CASE REPORT

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The Coexistence of *RAS* and *BRAF* Mutations in Metastatic Colorectal Cancer: A Case Report and Systematic Literature Review

Vlad-Adrian Afrăsânie[1], Bogdan Gafton[1,2], Mihai Vasile Marinca[1,2], Teodora Alexa-Stratulat[1,2], Lucian Miron[1,2], Cristina Rusu[1], Anca-Viorica Ivanov[1], Gheorghe G Balan[1,3], Adina-Emilia Croitoru[4,5]

1) Gr. T. Popa University of Medicine and Pharmacy, Iași
2) Oncology Dept., Regional Institute of Oncology, Iași
3) Gastroenterology Dept., Sf. Spiridon University Clinical Emergency Hospital, Iași
4) Titu Maiorescu University of Medicine, Bucharest
5) Oncology Dept., Fundeni Clinical Institute, Bucharest, Romania

Address for correspondence:
Bogdan Gafton
Gr. T. Popa University of Medicine and Pharmacy, Oncology Dept., Regional Institute of Oncology, Iași, Romania
gaftonbogdan@yahoo.com

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ABSTRACT

Background & Aims: The coexistence of *RAS* and *BRAF* mutations is extremely rare, occurring in approximately 0.05% of patients with metastatic colorectal cancer (mCRC). Starting from a case presentation, this review aims to examine the prevalence, clinical, histopathological and molecular features of tumors with concomitant mutations.

Methods: Case report and systematic review. We performed a systematic literature search in PubMed and EMBASE using the following MeSH terms: "coexistence" OR "concomitant" AND "*RAS*" AND "*BRAF*" AND "colorectal cancer" from the inception of the databases onwards.

Results: We present the case of a 53-year-old man diagnosed with metastatic rectal adenocarcinoma with both a *KRAS* and a *BRAF* mutation. The review included eleven papers reporting on a total of 30 mCRC cases with concomitant *RAS* and *BRAF* mutations. The male/female ratio was 11/5. The average age was 58.5 years. The tumor was located in nine cases on the right colon and in two cases in the left colon. 43.3% of subjects had liver metastases, and 6.6% had lung metastases. Next-generation sequencing (NGS) was used in 36.6% of cases and polymerase chain reaction (PCR) in 16.6% of cases. *KRAS* mutations were present in 83.3% of patients and *NRAS* mutations in 16.6% of patients. Survival could be assessed in 10 patients and the median was 21.1 months (about 30% lower than the survival in the general mCRC population).

Conclusion: The results of this systematic review suggest the need to design a cohort study (either prospective or retrospective) to better characterize the patients with concomitant *RAS* and *BRAF* mutations and to establish the optimal treatment for this rare situation.

Key words: metastatic colorectal cancer – concomitant mutations – *RAS* – *BRAF*.

Abbreviations: *BRAF*: v-raf murine sarcoma viral oncogene homolog B1; CRC: colorectal cancer; CT: computed tomography; EGFR: epithelial growth factor receptor; FU: fluorouracil; *KRAS*: Kirsten rat sarcoma viral oncogene homolog; mCRC: metastatic CRC; MEK inhibitor: mitogen-activated protein kinase inhibitor; MSI: microsatellite instability; *NRAS*: neuroblastoma rat sarcoma viral oncogene homolog; NGS: next-generation sequencing; OS: overall survival; PCR: polymerase chain reaction; PFS: progression-free survival; *RAS*: rat sarcoma.

INTRODUCTION

Colorectal cancer (CRC) has a central role in cancer pathology worldwide; it is the second most common cancer in women and the third in men [1]. Over the last 20 years, significant therapeutic progress has been achieved in the metastatic stage of the disease, which has resulted in increased survival rates [2, 3]. Currently, rat sarcoma (*RAS*) and v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutations are known to be crucial mediators on the epithelial growth factor receptor (EGFR) signaling pathway, and essential factors in colorectal carcinogenesis [4,5]. *RAS* mutations occur in approximately 40% of mCRC patients, while *BRAF* mutations are present in 8-12% of cases [6]. *RAS* mutations are predictive factors for primary resistance to anti-EGFR biological therapies. It was shown that the addition of anti-EGFR agents to standard chemotherapy regimens, such as FOLFOX (leucovorin, 5-Fluorouracil (5-FU) and oxaliplatin) or FOLFIRI (leucovorin, 5-FU and irinotecan), does not produce benefits for patients with *RAS* mutations [7-10]. The data is insufficient and less clear in order to draw a conclusion regarding the predictive value of *BRAF* mutations for anti-EGFR therapies [11]. It is widely accepted that *RAS* and *BRAF* are mutually exclusive in almost all cases. Rarely, however, CRC

patients may present with concomitant Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and *BRAF* mutations (0.05%). The clinical outcomes and prognostic role of concomitant mutations in CRC patients are yet to be fully understood and quantified [4].

