*research article*

# The five-year KRAS, NRAS and BRAF analysis results and treatment patterns in daily clinical practice in Slovenia in 1st line treatment of metastatic colorectal (mCRC) patients with *RAS* wild-type tumour (wt*RAS*) - a real- life data report 2013–2018

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**Background.** We preformed a Phase IV non-interventional study to assess KRAS, NRAS and BRAF status in metastatic colorectal cancer (mCRC) patients suitable for 1st line treatment and to evaluate the decisions for 1st line treatment considering the treatment goals in the RAS wild type (wt) patients. The aim of our study was also to evaluate the influence of a waiting period for biomarkers analysis on the start of first-line treatment.

**Patients and methods.** Patients with histologically confirmed mCRC adenocarcinoma suitable for first-line treatment fulfilling all inclusion criteria were included in the study. The KRAS, NRAS and BRAF analysis was performed from tissue samples of primary tumor site or metastatic site. All included patients have given consent to participate in the study by signing the informed consent form.

**Results.** From April 2013 to March 2018 at the Institute of Oncology Ljubljana 650 patients were included, 637 of them were treated with first- line systemic treatment according to RAS and BRAF status. Remaining 13 patients with mCRC did not receive systemic first-line treatment. The distribution of patients with KRAS mutated and wild-type tumors, was almost equal, 48.8% and 47.9% respectively, 89 % of the patients had wt NRAS tumours and 86.1% had wt BRAF tumours. The most frequently prescribed treatment was bevacizumab-based therapy (53.1%), either in combination with doublet chemotherapy or with mono-chemotherapy. EGFR inhibitors cetuximab and panitumumab were prescribed in wt RAS mCRC patients (30.9%). The waiting period for biomarkers analysis was two weeks.

**Conclusions.** Our real-world data, single centre 5-year analysis showed that the distribution between wild type and mutated type tumors of the patients with mCRC was approximately the same, as worldwide, so the Slovenian population with mCRC has the same ratio distribution of KRAS, NRAS and BRAF wild and mutated genes. We concluded that a two-week waiting period for biomarkers analysis did not influence the first line treatment decision, so it was in the accordance with the worldwide treatment guidelines based on evidence-based medicine.

Key words: metastatic colorectal cancer; RAS and BRAF biomarkers; systemic treatment

## Introduction

Colorectal cancer (CRC) is one of the most common cancers and one of the leading causes of cancer death in the world and also in Slovenia. According to the Cancer Registry of Slovenia 2021, 1349 new patients were diagnosed with CRC in 2018.[1] Approximately 25% of patients present with

metastatic disease at diagnosis, and about 50% of patients with CRC will eventually develop metastases.[2,3] Metastatic disease is still incurable, with 5% five-year overall survival (OS) without treatment. Until 1996 five-fluorouracil (5-FU) was the only approved drug for this disease. Since then, five new agents have been approved in the United States and in Europe for this disease, among them irinotecan, oxaliplatin and the targeted therapies cetuximab, bevacizumab and panitumumab.[2-16] With the current management of metastatic disease, with chemotherapy with oxaliplatin and irinotecan in combination with biologicals, targeting epidermal growth factor mediated growth regulatory pathway and the vascular endothelial growth factor mediated angiogenesis pathway, the progression-free survival (PFS) and OS of these patients can be prolonged. The CRC-related 5-year survival rate approaches 60%.[2,3]

CRC represents a heterogeneous group of diseases. They are promoted by environmental risk factors and various molecular pathways, which influence individual susceptibility to cancer. About 70% of CRCs are sporadic, while 20–30% have a hereditary component, such as Lynch syndrome and familial adenomatous polyposis (FAP).[2,3,15-17] Classification of CRC can be divided in anatomic, genetic and molecular transcriptomic classification. The race, foods, nutrients, carcinogenic agents and increasingly more important the gut microbiome act in a specific manner determined by the primary tumour location to promote carcinogenesis right-sided tumours (RCC) account about to 35% of cases, while left-sided (LCC) and rectal cancer represent about 65% of cases.[17,18] According to genetic classification approximately 85% of CRC, comprises of non-hypermutated, microsatellite stable (MSS) tumours with chromosomal instability with a high frequency of DNA somatic alterations.[17,18]

