heterogeneity. Cell J 19(1): 113-117.

41. Shen Y, Wang J, Han X, Yang H, Wang S, et al. (2013) Effectors of epidermal growth factor receptor pathway: the genetic profiling of KRAS, BRAF, PIK3CA, NRAS mutations in colorectal cancer characteristics and personalized medicine. PloS One 8(12): e81628.

42. Chen Jing, Fang Guo, Xin Shi, Lihua Zhang, Aifeng Zhang, et al. (2014) BRAF V600E mutation and KRAS codon 13 mutations predict poor survival in Chinese colorectal cancer patients. BMC cancer 14(1): 802.

43. Jakovljevic K, Malisic E, Cavic M, Krivokuca A, Dobricic J, et al. (2012) KRAS and BRAF mutations in Serbian patients with colorectal cancer. J BUON 17(3): 575-580.

44. Lee Joon Hyop, Jiyoung Ahn, Won Seo Park, Eun Kyung Choe, Eunyoung Kim, et al. (2019) Colorectal cancer prognosis is not associated with BRAF and KRAS mutations-a STROBE compliant study. J Clin Med 8(1): 111.

45. Tian An Guo, Yu Chen Wu, Cong Tan, Yu Tong Jin, Wei Qi Sheng, et al. (2019) 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 145(6): 1625-1634.

46. Kadowaki Shigenori, Miho Kakuta, Shuhei Takahashi, Akemi Takahashi, Yoshiko Arai, et al. (2015) Prognostic value of KRAS and BRAF mutations in curatively resected colorectal cancer. World J Gastroenterol 21(4): 1275-1283.

47. Wojas Krawczyk K, Kalinka Warzocha E, Reszka K, Nicoś M, Szumiło J, et al. (2019) Analysis of KRAS, NRAS, BRAF, and PIK3CA mutations could predict metastases in colorectal cancer: A preliminary study. Advan Clin Experimen Med 28(1): 67-73.

48. Hao YX, Li YM, Ye M, Guo YY, Li QW, et al. (2017) KRAS and BRAF mutations in serum exosomes from patients with colorectal cancer in a Chinese population. Oncol Lett 13(5): 3608-1316.

49. Pu Xingxiang, Zhizhong Pan, Ying Huang, Ying Tian, Hongqiang Guoet, et al. (2013) Comparison of KRAS/BRAF mutations between primary tumors and serum in colorectal cancer: biological and clinical implications. Oncol Lett 5(1): 249-254.

50. Thierry AR, Mouliere F, El Messaoudi S, Rolet F, Lamy PJ, et al. (2014) Circulating DNA analysis and concordance with tumor section analysis in the detection of KRAS and BRAF point mutations from metastatic colorectal cancer. Nat Med 20(4): 430-435.

51. Liu XN, Tian Z, Wei XF, Wang Q, Zhang JX, et al. (2019) Combined detection of KRAS, NRAS, BRAF and PIK3CA mutations in the plasma and tumor tissues of colorectal cancer patients. Zhonghua Bing Li Xue Za Zhi 48(5): 373-377.

52. Calistri Daniele, Claudia Rengucci, Ian Seymour, Elena Leonardi, Mauro Truini, et al. (2006) KRAS, p53 and BRAF gene mutations and aneuploidy in sporadic colorectal cancer progression. Cell Oncol 28(4): 161-166.

53. Holck S, Jesper B, Helle P, Anja AP, Amita C, et al. (2016) Localization of active, dually phosphorylated extracellular signal-regulated kinase 1 and 2 in colorectal cancer with or without activating BRAF and KRAS mutations. Hum Pathol 54: 37-46.

54. Lucchetti J, Formica V, Doldo E, Palmieri G, Orlandi A, et al. (2020) Value of liquid biopsy using high-affinity plasma DNA binding magnetic beads and qualitative real-time PCR for KRAS, NRAS and BRAF mutations in metastatic colorectal cancer patients. Ann Oncol 31(S3): 217-219.

55. Spindler KL, Pallisgaard N, Vogelius I, Jakobsen A (2012) Quantitative cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. Clin Cancer Res 18(4): 1177-1185.

56. Pallisgaard N, Spindler KG, Vogelius IS, Jakobsen AK (2011) Cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer treated with third line cetuximab and irinotecan. J Clin Oncol 29(15): 3599.