CASE REPORT

A 53-year-old Caucasian male, accountant, presented at our hospital with severe rectal bleeding. The patient had no relevant medical or family history, did not smoke, and drank alcohol occasionally. His performance status was 1 on the ECOG (Eastern Cooperative Oncology Group) scale. Clinical examination revealed hepatomegaly. The biological analysis indicated iron deficiency anemia with a hemoglobin level of 10.6 g/dL. The colonoscopy detected a vegetative tumor located 6 cm from the anal verge, and histological analysis showed adenocarcinoma. Pelvic magnetic resonance imaging (MRI) identified a 67 mm-long irregular circumferential thickening of the lower and median rectal wall at 40 mm from the anus, extending into the perirectal fatty area. The abdominal computed tomography (CT) scan showed an enlarged liver with multiple nodules ranging between 2 mm and 106 mm in size, suggestive for liver metastases. The case was discussed in our multidisciplinary tumor board and rectal surgery (Hartmann surgical approach) was decided for severe rectal bleeding. The pathology exam indicated pT3pN1b G2 rectal adenocarcinoma. The mutational status of the *RAS* and *BRAF* genes was analyzed by polymerase chain reaction (PCR) from the surgical specimen. *KRAS* mutation (G12D) and *BRAF* mutation (position 600 of exon 15 (V600E)) were identified. Immunohistochemistry testing for microsatellite instability (MSI) did not show a MSI-high profile. Two months later, the patient was admitted in Medical Oncology Department and received first-line palliative chemotherapy with the FOLFOX-4 regimen (oxaliplatin 85 mg/m^2 iv on day 1, 5-FU 400 mg/m^2 iv bolus followed by 600 mg/m^2 iv 22-hour continuous infusion on days 1 and 2, and leucovorin: 200 mg/m^2 iv on days 1 and 2) plus bevacizumab (5 mg/kg) on a two-week schedule. After twelve cycles, the patient developed grade 3 sensitive peripheral neuropathy. Oxaliplatin was discontinued, and treatment was continued with capecitabine (1000 mg/m^2 day 1 to 14) and bevacizumab 7.5 mg/kg every three weeks.

At six months after starting the chemotherapy, the disease was stable according to response evaluation criteria in solid tumors (RECIST) 1.1. Over the next six months, the patient continued on capecitabine associated with bevacizumab, but the subsequent CT examination revealed progressive disease (increased number and size of liver metastases) and a new 67/67/77 mm rectal lesion that invaded the anal sphincter. The chemotherapy was switched to FOLFIRI (irinotecan 180 mg/m^2 iv on day 1, 5-FU: 400 mg/m^2 iv bolus on day 1, followed by 2400 mg/m^2 iv continuous infusion for 46 hours, and leucovorin: 400 mg/m^2 iv on day one every two weeks). Bevacizumab was interrupted due to persistent grade 3 proteinuria. We proposed palliative radiotherapy, but the patient refused. After four cycles of FOLFIRI, imaging studies revealed that the patient had progressive disease at the hepatic site. The patient died due to liver failure, 23 months after diagnosis.

SYSTEMATIC REVIEW OF THE LITERATURE

An extensive literature search was done based on a pre-drafted protocol, including eligibility criteria, search strategies, criteria for study selection and methods for extracting data according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.

Search methods

Previously published articles indexed in PubMed and EMBASE were searched using the following MeSH terms and keywords: "coexistence" OR "concomitant" AND "*RAS*" AND "*BRAF*" AND "colorectal cancer". Data was gathered from the

