VIA MEDICA

REVIEW ARTICLE

Paweł M Potocki, Piotr J Wysocki

Oncology Department, Jagiellonian University Medical College, Cracow

BRAF — a new therapeutic target in colorectal cancer

Address for correspondence:
Lek. Paweł Potocki
Klinika Onkologii, Szpital Uniwersytecki
ul. Śniadeckich 10, 31–501 Kraków
Phone: +48 12 424 89 12
e-mail: pawel.potocki@uj.edu.p

Oncology in Clinical Practice
2018, Vol. 14, No. 2, 86–95
DOI: 10.5603/OCP.2018.0013
Translation: dr n. med. Dariusz Stencel

ISSN 2450–1654

ABSTRACT

The *BRAF V600E* mutation, already a well-established biomarker in the treatment of metastatic melanoma, has been extensively studied in patients with metastatic colorectal cancer (CRC) in recent years. It was revealed that this mutation occurs in about 10% of CRC patients. It has been proven to be a negative prognostic factor, although more recent studies indicate a complex association of this effect with the state of genes responsible for the repair of "mismatch" DNA damage. Although the predictive value of the *BRAF V600E* mutation for chemotherapy and targeted treatment remains the subject of controversy, the guidelines of international scientific societies highlight the need for a different approach to systemic treatment of patients in this population. Numerous treatment options are currently evaluated: from the intensification of the classic chemotherapy regimens administered in the first-line setting to the innovative combinations of targeted drugs aimed at eliminating the influence of *BRAF V600E* mutation on signal transduction pathways that are crucial for carcinogenesis. The following review is intended to bring this complex topic to the attention of oncologists who deal with the treatment of gastrointestinal cancer in clinical practice.

Key words: colorectal cancer, BRAF, V600E, FOLFOXIRI

Oncol Clin Pract 2018; 14, 2: 86–95

Introduction

Colorectal cancer (CRC) is diagnosed every year in approximately 1.4 million patients worldwide, including more than 18,000 patients in Poland [1, 2]. Because five-year survival rates are highly unsatisfactory (approximately 60% in Europe, approximately 50% in Poland), there is huge demand for the development of new, more effective treatment options.

Over the last three decades, personalised oncology has been introduced and intensively developed. Along with a better understanding of the mechanisms behind cancer growth, its invasive nature, and complex interactions with the microenvironment, new therapeutic options have been increasingly adapted according to the unique profile of the patient. Increased knowledge about prognostic factors allowed the prediction of the dynamics of disease and adjust the intensity of treatment accordingly. The growing number of predictive biomarkers potentially reduces the chance of therapy failure.

All these advances have prolonged the median survival of patients with advanced CRC from the 16 months offered by the cytotoxic doublet 10 years ago [3] to over 30 years with use of modern strategies of combination therapy, including drugs targeting epithelial growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) [4]. Improvement of the results, however, does not affect all patients equally. Similarly to other malignancies, attention of researchers is focused on identified groups of patients with particularly poor prognosis.

The importance of the role of proto-oncogenic BRAF kinase (type B rapid accelerated fibrosarcoma) in carcinogenesis was established in 2002 by the Cancer Genome Project group research [5]. BRAF deregulation, common in various cancers, and the most frequent in melanoma, colorectal cancer, and gliomas, has been the subject of intensive research. BRAF acts mainly as a regulatory kinase, one of the transducers of the signals from growth factor membrane receptors. Specifically, it is one of transmitters in mitogen-activated protein kinase

signalling pathway (MAPK, or RAS-RAF-MEK-ERK), being a crucial pathway regulating the proliferative activity of epithelial cells (Fig. 1). The *BRAF* gene can be the subject of different activating mutations. The mutations in codon 600 have been specifically associated with strong activation of the MAPK pathway, which is known to drive key processes for carcinogenesis: proliferation, invasion, and angiogenesis. The development of targeted molecules against mutant BRAF (vemurafenib, dabrafenib) as well as against MEK kinase activated directly by BRAF (trametinib, cobimetinib) led to a significant improvement in the prognosis of patients with metastatic melanoma with the *BRAF V600* mutation. Spectacular effects of BRAF inhibition in melanoma drew oncologists' attention to other cancers, characterised by a high frequency of this mutation. In this respect, one of the most promising appeared to be metastatic CRC (mCRC).