Several oncogenes play key roles in promoting colorectal cancer.[17-20] Among them firstly in clinical practice of mCRC management, oncogenic mutations of Rat sarcoma virus (RAS) and b-Raf murine sarcoma viral oncogene homolog B (BRAF), which activate the mitogen-activated protein kinase (MAPK) signalling pathway, are the most important.[21-24] Oncogenic RAS mutations have historically been present in approximately 40–50% of CRC patients. According to additional subsequent analysis, the prevalence of RAS mutations in mCRC has been shown to be higher, in 55.9% with mutations in KRAS exon 2 being the most common, they represent 42.6%, followed by KRAS exon 3 in 3.8%, KRAS exon 4 in 6.2%, NRAS exon 2 in 2.9%, NRAS exon3 in 4.2% and NRAS exon4 in 0.3% mutations.[16] NRAS mutant tumours are more frequently in older patients and located on the proximal colon. KRAS and NRAS mutant tumours exhibit similar metastasis patterns. *RAS* mutations are encountered in approximately 50% of the total population worldwide but it also demonstrates geographical variations. Therefore, such a country-by-country determination of the mutation status of the RAS gene in mCRC patients would be meaningful for personalised treatment decision-making.

Selection of patients for anti-epidermal growth factor receptor (EGFR) antibodies based on molecular characteristics of the tumour is very important, because the activity of the anti-EGFR antibodies was confined to wtKRAS tumours (traditionally mutations on codon 12 and 13 of exon 2).[22-26] Than the testing was expanded to the other more rare RAS mutations: codon 61 of exon 3 and codon 117 and 146 of exon 4 of KRAS and exons 2, 3 and 4 of N-Rat sarcoma virus (NRAS), which are also predictive to response to anti-EGFR antibodies.[2] Exon 2 KRAS mutations occur in ~40% of CRC cases, and the other KRAS and NRAS mutations in ~10%–15% of CRC patients. Those mutations influence the response to the combinations of cetuximab or panitumumab alone or with irinotecan- and oxaliplatin-based regimens.[27-42] Treatment with anti-EGFR antibodies may even harm patients with a RAS mutation, especially when combined with oxaliplatin.[4]

BRAF mutations (mt BRAF) are present in about 10–15% of CRCs, with mt BRAFV600E mutations being the most frequent.[15,16] BRAF encodes a serine/threonine protein kinase, a downstream effector of the KRAS protein, and mt BRAF results in constitutive activation of the MAPK signalling pathway. RAS mutations (mt RAS) and mt BRAF mutations are usually mutually exclusive. The most common mutation of the BRAF gene is V600E.[15-18] The same was reported in our previous study carried on Slovenian patients with CRC where the mt BRAF V600E was found in 5.1% of patients.[22,43,44] A mt BRAF is a strong negative prognostic biomarker. The patients with a mt BRAF mCRC have a very poor prognosis. In prognostic analyses, patients with mt BRAF had significantly shorter PFS (7.0 months *vs.* 12.2 months, HR 2.78; $p < 0.001$) and OS (13.4 months *vs*. 37.9 months, HR 5.67; $p < 0.001$) compared with BRAF wild-type (wt BRAF) patients, whilst patients with mt RAS experienced no difference in PFS (11.0 months *vs.*

12.2 months, HR 1.15; p = 0.241); however, OS was significantly shorter compared with wtRAS patients (26.3 months *vs.* 37.9 months, HR 1.44; p = 0.015).[2,3,41,44] Other, non-V600 mt BRAF are a very rare and occur in 2% of metastatic CRC patients, most frequently in younger male patients, with patients, mostly males. Tumours are generally well differentiated tumours, more frequently harbour concurrent mt RAS. Survival of these patients was shown to be improved compared to metastatic CRC patients with V600E mt BRAF or RAS wild-type (wt RAS).[15,16]