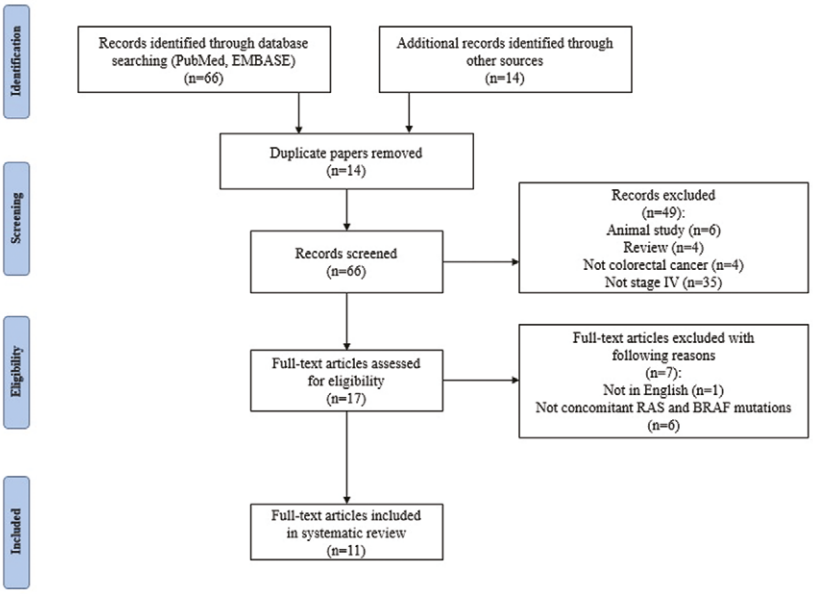

Fig. 1. PRISMA flowchart of literature search.

inception of the databases onwards. The search was restricted to papers with full text available in English or at least with access to an abstract in English. We also included abstracts from conferences, and we evaluated the reference lists of articles to include the potentially appropriate articles. Reviews and articles that were not referring to CRC and stage IV of disease were excluded. Only reports in humans were included for this systematic review. Fig. 1 shows the flowchart of selecting the studies according to PRISMA guidelines.

Eligibility criteria

Studies were included only if the authors concluded that *RAS* and *BRAF* mutations were coexistent in one or more mCRC patients, and the results were written in English.

RESULTS

Eleven full text-articles were included, covering 30 cases in total. Gender, age, primary tumor site, location of metastases, method of determination, *RAS* mutation, *BRAF* mutation, location of mutations in codons, first-line treatment, response and survival are detailed in Supplementary Table I. However, the data collected for the studied parameters in every patient were not complete for all the 30 cases identified in the literature. The percentages are calculated to a total of 30 patients. For each parameter, we reported the percentage of missing data.

Clinical characteristics

The ratio between males and females was 11/5 (36.6%/16.6%) and the average age of patients was 58.5 years; however, data were not available in 46.6% of cases. In nine cases (30%) the tumor was located on the left colon and in only two cases (6.6%) on the right colon, for the other nineteen cases (63.3%) the tumor location was not described. Thirteen (43.3%) patients had liver metastases, two (6.6%) had lung metastases, and in 15 (50%) cases, the site of the secondary lesion was not described.

Molecular characteristics

The method used to detect the mutations were: PCR in 16.6% of subjects (n=5) and next-generation sequencing (NGS) in 36.6% (n=11) of cases; in 46.6% (n=14) data regarding the used technique was missing. *KRAS* mutations were present in 83.3% (n=25) of cases and 16.6% (n=5) of patients had neuroblastoma rat sarcoma viral oncogene homolog (*NRAS*) mutations. One patient had coexistent *KRAS* and *NRAS* mutation, and in one subject the type of *RAS* mutation was not described. *KRAS* mutations were most frequently found at codons G12D (26.6%, n=8), G13D (16.7%, n=5), G12V (10%, n=3) and other mutation types were found in every other patient and counted for 30% (n=9). *NRAS* mutations were identified in different codons: G12A (n=1), G12D (n=2), G13S (n=1), Q61Q (n=1). *BRAF* mutations were most frequently located in codons V600E (50%, n=15), V600D (6.6%, n=2), D594G (6.6%, n=2), G466V (6.6%, n=2).

Management and outcome

Information about treatment was available only for ten patients (33.3%), in whom FOLFOX (20%, n=6), FOLFIRI (10%, n=3) and IFL (irinotecan, leucovorin and 5-FU) (3.3%, n=1) were the most commonly used chemotherapy regimens. Bevacizumab was administered in association with chemotherapy only in 4 patients. The responses were mixed, and no conclusion could be drawn regarding the best therapeutic approach. Survival data was available only for ten patients, averaging 21.6 months [4, 5, 6, 11-19].

DISCUSSION

The *RAS* protein is a crucial factor in regulating cellular mitosis, and its expression and activity are amplified by activating mutations in its coding gene. *KRAS* mutations occur in approximately 40% of cases, especially in exon 2, codons 12 (70-80%) and 13 (15-20%), while *NRAS* mutations occur in exons 2, 3 and 4 [20, 21]. *BRAF* also plays an essential role in the MAP-kinase (mitogen-activated protein kinase) pathway activation which contributes to cellular growth, proliferation, and differentiation, as well as to other vital cellular processes such as migration, apoptosis and cellular survival. Approximately 90% of *BRAF* activating mutations occur in exon 15 [22, 23].