57. Moradi Marjaneh, Fereshteh Asgharzadeh, Elnaz Khordad, Mahdi Moradi M, et al. (2020) The clinical impact of quantitative cell-free DNA, KRAS, and BRAF mutations on response to anti-EGFR treatment in patients with metastatic colorectal cancer. Curr pharm Des 27(7): 942-952.

58. Liu LL, Tong Z, Yan C, Zhang H, Jiang W, et al. (2018) Dynamic monitoring of KRAS, NRAS, BRAF and PIK3CA mutations in circulating cell-free DNA for metastatic colorectal cancer patients treated with cetuximab. Ann Oncol 29(8): 185.

59. Kidess Sigal, Haiyan E Liu, Melanie M Triboulet, James Che, Vishnu C Ramani, et al. (2016) Enumeration and targeted analysis of KRAS, BRAF and PIK3CA mutations in CTCs captured by a label-free platform: Comparison to ctDNA and tissue in metastatic colorectal cancer. Oncotarget 7(51): 85349-85364.

60. Lisa Simi, Nicola Pratesi, Marina Vignoli, Roberta Sestini, Fabio Cianchi, et al. (2008) High-resolution melting analysis for rapid detection of KRAS, BRAF, and PIK3CA gene mutations in colorectal cancer. Am J Clin Pathol 130(2): 247-253.

61. Hsieh Li Ling, Tze Kiong Er, Chih Chieh Chen, Jan Sing Hsieh, Jan Gowth Chang, et al. (2012) Characteristics and prevalence of KRAS, BRAF, and PIK3CA mutations in colorectal cancer by high-resolution melting analysis in Taiwanese population. Clin Chim Acta 413(19-20): 1605-1611.

62. Li Ling Hsieh, Tze Kiong Er, Chih Chieh Chen, Jan Sing Hsieh, Jan Gowth C, et al. (2012) Characteristics; prevalence of KRAS; BRAF; and PIK3CA mutations in colorectal cancer by high-resolution melting analysis in Taiwanese population. Clin Chim Acta 413(19-20): 1605-1611.

63. Robešová B, Monika B, Alžběta V, Lenka O, Jitka H, et al. (2013) Molecular biological diagnostics of KRAS and BRAF mutations in patients with colorectal cancer-laboratory experience. Klinicka onkologie: casopis Ceske a Slovenske onkologicke spolecnosti 26(1): 25-30.

64. Nguyen Ha T, Do Thanh L, Quan HD, Vinay Bharadwaj T, Bang Van N, et al. (2021) High frequency of microsatellite instability and its substantial co-existence with KRAS and BRAF mutations in Vietnamese patients with colorectal cancer. Oncol Lett 21(1): 41.

65. Mancini I, Santucci C, Sestini R, Simi L, Pratesi N, et al. (2010) The use of COLD-PCR and high-resolution melting analysis improves the limit of detection of KRAS and BRAF mutations in colorectal cancer. J Molec Diagnost 12(5): 705-711.

66. Pisareva EE, Ljubchenko LN, Kovalenko SP, Shamanin VA (2016) Analysis of mutations in kras and braf genes in colorectal cancer in Russian patients. Siberian journal of oncology15(2): 36-41.

67. Seth R, Crook S, Ibrahem S, Fadhil W, Jackson D, et al. (2009) Concomitant mutations and splice variants in KRAS and BRAF demonstrate complex perturbation of the Ras/Raf signalling pathway in advanced colorectal cancer. Gut 58(9): 1234-1241.

68. Li X, Yang T, Li CS, Song Y, Lou H, et al. (2018) Surface enhanced Raman spectroscopy (SERS) for the multiplex detection of Braf, Kras, and Pik3ca mutations in plasma of colorectal cancer patients. Theranostics 8(6): 1678-1689.

69. Lilleberg SL, Durocher J, Zhi C, Walters V, Sanderset C (2004) A comprehensive panel of mutation scanning assays detects mutations in the APC, TP53, KRAS, BRAF genes and hypermethylated APC and MLH1 DNA in plasma of patients with various stages of colorectal cancer: Utility for early detection, prognosis, and disease monitoring. J Clin Oncol 22(14): 3765-3765.