As the role of targeted therapy for treatment of advanced or mCRC has become increasingly imported, so currently, determination of tumour gene status for KRAS/NRAS and BRAF mutations, as well as HER2 amplifications and MSI/MMR status, neurotrophic tyrosine receptor kinase (NTRK) fusions are recommended for patients with mCRC. Testing may be carried out for individual genes or as part of an NGS panel.[2,3]

In order to get the real-life data for Slovenian population with mCRC treated at the Oncology Institute of Ljubljana from 30 April 2013 to 5 March 2018 we have performed a Phase IV non-interventional study to assess KRAS, NRAS and BRAF status in mCRC patients suitable for 1$^{st}$ line treatment.

This prospective cohort study was approved by the Institutional Review Board Committee (Approval number: KESOPKR-6 and 03-Z/KESOPKR-6) and was conducted following the ethical standards defned by the Declaration of Helsinki. The study was conducted with the understanding and the consent of the patients. Prior to treatment patients have signed an informed consent for treatment and that their data could be used for scientific purposes.

The aim of this prospective cohort study was to determine in our mCRC patient population the biomarkers status and distribution in the RAS and BRAF genes, and to determine the time from receipt of the histological sample to the determination of the molecular status.

## Patients and methods

We performed a prospective, single-arm, single-centre, non-comparative, case-based, observational study from 30 April 2013 to 5 March 2018. The study was comprised of one centre which covers the treatment of all mCRC patients and reflects Slovenian demographic distribution. Originally, all samples for biomarker examination were to be evaluated for KRAS status by the certificated Molecular laboratory at the Institute of Oncology Ljubljana. The data was to be recorded in a collective data base to provide reliability. As from 2013 before initiating treatment with cetuximab, the testing for RAS status included not only KRAS, but also NRAS mutations by an experienced laboratory using validated test methods for detection of KRAS and NRAS. According to this in November 2013 in addition to KRAS we included also NRAS mutations analysis in all patients included in this study. RAS testing had previously been performed for all patients already included.

In this study, 650 mCRC patients treated at the Institute of Oncology Ljubljana were enrolled.

Initially, the planned number of patients for the study was 300. Between April 2013 and February 2014, 300 patients were recruited and underwent KRAS testing. The protocol was then amended in view of full RAS testing becoming standard clinical practice. The protocol was amended again in November 2015, to include an extra 350 above the original plan – a total of 650, and BRAF testing was added to the list of biomarkers analysed. From November 2015 until March 2018, 350 additional patients were recruited, making a total recruitment of 650. Thirteen patients that were included and signed consent to be part of this study, and underwent RAS and BRAF testing, did not present for the first-line systemic treatment afterwards, resulting in complete data for 637 patients.

The basic data about each patient were recorded, as date of birth, gender, performance status Eastern Cooperative Oncology Group (ECOG) performance status and smoking habits. The disease characteristics were also included, including date of diagnosis of the primary tumour, tumour location – colon or rectum, initial stage at the time of diagnosis (according to the TNM (tumour T, nodes N, and metastases M) classification system), the treatment option used for the primary tumour, (radiotherapy or surgery, followed or not by adjuvant chemotherapy, with the duration time treatment and the type of the adjuvant chemotherapy (capecitabine, 5-FU or oxaliplatin). The date of metastatic diagnosis was also included in the analysis, the sites of metastatic spread and the treatment option used, as surgery and systemic treatment, with systemic treatment comprising the treatment goals – curative, potentially curative or palliative, liver and/or lung metastases potentially respectable, depending upon tumour burden. The systemic first line treatment options included vari-