Some negative effects of the *BRAF V600* mutation on the prognosis of CRC patients have been demonstrated, and its occurrence has been associated with potential resistance to some forms of systemic treatment. There are also data on possible intensification of therapy, as well as attempts of causal interventions being effective in this group of patients with poor prognosis. As new discoveries are translated into clinical practice, BRAF is gaining importance as another factor influencing the optimal therapeutic strategy.

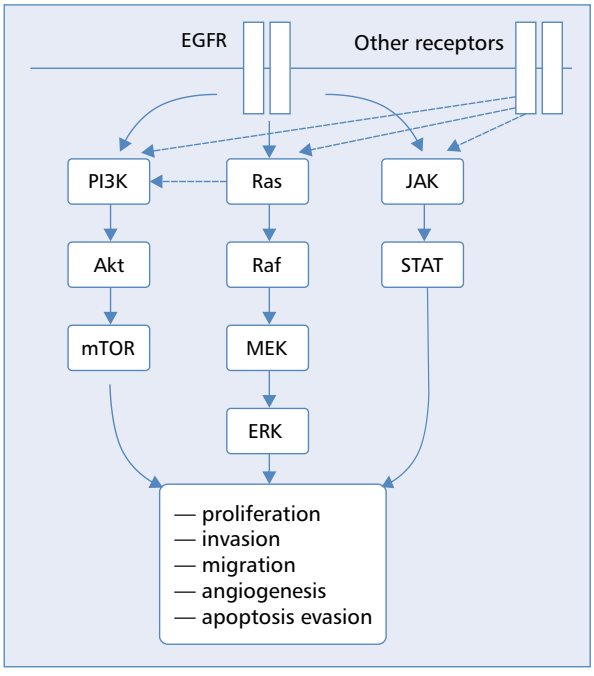

Figure 1. Simplified scheme of the most important signalling pathways regulating the growth and differentiation of epithelial tumours

This article aims to summarise current knowledge and make oncologists treating mCRC patients familiar with BRAF clinical potential.

Role in carcinogenesis

The adenoma-adenocarcinoma sequence, now considered the classic pathway of colon cancer carcinogenesis, is initiated by the knock-out of the *APC* gene and the development of chromosomal instability. Other changes, such as *TP53* knock-out and KRAS or BRAF activation, take place in subsequent stages [6].

An alternative route of carcinogenesis has been proposed based on the genome analysis of serrated adenocarcinomas of which serrated adenomas has been shown to be precorsor lesions. It has been revealed that hyperactivation of BRAF, as well as the acquisition of CpG island methylator phenotype (CIMP), are the early stages of their pathogenesis [7]. Subsequent deactivating mutations *CDKN2A* (p16) and *TP53* and activation of WNT/β-catenin pathway lead to the formation of adenocarcinoma with specific features.

The importance of the MAPK pathway in the pathophysiology of colorectal cancer is widely recognised. The *BRAF* gene located on chromosome 7 encodes one of the three RAF family serine-threonine kinases (the other two are ARAF and CRAF). The best described consequence of kinase activation is MAPK-dependent stimulation of growth and proliferation. However, the activation of parallel regulatory pathways regulated by RAF family proteins has also been described, resulting in the occurrence of crucial features of tumorigenesis: increased mobility and the development of resistance to mechanisms inducing cell death [8].

Occurrence and predictive value

BRAF mutations occur in about 15% of all human tumours [5] and in about 10% of mCRC cases (Table 1) [9–13]. The most common mutation is located in exon 15 of the gene, resulting in the substitution of a small hydrophobic valine by the larger, polar glutamic acid in codon 600 (V600E). The resultant altered kinase conformation is characterised by activity around 10 times stronger compared to the wild type [5]. It is worth noting that in mCRC this mutation almost never coexists with *KRAS* and *NRAS* mutations, but it may coexist with mutations in the *PIK3CA* gene (catalytic subunit alpha of phosphatidylinositol kinase).