70. Franczak C, Witz A, Geoffroy K, Demange J, Rouyer M, et al. (2020) Evaluation of KRAS, NRAS and BRAF mutations detection in plasma using an automated system for patients with metastatic colorectal cancer. PloS One 15(1): e0227294.

71. Gilson P, Franczak C, Dubouis L, Husson M, Rouyer M, et al. (2019) Evaluation of KRAS, NRAS and BRAF hotspot mutations detection for patients with metastatic colorectal cancer using direct DNA pipetting in a fully automated platform and next generation sequencing for laboratory workflow optimisation. PLoS One 14(7): e0219204.

72. Kiran MD, Alvin Concepcion, Jessica Shea, Smriti Gupta, Lilly Kong (2016) Sensitive, specific, and modular KRAS, NRAS, and BRAF assays for simultaneous detection of 30 important point mutations in colorectal cancer specimens. J Clin Oncol 34(4): 547-547.

73. Balschun Katharina, Ann Kathrin Wenke, Christoph Röcken, Jochen Haag (2011) Detection of KRAS and BRAF mutations in advanced colorectal cancer by allele-specific single-base primer extension. Expert Rev Mol Diagn 11(8): 799-802.

74. Bagci Binnur, Musa Sari, Kursat Karadayi, Mustafa Turan, Ozturk Ozdemir, et al. (2016) KRAS, BRAF oncogene mutations and tissue specific promoter hypermethylation of tumor suppressor SFRP2, DAPK1, MGMT, HIC1 and p16 genes in colorectal cancer patients. Cancer Biomarkers 17(2): 133-143.

75. Emelyanova MA, Amossenko FA, Semyanikhina AV, Aliev VA, Barsukov YA, et al. (2015) Biochip detection of KRAS, BRAF, and PIK3CA somatic mutations in colorectal cancer patients. Mol Biol 49(4): 617-627.

76. Santos C, Garcia Carbonero R, Garcia Alfonso P, Azuara D, Varela M, et al. Comparison of three molecular methods to detect mutations in KRAS, NRAS, BRAF and PIK3CA in Metastatic Colorectal Cancer (mCRC). J Clin Oncol 34(14): 3559-3559.

77. Levin Sparenberg E, Bylsma LC, Lowe K, Sangare L, Fryzek JP, et al. (2020) A systematic literature review and meta-analysis describing the prevalence of KRAS, NRAS, and BRAF gene mutations in metastatic colorectal cancer. Gastroenterol Res 13(5): 184-198.

78. Ma B, Mo F, Tong JH, Wong A, Wong SC, et al. (2015) Elucidating the prognostic significance of KRAS, NRAS, BRAF and PIK3CA mutations in C hinese patients with metastatic colorectal cancer. Asia Pac J Clin Oncol 11(2): 160-169.

79. Loupakis F, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, et al. (2009) KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 101(4): 715-721.

80. Marta Herreros Villanueva, Noemí Gomez Manero, Pilar Muñiz, Carlos García Girón, Maria Jesús Coma del Corral (2011) PIK3CA mutations in KRAS and BRAF wild type colorectal cancer patients. A study of Spanish population. Molecular biology reports 38(2): 1347-1351.

81. De Roock Wendy, Bart Claes, David Bernasconi, Jef De Schutter, Bart Biesmans, et al. (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11(8): 753-762.

82. Pechanska P, Mayr T, Hinzmann B, Klaman I, Daniel Abd el Messih S, et al. (2012) Use of wild-type (wt) KRAS, BRAF, and PIK3CA, combined with KRAS Mutations (mut) in codon 13 and RNA expression level of Amphiregulin (AREG) and Epiregulin (EREG), to predict Cetuximab (CE) response with high accuracy in colorectal cancer of all four Dukes' stages. J Clin Oncol 30(4): 517-517.

83. Normanno N, Rachiglio AM, Lambiase M, Martinelli E, Fenizia F et al. (2015) Heterogeneity of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer and potential effects on therapy in the CAPRI GOIM trial. Ann Oncol 26(8): 1710-1714.

84. Miyake Y, Iwamoto S, Hazama S, Goda F, Matsuda C, et al. (2012) Phase II multicenter study of second line FOLFIRI plus cetuximab (FLIER) in patients with KRAS wild-type metastatic colorectal cancer: Final analysis of survival and additional analysis of BRAF, PI3CA mutations. J Clin Oncol 30(4): 481.