**TABLE 1.** Patients baseline characteristics

| Patients baseline characteristics | Number (%) |
|---|---|
| Gender | |
| female | 233 (36.6) |
| male | 404 (63.4) |
| WHO clasiffication: | |
| 0 | 241 (37.8) |
| 1 | 300 (47.1) |
| 2 | 72 (11.3) |
| Tumor location: | |
| colon | 387 (60.8) |
| rectum | 250 (39.2) |
| Clinical signs of the primary colorectal cancer present before pathological confirmation | 178 (20.9) |
| Primary metastatic | 361 (56.7) |
| Liver metastases | 218 (34.3) |

* Some pathohistological reports from external hospital had unclear description, about the TNM status and risk factors

ous combinations of irinotecan, oxaliplatin based therapy, 5-FU, capecitabine, FOLFOXIRI protocol, cetuximab in a weekly or biweekly manner, bevacizumab, panitumumab or other.

The tumour characteristics were assessed regarding with KRAS/RAS and BRAF status. The date of the request for the biomolecular analysis was included, and the date of the analysis receipt.

DNA for molecular analysis was extracted from formalin-fixed, paraffin-embedded tumour tissue of primary tumours or metastases with at least 70% of tumour cells. Molecular testing was performed with RT-PCR KRAS Mutation Analysis Kit (EntroGen, Inc.), RT-PCR NRAS Mutation Analysis Kit (EntroGen, Inc.) and RT-PCR BRAF Mutation Analysis Kit (EntroGen, Inc.), according to manufacturer's instructions. The KRAS and NRAS analyses were assessed on exons 2, 3 and 4, and for BRAF on exon 15.

**TABLE 2.** Disease characteristics

| Disease characteristics | Number (%) |
|---|---|
| pT4 of primary tumor | 186 (29.2) |
| Affected regional lymph nodes (N): | |
| N0 (no affected regional lymph nodes) | 110 (17.3) |
| N1 (1 to 3 affected regional lymph nodes) | 209 (32.8) |
| N2 (more than 3 affected regional lymph nodes) | 239 (37.5) |
| Missing data* | 62 (11.3) |
| Vascular invasion | 114 (17.9) |
| Perineural invasion | 95 (27.9) |
| Lymphangiosis | 115 (18.1) |
| Grade of differentiation: | |
| G1 (well) | 19 (3) |
| G2 (medium) | 162 (25.4) |
| G3 (poorly) | 53 (8.3) |
| G4 (no differentiated) | 1 (0.2) |
| Missing data* | 402 (63.2) |
| Resection of primary tumour | |
| R0 | 63 (9.9) |
| R1 | 10 (1.6) |
| R2 | 16 (2.5) |

* Other metastatic locations: brain, ovaries, suprarenal glands, local recurrence, muscular dissemination

## Statistical methods

In this study 95% confidence intervals were calculated to indicate the degree of certainty of KRAS, NRAS and BRAF status frequency as being wild type or mutant type.

A sample size of 551-634 patients was necessary for a two-sided 95% confidence interval with a width of 4% anticipating a *BRAF* mutant rate of 5.5% to 6.5%. The planned sample size was increased to 650 patients, to consider non-evaluable biomarker test results and missing data.

# Results

In total, 650 patients of the whole mCRC patients treated at Oncology Institute of Ljubljana were enrolled in this prospective clinical study.

## Patient's characteristics and treatment

The demographic data about each patient were recorded, such as date of birth, gender, performance status (WHO) and smoking habits. Two thirds of the patients were male and most (84.9%) of the patients initially had a very good (ECOG 0–1) performance status. The main localization of the primary tumour was colon in 60.8% of the patients. All of included patients in this study had pathological confirmation of the carcinoma of the colon or rectum. Almost one third (27.9%) of the patients had clinical signs for CRC, before being pathologically confirmed, including occult bleeding or were asymptomatic and detected in the Slovenian national program for the early detection of the colorectal cancer named SVIT. For more than half (56.7%) of patients the diagnosis was confirmed pathologically, after biopsy was performed at colonoscopy, and for the others after the surgery.