Concomitant *RAS* and *BRAF* mutations are rare: one study on mCRC patients indicated an incidence of 0.2% in a population of 6,633 patients, and in another 6,251-patient series the incidence was 0.064% [4, 13]. Thirty cases of concomitant *BRAF* and *KRAS* mutations in mCRC patients have been described in the literature so far. However, the detection rate for these genetic lesions depends on the used technique. Real time PCR has been traditionally used for molecular characterization of CRC, but the modern NGS method has the potential to more precisely identify cu identify more precisely the incidence of concomitant *RAS* and *BRAF* mutations, which might be higher than previously estimated [16]. Given their rarity, it is not yet clear if tumors with concomitant *RAS* and *BRAF* mutations have a different tumor biology and natural history than those with individual *RAS* or *BRAF* mutations, and which of these two mutations play a more significant role in tumor invasion and aggressiveness [5]. Molecular profiling has demonstrated that dual *RAS* and *BRAF* mutant tumors have different genetic signatures suggesting that different signaling pathways are activated [24]. Moreover, it is still not known if *RAS*, *BRAF* or both are driver mutations [4]. It is believed that colorectal tumors originate in two clonal populations with the coexistence of two mutations at the same genetic level [5, 25]. Several theories have attempted to explain the coexistence of these two mutations, but none is definitively proven. One possibility is that redundant oncogenic stimuli are activated. Another hypothesis states that these mutations exert a synergistic role in the stimulation of the disease progression [26, 27].

In their research, Oliveira et al. [27] were able to associate concurrent *RAS* and *BRAF* mutations with more advanced stages, observing an increased likelihood of lymph node invasion, distant metastases, and poor prognosis in these cases. However, other studies did not confirm this to be a consistent pattern. Because only 30 cases have been described in the literature up to the present time, we cannot conclude on clinical, pathological and molecular correlations. Relevant features of these 30 reported cases are summarized in Supplementary Table I [4-6, 12-19].

In these cases, we identified two molecular subtypes of *BRAF*-mutated mCRC: one with V600E mutation and the other with non-V600 mutation. Recent studies discuss these two phenotypes, and they show that they have indeed different clinical, molecular, histopathological and prognostic features [28]. Tumors with *BRAF* V600E mutations are most often located in the right colon and are prevalent in women and older patients (usually over 60 years), are poorly differentiated and are associated with mucinous histological type and MSI-high [29]. Non-V600 mutations most often affect codons 594 and 596 and are less frequent (2.2% of mCRC). These tumors occur more often in men, at younger ages, are well-differentiated, located in the left colon, and are associated with *RAS* mutation and rarely present MSI-high. This suggests that, at least in some cases, non-V600 *BRAF* mutations have a better prognosis than V600E [28, 30]. At the same time, considering the existing molecular data, the following classification of *BRAF* mutations has been proposed [30]: class 1 - *BRAF RAS*-independent mutations - with signaling as monomers; class 2 - *BRAF RAS* mutations – with independent signaling as dimers; class 3 - *BRAF RAS* dependent mutations - with impaired kinase activity.

Schirripa et al. [31] have demonstrated a correlation between these three classes and clinical characteristics. Class 3 was associated with left tumors, no lymph node and no peritoneal metastases (as opposed to class 1), while class 2 was similar to class 1. Prognosis was different between subclasses - classes 1 and 2 had similar median overall survival (OS): 21 and 23 months, while class 3 and *BRAF* WT had a median OS of 44.5 months. This study confirms the significant survival differences between *BRAF* non-V600E mutations and *BRAF* V600E mutations, highlighting the less aggressive behavior of the former [31].

Data from literature are insufficient to guide choices of the appropriate type of systemic treatment in such cases [13]. Of the 30 patients, only 10 received chemotherapy with or without targeted therapy. Only one study included mCRC patients with concurrent *KRAS*/*BRAF* mutations who were treated exclusively by the surgical removal of the metastatic lesions, but the results were inconclusive.