85. Modest DP, Jung A, Moosmann N, Laubender RP, Giessen C, et al. (2012) The influence of KRAS and BRAF mutations on the efficacy of cetuximab-based first-line therapy of metastatic colorectal cancer: An analysis of the AIO KRK-0104-trial. Int J cancer 131(4): 980-986.

86. Di Bartolomeo M, Pietrantonio F, Perrone F, Dotti KF, Lampis A, et al. (2014) Lack of KRAS, NRAS, BRAF and TP53 mutations improves outcome of elderly metastatic colorectal cancer patients treated with cetuximab, oxaliplatin and UFT. Target Oncol 9(2): 155-162.

87. Innocenti Federico, Sara R Selitsky, Joel S Parker, James Todd Auman, Kelli Hammond, et al. (2020) Association of KRAS and BRAF mutations with Progression-Free Survival (PFS) with second line FOLFIRI+/-regorafenib in Metastatic Colorectal Cancer (mCRC). J Clin Oncol 38(4): 196-196.

88. Ruzzo A, Cremolini C, Loupakis F, Fornaro L, Santini D, et al. (2009) Association of BRAF mutations and EGFR Intron-1 L/L genotype with resistance to cetuximab plus irinotecan treatment in KRAS wild-type metastatic colorectal cancer patients. J Clin Oncol 27(15): 4058-4058.

89. Garcia CN, Martinez U, Li W, Orta A, Perez N, et al. (2020) KRAS and BRAF mutations as prognostic and predictive biomarkers for standard chemotherapy response in metastatic colorectal cancer: a single institutional study. Cells 9(1): 219.

90. Yun Jihye, Edouard Mullarky, Changyuan Lu, Kaitlyn N Bosch, Adam Kavalier, et al. (2015) Vitamin C inhibits the survival and growth of colorectal cancer cells with KRAS or BRAF mutations. Sci 350(6266): 1391-1396.

91. Spindler KG, Pallisgaard N, Jakobsen AK (2010) The predictive value of KRAS and BRAF mutations for outcome in second-line irinotecan monotherapy in Metastatic Colorectal Cancer (mCRC). J Clin Oncol 28(15): e14062-e14062.

92. Dolatkhah, Roya (2015) Common KRAS and BRAF mutations in colorectal cancer patients. Govaresh 20(1): 27-33.

93. Tejpar S, De Roock W, Claes B, Fountzilas G, Kalogeras K, et al. (2010) Pik3ca, braf, nras and kras mutations and outcome prediction in chemo refractory Metastatic Colorectal Cancer (mcrc) patients treated with egfr-targeting Monoclonal Antibodies (moabs): results of a european consortium. Ann Oncol 21: 6.

94. Negru Serban, Eirini Papadopoulou, Angela Apessos, Dana Lucia Stanculeanu, Eliade Ciuleanu, et al. (2014) KRAS, NRAS and BRAF mutations in Greek and Romanian patients with colorectal cancer: a cohort study. BMJ Open 4(5): e004652

95. Vaughn Cecily P, Scott D Zobell, Larissa V Furtado, Christine L Baker, Wade S Samowitz (2011) Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. Genes Chromosomes Cancer 50(5): 307-312.

96. Kawazoe Akihito, Kohei Shitara, Shota Fukuoka, Yasutoshi Kuboki, Hideaki Bando, et al. (2015) A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. BMC Cancer 15(1): 258.

97. Emi Y, Oki E, Saeki H, Kitao H, Samura H, et al. Analysis of KRAS/NRAS, PI3CA, and BRAF mutations in the phase II KSCC0901 study of cetuximab plus S-1 as third-line treatment for metastatic colorectal cancer in Japanese patients. J Clin Oncol 32(3): 587.

98. Lambrechts D, De Roock W, Prenen H, De Schutter J, Jacobs B, et al. (2009) The role of KRAS, BRAF, NRAS, and PIK3CA mutations as markers of resistance to cetuximab in chemorefractory metastatic colorectal cancer. J Clin Oncol 27(15): 4020.