**TABLE 3.** The most frequent metastatic sites

| The most frequent metastatic sites | Number (%) |
|---|---|
| **Total** | **637 (100)** |
| Liver | 218 (34.3) |
| Lungs | 70 (11.0) |
| Lymph nodes | 34 (5.3) |
| Peritoneum | 115 (18.1) |
| Bones | 4 (0.6) |
| Other* | 18 (2.8) |
| Multiple locations | 261 (41.0) |

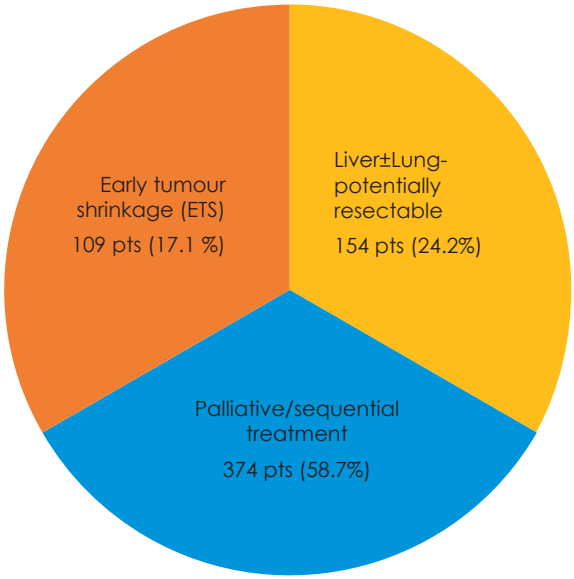


**FIGURE 1.** Treatment goal options at presentation of the metastatic colorectal cancer (mCRC) patients.

Complete resection (R0) of operated primary tumour was achieved in 9.9%. Most of the included patients were non-smokers (62.6%).

According to the TNM characteristics, initially at the first presentation of the primary CRC, in the external hospitals, 53.7% of patients had T3 tumours, 31.6% of patients had N1 (31,6%) and 34.5% of patients had N2. One third of patients had initially no metastatic disease (33.4%). Mostly, the primary CRC had pathohistological characteristics as follows: grade 2, with one third having vascular invasion, perineural invasion or lymphangiosis. Primary metastatic disease was confirmed in 56.7% of patients. The most common sites of metastases were liver and lung. Some of patients with mCRC in liver and lungs had surgery of the metastasis before the mCRC treatment, 6.8% and 2.0% respectively. In 10% of the mCRC patients R0 resection (70.8% of the total number of resections) was achieved. The metastatic sites were included in the analysis, and the main sites of CRC dissemination were liver (34.2%), lungs (11.0%), lymph nodes (5.3%), peritoneum (5.0%). Sixty-two patients were no previous smokers, and only 9% of patients were smoking at the time of our analysis. Patients' baseline characteristics and are shown in Table 1, disease characteristics are shown in Table 2, the most frequent metastatic site are shown in Table 3, smoking habits of included patients are shown in Table 4.

The treatment goals were in oligometastatic disease as follows: respectable and potentially resectable, or palliative for patients with high burden of the disease. More than a half (58.7%) of the patients were initially candidates for the palliative treatment, and 24.2% were potentially resectable at presentation. Treatment goal options at presentation of the mCRC patients are shown in Figure 1.

More than half (58.7%) of patients were initially candidates for palliative systemic treatment, and 24.2% were potentially resectable at presentation.