The phase III TRIBE study included mCRC patients who received first-line treatment with either FOLFIRI plus bevacizumab or FOLFOXIRI plus bevacizumab. The results showed that patients with mCRC and *BRAF* mutation could benefit more from an intensive chemotherapy regimen. However, this study included a small number of patients (n=28) and gains on progression-free survival (PFS) and OS, although present, were not statistically significant [32]. Recently, TRIBE 2, a prospective randomized phase III trial included patients with treatment-naïve mCRC in two arms: in one arm subjects received FOLFOX and bevacizumab, followed by FOLFIRI at disease progression, and in the other arm FOLFOXIRI and bevacizumab, which was re-introduced at progression. The trial included 66 patients with *BRAF* mutations, 33 in each arm. The lack of benefit from the more intensive treatment observed in the *BRAF* mutated subgroup could be explained by the clinical heterogeneity of *BRAF* mutant tumors or by a different comparator used in the TRIBE study (FOLFIRI) than in the TRIBE 2 study (FOLFOX). However, it seems that the effect of a more aggressive treatment in tumors with *BRAF* mutation is different depending on the location of the primary tumor: subjects with tumors located on the right side seem to benefit most from the triplet, as the subgroup analyzes in this trial have shown [33].

Another therapeutic alternative in patients with *BRAF* mutation is the inhibition of the RAF pathway. Various studies have shown modest activity of *BRAF* inhibitors in monotherapy, but their use in combination with MEK inhibitor or EGFR inhibitors has yielded better results [34, 35]. A triple combination between encorafenib, cetuximab and a PIK3CA inhibitor, alpelisib, was explored in a phase I and in a phase II study but the results were not promising, and this strategy was abandoned [36, 37]. Other triple combinations between *BRAF* inhibitors, MEK inhibitors, EGFR inhibitors, and/or chemotherapy were studied, and phase I and II studies created the rationale for a large phase III trial conducted by Kopetz et al. (BEACON study) [38, 39, 40]. Six hundred and sixty-five mCRC patients with *BRAF* V600E mutation who had progressive disease after one or two lines of treatment were enrolled. The study had 3 arms: one arm received encorafenib, binimetinib and cetuximab, one arm encorafenib and cetuximab, and the control group cetuximab and irinotecan or cetuximab and FOLFIRI (at the investigator's choice). Patients who received the triplet had a better outcome with an OS of 9 months, versus those who received doublet (8.4 months) and those in the control group (5.4 months) ($p < 0.001$) [40].

The TRIBE, TRIBE2 and BEACON studies analyzed the efficacy of different treatments in the population of *BRAF* V600E mutation patients, but there were no patients enrolled with concomitant *RAS* and *BRAF* mutations [32, 33, 40]. Although these treatments may be effective for patients with coexisting mutations, there is no data to support this idea, so new therapeutic strategies may be studied in the future for this very rare category of patients [11].

The role of *RAS* and *BRAF* mutations as prognostic factors has been evaluated by many researchers. *RAS* mutations are considered a negative prognostic factor in multiple trials, but some data contradicts this result [42-44]. *BRAF* mutations are associated with a shorter OS (10-16 months) in mCRC patients. Multiple prospective, retrospective studies and a meta-analysis have confirmed these findings [42, 45-49]. Although some retrospective studies view concomitant mutations as a poor prognosis factor, more data on a more significant number of patients are required to adequately define the prognostic role of such a rare condition [13]. Compared to other cases in which survival was reported, the 23-month survival of our patient exceeded the 21.1-month median. Also, the survival of our patient was superior to the 18.5-month median OS reported for *BRAF* mutated patients but was inferior to the 25.6-month survival of patients with *RAS* mutations [50].

Our case shares many features with those previously reported, but very few with the *BRAF* V600E mutant patients: clinical (male, 51 years old), pathological (rectal adenocarcinoma) and molecular (*KRAS* G12D mutation and *BRAF* V600E mutation). On the other hand, the patient had similar survival with the average mentioned in the literature: 23 months following diagnosis. This could suggest that concomitant *KRAS* and *BRAF* mutations may not necessarily

be a poorer prognostic factor than *BRAF* mutations. There is still a plethora of other factors that could have impacted the prognosis of our patient: the rectal localization of the primary tumor, the fact that the patient was not tested by NGS for other mutations, the genetic heterogeneity of CRC tumors, or the consensus molecular subtype [5].

CONCLUSIONS

Prospective or retrospective cohort studies on a large number of patients are necessary in order to adequately assess the clinical, pathological and molecular characteristics of patients with mCRC and concomitant *KRAS* and *BRAF* mutations. Such studies would provide useful insights informing appropriate treatment choices in accordance with the patient profile and prognosis data.

Conflicts of interest: None to declare.

Authors' contributions: V.A.A. conceived and designed the article. M.V.M., T.A.S. data analysis and drafted the manuscript. T.A.S., A.V.I. searched in the literature. A.E.C., B.G., L.M., R.C supervised and revised the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work.

Supplementary material: To access the supplementary material visit the online version of the *J Gastrointestin Liver Dis* at http://dx.doi.org/10.15403/jgld-1003

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