99. Saridaki Z, Tzardi M, Papadaki C, Sfakianaki M, Pega F, et al. (2011) Impact of KRAS, BRAF, PIK3CA Mutations, PTEN, AREG, EREG Expression and Skin Rash in≥2nd Line Cetuximab-Based Therapy of Colorectal Cancer Patients. PloS One 6(1): e15980.

100. Yungmae M, Gregory P, Philip W, Youyan Z, Robert D, et al. (2017) Mouse PDX trial suggests synergy of concurrent inhibition of RAF and EGFR in colorectal cancer with BRAF or KRAS mutations. Clin Cancer Res 23(18): 5547-5560.

101. Jia YT, Yang DH, Zhao Z, Bi XH, Han WH, et al. (2018) Effects of PHA-665752 and cetuximab combination treatment on in vitro and murine xenograft growth of human colorectal cancer cells with KRAS or BRAF mutations. Current cancer drug targets 18(3): 278-286.

102. Oliner K, Douillard JY, Siena S, Tabernero J, Burkes R, et al. (2013) Evaluation of KRAS, NRAS, and BRAF mutations in PRIME: panitumumab with FOLFOX4 as first-line treatment in Metastatic Colorectal Cancer (mCRC). Annals of Oncology 24: 23-24.

103. Oliner KS, Douillard JY, Siena S, Tabernero J, Burkes RL, et al. (2015) Analysis of KRAS/NRAS and BRAF mutations in the phase III PRIME Study of Panitumumab (pmab) plus FOLFOX versus FOLFOX as first-line treatment (tx) for Metastatic Colorectal Cancer (mCRC). Clin Cancer Res 21(24): 5469-5479.

104. Peeters M, K Oliner, T Price, A Cervantes, A Sobrero, et al. (2014) KRAS/NRAS and BRAF Mutations in the 20050181 Study of Panitumumab+FOLFIRI for the 2ND-Line Treatment of Metastatic Colorectal Cancer: Updated Analysis. Ann Oncol 25(2): 115.

105. Peeters Marc, Kelly Smith Oliner, Timothy Jay Price, Andres Cervantes, Alberto F Sobrero, et al. (2014) Updated analysis of KRAS/NRAS and BRAF mutations in study 20050181 of Panitumumab (pmab) plus FOLFIRI for second-line Treatment (tx) of Metastatic Colorectal Cancer (mCRC) pp.3568-3568.

106. Phua Lee C, Hui Wen, Angie Hui, Elya Chen, Michelle Shu, et al. (2015) Prevalence of KRAS, BRAF, PI3K and EGFR mutations among Asian patients with metastatic colorectal cancer. Oncol lett 10(4): 2519-2526.

107. S Tian, I Simon, P Roepman, J Tabernero, M Snel, et al. (2011) The role of activating mutations of KRAS, BRAF, and PIK3CA pathway convergence at the transcriptional level and prediction of treatment response to cetuximab in colorectal cancer. J Clinic Oncol 29(15): 3534-3534.

108. Behl Ajay, Katrina A, Thomas J Flottemesch, David Veenstra, Richard T Meenan, et al. (2012) Cost-effectiveness analysis of screening for KRAS and BRAF mutations in metastatic colorectal cancer. JNCI: J Natl Cancer Inst 104(23): 1785-1795.

109. Behl Ajay (2011) PS-30: Cost-Effectiveness Analysis of Screening for Kras and Braf Genetic Mutations in Metastatic Colorectal Cancer. Clin Med Res 9(3-4): 174-174.

110. Ličar Alenka, Petra Cerkovnik, Srdjan Novaković (2011) Distribution of some activating KRAS and BRAF mutations in Slovene patients with colorectal cancer. Med Oncol 28(4): 1048-1053.

111. Guglielmini, Pamela Francesca, Federica Grosso, Sara Orecchia, Marco Galliano, et al. (2013) Double KRAS and BRAF mutations in colorectal cancer in a single oncologic department series. J Clin Oncol 31(15): e14657-e14657.

112. Kima, Richard, Brooke Phillipsa, Wasif Saifb M (2010) Assessment of KRAS and BRAF Mutations in Metastatic Colorectal Cancer: A Step Toward Personalized Medicine. Gastrointesti Malignancies 1(1): 9.