Of all 637 mCRC patients had the following possible first line systemic treatment options: chemotherapy - fluoropyrimidine based systemic therapy combined with oxaliplatin and/or irinotecan plus, for subjects with KRAS/RAS wild type tumours, the EGFR inhibitors cetuximab or panitumumab, or the VEGF inhibitor bevacizumab for those with KRAS/RAS mutated tumours. Most

**TABLE 4.** Treatment regimen decision for the first line treatment of the metastatic colorectal cancer (mCRC)

| Systemic therapy options | Number (%) |
|---|---|
| Fluoropirimidines/Irinotecan/Cetuximab* | 73 (11.4) |
| Fluoropirimidines/Oxaliplatin/Cetuximab* | 49 (7.7) |
| Fluoropirimidines/Irinotecan/Panitumumab* | 17 (2.7) |
| Fluoropirimidines/Oxaliplatin/Panitumumab* | 12 (1.9) |
| EGFR inhibitors other** | 46 (7.2) |
| Fluoropirimidines/Irinotecan/Bevacizumab | 126 (19.8) |
| Fluoropirimidines/Oxaliplatin/Bevacizumab | 191 (30.0) |
| VEGF inhibitor other*** | 21 (3.3) |
| Chemotherapy only | 102 (16.0) |

*EGFR inhibitors for RAS (NRAS and KRAS) wild type only
**EGFR inhibitors monotherapy ± one chemotherapy
***VEGF inhibitor monotherapy ± one chemotherapy

**TABLE 5.** The RAS/KRAS and BRAF status distribution

| The RAS/KRAS and BRAF status distribution | Number (%) |
|---|---|
| **KRAS testing** | 637 (100) |
| KRAS mutated | 311 (48.8) |
| KRAS wild type | 305 (47.9) |
| Not possible | 21 (3.3) |
| **NRAS testing** | 637 (100) |
| NRAS mutated | 57 (9.0) |
| NRAS wild type | 567 (89.0) |
| Not possible | 13 (2.0) |
| **BRAF testing** | 637 (100) |
| BRAF mutated | 84 (13.2) |
| BRAF wild type | 548 (86.1) |
| Not possible | 5 (0.7) |

**TABLE 6.** Methods used for KRAS/RAS and BRAF analysis

| Methods used for KRAS/RAS and BRAF analysis | Number (%) |
|---|---|
| Direct sequencing | 223 (35.0) |
| Pyrosequencing | 322 (50.5) |
| qPCR | 92 (14.5) |

frequently used was bevacizumab-based therapy (53.1%), either in combination with doublet chemotherapy or with one cytostatic or as monotherapy. EGFR inhibitors cetuximab and panitumumab were used in RAS (KRAS/NRAS) wild type mCRC subjects (30.9%). Data presented in the Table 9. Fifty-one subjects with RAS (KRAS/NRAS) wild type tumours received chemotherapy alone or in combination with Bevacizumab, as first line treatment. The first line treatment decision is made by the oncologist in accordance with NCCN and ESMO guidelines and consider the patient's preferences. Some patients found skin side effects and higher parenteral application frequency important parameters for a possible decrease of their quality of life. Most frequently prescribed treatment protocols are shown in Table 4.

### Biomarker status

The assessment of the KRAS/RAS and BRAF status was performed for all 650 patients. The RAS and BRAF status analysis were performed simultaneously, and for the group of subjects from the first Amendment, after the Amendment II was added to the protocol, the NRAS and BRAF data were added to the database. Data of the RAS/KRAS and BRAF status distribution are shown in Table 5. The distribution of patients with KRAS mutated and wild-type tumours, was almost equal, 48.8% and 47.9% respectively. For 3.3% of patients of the assessed population the KRAS status analysis was unsuccessful, due to unrepresentative tumour samples (Table 6). For 2.0% and 0.7% of the subjects the NRAS and BRAF genotyping was not possible due to unrepresentative tumour samples. The KRAS and NRAS analyses were assessed on exons 2, 3 and 4, and for BRAF on exon 15. The most frequent mutation was in KRAS in codon 12 and codon 13, and the same was true for NRAS and for the BRAF V600E mutation. The most frequent mutations, as follows were: pGly12Asp (c35 G>A) 7.8%, pGly 12Val (c35G>T) 6.6%, pGly13Asp (c38 G>A) 4.7% and pGly12Cys (c34 G>T) 2.4%.