113. Ntavatzikos A, A Spathis, P Patapis, I Papadopoulos, A Koumarianou, et al. (2018) Implications of thymidylate synthase gene polymorphisms, KRAS and BRAF mutations in the survival of patients with colorectal cancer treated with adjuvant chemotherapy. Annals Oncol 29: 176.

114. Yun Jihye (2009) Glucose deprivation selects for KRAS and BRAF mutations in colorectal cancer. Johns Hopkins University.

115. Artale Salvatore, Andrea Sartore, Silvio Marco, Valentina Gambi, Carolina Silvia S, et al. (2008) Mutations of KRAS and BRAF in primary and matched metastatic sites of colorectal cancer. J Clin Oncol 26(25): 4217-4219.

116. Bergamo Francesca, Marta Schirripa, Fotios Loupakis, Chiara Cremolini, Mariaelena Casagrande, et al. (2014) BRAF and KRAS Mutations in Liver-Resected Metastatic Colorectal Cancer (mCRC) Patients (pts). Journal of Clinical Oncol 32(3): 476-476.

117. Rebersek M, Boc M, Cerkovnik P, Benedik J, Hlebanja Z, et al. (2011) Efficacy of first-line systemic treatment in correlation with BRAF V600E and different KRAS mutations in metastatic colorectal cancer-a single institution retrospective analysis. Radiol oncol 45(4): 285-291.

118. Saridaki, M Tzardi, Chara Papadaki, Fragka Pegka, M Sfakianaki, et al. (2011) Impact of KRAS, BRAF, and PIK3CA mutations, PTEN, AREG, and EREG expression, and skin rash in metastatic colorectal cancer patients treated with cetuximab-containing salvage treatment. J Clinical Oncol 29(4): 445-445.

119. Saridaki Zacharenia, Maria Tzardi, Chara Papadaki, Maria Sfakianaki, Fraga Pega, et al. (2011) Impact of KRAS, BRAF, PIK3CA Mutations, PTEN, AREG, EREG Expression and Skin Rash in [greater than or equal to] 2 Line Cetuximab-Based Therapy of Colorectal Cancer Patients. PLoS One 6(1): e15980-e15980.

120. Rebersek Martina, Marko Boc, Zvezdana Hlebanja, Maja Ebert, Janja Ocvirk, et al. (2012) PD-0015 Influence of BRAF V600E and Different Kras Mutations on Outcome of Metastatic Colorectal Cancer Patients Treated with First-Line Therapy. Annals Oncol 23(4): 24.

121. Renaud S, Romain B, Falcoz PE, Olland A, Santelmo N, et al. (2015) KRAS and BRAF mutations are prognostic biomarkers in patients undergoing lung metastasectomy of colorectal cancer. Br J Cancer 112(4): 720-728.

122. Larsen SG, Dueland S, Goscinski M, Steigen S, Hofsli E, et al. (2019) Survival according to mutations in BRAF, KRAS, or Microsatellite Instability (MSI-H) after cytoreductive surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) in patients with peritoneal metastases from colorectal cancer. J Clinic Oncol 37(7): 3565-3565.

123. Soeda H, Shimodaira H, Watanabe M, Suzuki T, Gamoh M, et al. (2013) Clinical usefulness of KRAS, BRAF, and PIK3CA mutations as predictive markers of cetuximab efficacy in irinotecan-and oxaliplatin-refractory Japanese patients with metastatic colorectal cancer. Int J clinic Oncol 18(4): 670-677.

124. Modest Dominik P, Ingrid Ricard, Volker Heinemann, Susanna Hegewisch B, Wolff Schmiegel, et al. (2016) Influence of KRAS exon 2 mutation variants as well as NRAS-and BRAF-mutations on outcome of patients with Metastatic Colorectal Cancer (mCRC) receiving combination chemotherapy with or without bevacizumab. J Clinical Oncol 34(15): 3551-3551.

125. Li Hong, Yuan Yuan Lu, Yan Xin An, Xin Wang, Qing Chuan Z, et al. (2011) KRAS, BRAF and PIK3CA mutations in human colorectal cancer: relationship with metastatic colorectal cancer. Oncol Rep 25(6): 1691-1697.