The median time for biomarkers status assessment was 14 days.

Mostly direct sequencing was used for KRAS/RAS and BRAF status assessment, but also other methods, as pyrosequencing and qPCR. The Entrogen RT PCR KRAS/RAS and BRAF mutation Kit was used. Methods used for KRAS/RAS and BRAF analysis are shown in Table 6.

## Discussion

The aim of the observational mCRC population-based study was to determine the biomarker status in the RAS and BRAF genes, to determine their distribution in the mCRC patient Slovenian population, and to determine the time from receiving the histological sample to the determination of the biomarker status and its impact on the initiation of systemic oncological therapy.

This study was performed in order to achieve a real perspective of the bio-markers status of the patients with metastatic colorectal cancer in comparison with worldwide data. The analysis of KRAS/NRAS and BRAF status was a relatively new approach in the treatment of the patients with metastatic colorectal cancer at the time of conducting of our clinical study, so we also wanted to get real insight of the time frame needed for such analysis and its possible effect on the initiation of the first-line treatment.

The status of mutations in the RAS gene is a molecular predictive factor for response to treatment with EGFR inhibitors inmCRC. Determination of mutational status in KRAS gene has been standard clinical practice since 2008. Additional mutations in the codons of 61 and 146 of KRAS gene, and in codons 12, 13, 61 and 146 of NRAS gene are determined at our Institute of Oncology Ljubljana

since autumn 2013 and are being standard in international clinical practice since 2014. According to the literature data, are about 15%.

In our prospective cohort study KRAS mutations were found in 48.8% of patients, and additionally 9% of NRAS mutations were determined. So approximately 60% of patients had *RAS* mutated mCRC, which is consistent with reports from previous literature.[2,3,22,45] In our retrospective analysis we found 17% of additional mutations in RAS gene, which is a higher percentage than in our prospective cohort study, probably due to characteristics of patient population included.[45]

Also, higher, 13.2% BRAF mutations, were determined in our cohort study. According to the literature, the frequency of this mutation is from 5 to 10%. [2,3,24] According to our previous retrospective analysis, the V600E mutation in the BRAF gene was also present at a similar percentage of patients, in 7.4%.[46] It is assumed that this difference in the percentage of BRAF mutations in clinical studies is due to the characteristics of the patients included in the analysis.

So, according to our study analysis data, the distribution between wild type and mutated type tumours of mCRC patients was approximately the same, as worldwide, so the Slovenian population with mCRC has the same ratio distribution of KRAS, NRAS and BRAF wild and mutated genes.

The two-week time period from the initial presentation of the patient until the biomarker status analysis report did not affect the starting of the systemic treatment or the treatment decision, as usually mCRC patients are given a brief period of time for psychical and physical preparation for the systemic treatment, at which time supportive care – nutritional and symptomatic support, is given.

As for the time spent on biomarkers analysis, we concluded that the two-week period was not influencing the first line treatment decision, because patients started with systemic chemotherapy immediately and after 2 weeks have already received a biologic drug at the 2nd cycle of chemotherapy according to molecular genetic testing, And also in Slovenia we do not have waiting lists and cancer patients, who need treatment are referred to the oncology treatment facility shortly after the diagnosis, so this brief period of time is usual for psychical and physical preparation for the systemic treatment, at which time supportive care – nutritional and symptomatic support, is given.

In conclusion, we have proven that the biomarkers distribution in Slovenian population with mCRC was approximately the same as worldwide, so the decision treatment approach should be in the accordance with the worldwide treatment guidelines based on evidence-based medicine. Secondly, the laboratory for the molecular analysis that we have in the Institute of Oncololgy Ljubljana was performing the needed biomarkers analysis in an acceptable time that didn't affect the treatment decision or delay the needed cancer treatment.

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