126. Reggiani Bonetti L, Barresi V, Maiorana A, Manfredini S, Caprera C, et al. (2018) Clinical impact, and prognostic role of kras/braf/pik3ca mutations in stage I colorectal cancer. Dis Markers 2018: 2959801.

127. Barresi V, Bonetti LR, Bettelli S (2015) KRAS, NRAS, BRAF mutations and high counts of poorly differentiated clusters of neoplastic cells in colorectal cancer: observational analysis of 175 cases. Pathology 47(6): 551-556.

128. Gershon A, R Machado, H Trinh, C Julian, Z Shi, et al. (2020) 446P Real-World Data (RWD) survival analysis of First Line (1L) Metastatic Colorectal Cancer (mCRC) Patients (pts) with BRAF V600 and non-V600, KRAS G12 and G13, and NRAS mutations. Annals of Oncol 31(4): S409-S461.

129. Fujiyoshi K, Yamamoto G, Takahashi A, Arai Y, Yamada M, et al. (2017) High concordance rate of KRAS/BRAF mutations and MSI-H between primary colorectal cancer and corresponding metastases. Oncol Rep 37(2): 785-792.

130. Albitar Maher, Sucha Sudarsanam, Wanlong Ma, Shiping Jiang, Wayne Chen, et al. (2018) Association between microsatellite instability and BRAF, TP53, PTEN, and KRAS mutations in colorectal cancer. J Clinic Oncol 36(15): e15604-e15604.

131. Chan TL, Wong CW, Chan AS, Guo DL, Ho JW, et al. BRAF and KRAS mutations in mismatch repair deficient colorectal cancer.

132. Velho S, Moutinho C, Cirnes L, Albuquerque C, Hamelin R, et al. (2008) BRAF, KRAS and PIK3CA mutations in colorectal serrated polyps and cancer: primary or secondary genetic events in colorectal carcinogenesis? BMC cancer. 8: 255.

133. Hurtado C, Zarate A, Wielandt AM, Carrillo K, Yamagiwa K, et al. (2012) Somatic mutations in KRAS, BRAF and PIK3CA genes in Chilean patients with sporadic colorectal cancer: Correlation with clinical and histological features. 72(8)

134. Wallin U, Helgi Birgisson, Karolina Edlund, Lars Påhlman, Hanna Göransson Kultima, et al. (2015) Microsatellite instability, KRAS, BRAF and PIK3CA mutations in patients operated for stage II and III colorectal cancer. BMC Cancer 15: 125.

135. Roma AE, Modulation of colorectal cancer stem cell pool and its crosstalk with tumor microenvironment by KRas, BRaf, and PIK3CA mutations (Doctoral dissertation, 01100: Universidade do Porto).

136. De Vogel S, Weijenberg P, Herman G, Wouters A, De Goeij F, et al. (2009) MGMT and MLH1 promoter methylation versus APC, KRAS and BRAF gene mutations in colorectal cancer: indications for distinct pathways and sequence of events. Ann oncol 20(7): 1216-1222.

137. Nourizadeh (2017) An APC, BRAF and KRAS mutations, and MLH1, MGMT and CDKN2A expression analysis in Nepalese colorectal cancer patients. pp.24.

138. Ashktorab H, Kumar K, Brim H, Smoot DT, Lee EL (2009) Abstract A83: Distinct BRAF (V600E) and KRAS mutations in high microsatellite instability sporadic colorectal cancer in African Americans. Clin Cancer Res 15(4): 1155-1161.

139. Kumar Krishan, Hassan Brim, Francis Giardiello, Duane T, Mehdi Nouraie, et al. (2009) Distinct BRAF (V600E) and KRAS mutations in high microsatellite instability sporadic colorectal cancer in African Americans. Clin Cancer Res 15(4): 1155-1161.

140. Velho S, Moutinho C, Cirnes L, Albuquerque C, Hamelin R, et al. (2008) BRAF, KRAS and PIK3CA mutations in colorectal serrated polyps and cancer: primary or secondary genetic events in colorectal carcinogenesis? BMC Cancer 8(1): 255.

141. Oliveira C, Velho S, Domingo E, Preto A, Hofstra RM, et al. (2005) Concomitant RASSF1A hypermethylation and KRAS/BRAF mutations occur preferentially in MSI sporadic colorectal cancer. Oncogene 24(51): 7630-7634.