Characteristic clinical and pathological features of colorectal cancer associated with the *BRAF V600E* mutation include the following: more frequent occurrence in women and in older patients at diagnosis, proximal

Table 1. Incidence of *BRAF* mutations in large clinical trials

Publication (n)	Study/database	BRAF V600E	BRAF nonV600E
Seymour et al. (2013) [9] (n = 696)	PICCOLO	9.1% (n = 63)	N/A
Venderbosch et al. (2014) [10] (n = 3063)	CAIRO, CAIRO2, COIN, FOCUS	8.2% (n = 250)	N/A
Modest et al. (2016) [11] (n = 1239)	FIRE-1, FIRE-3, AIOKRK0207, AIOKRK0604, RO91	6.0% (n = 74)	N/A
Cremolini et al. (2015) [12] (n = 629)	Dedicated database of Italian centres	12.2% (n = 77)	1.6% (n = 10)
Jones et al. (2017) [13] (n = 9643)	Mayo Clinic, MD Anderson, Foundation Medicine	7.9% (n = 757)	2.2% (n = 208)
In total (n = 15,270)		**8.7% (n = 1221)**	**2.1% (n = 218)**

Table 2. Difference in treatment outcomes and survival parameters depending on the status of the *BRAF* mutation

Study	Median PFS (months)		Median OS (months)	
	BRAF WT	BRAFmut	BRAF WT	BRAFmut
Modest et al. (2016) [11] (n=239)	10.3	7.4	26,9	11,7
Pooled analysis FIRE-1; FIRE-3; AIO-KRK0604; AIO-KRK 0207; RO91	(P < 0.001)		(P < 0.001)	
First line, different cytotoxic regimens ± bevacizumab				
Venderbosch et al. (2014) [10] (n = 3063)	7.7	6.2	17.2	11.4
Polled analysis CAIRO; CAIRO2; COIN; FOCUS				
First line, different cytotoxic regimens ± bevacizumab	(P < 0.001)		(P < 0.001)	

(right-sided) primary tumour location, a higher clinical stage, tendency for intraperitoneal metastases (peritoneal carcinomatosis), more frequently mucous phenotype, lower differentiation grade, and often concomitant microsatellite instability (MSI) [14].

Data from the majority of large clinical trials indicate a negative prognostic value of the *BRAF V600E* mutation at all stages of the disease.

The results of the PETACC-3 study, as well as cohort studies [15–17] involving patients undergoing radical resection, indicate that the presence of the *BRAF* mutation adversely affects overall survival (OS), but not relapse-free survival (RFS).

Similarly, the negative prognostic value of *BRAF* mutation is observed in metastatic disease (Table 2). Polled analysis of data from large clinical trials recruiting mCRC patients with no prior systemic treatment (CAIRO 1 and 2, COIN, FOCUS; n = 3063) showed a significant effect of *BRAF* mutation on median OS: 11.4 *vs.* 17.2 months; HR 1.91 (1.66–2.19), P = 0.001 and median progression-free survival (PFS): 6.2 *vs.* 7.7 months; HR 1.34 (1.17–1.54), P < 0.001 [10]. A similar analysis of the AIO group data (n = 1239) also showed a negative effect of the *BRAF* mutation on OS: 11.7 *vs.* 26.9 months; HR 2.99 (2.1–4.25), P < 0.001 and mPFS: 7.4 *vs.* 10.3 months; HR 2.19 (1.59–3.02), P < 0.001 [11].

Finally, the pooled analysis of FOCUS, COIN, and PICCOLO trials (n = 2071) published in December 2016 showed a significant negative prognostic value of *BRAF* mutation for median OS 10.8 *vs.* 16.4 months; HR 1.49 (1.23–1.80), P < 0.001, without effect on PFS [18]. Disease control rate (DCR) in first-line treatment did not differ significantly between cancers with or without *BRAF* mutation: in the FOCUS trial: HR 1.01 (0.36–2.84), P = 0.97; in the COIN trial: HR 0.76 (0.49–1.20), P = 0.24. The rate of survival after progression differed significantly in favour of patients with normal *BRAF* gene — in the FOCUS trial: HR 1.65 (1.03–2.67), P = 0.038; in the COIN trial: HR 1.72 (1.35–2.19), P < 0.001. Based on these findings it was concluded that dynamic, symptomatic progression occurring during or after first-line treatment contributes to worse prognosis, rather than resistance to cytotoxic treatment.

The negative impact of *BRAF* mutations on prognosis is also observed in patients undergoing surgical treatment for oligometastatic disease. Metastatic resections are performed less frequently in this group, and PFS and OS after such procedures are significantly worse than in the general population [19–21]. However, more recent reports show that despite this negative effect, patients

with mutation treated by experienced multidisciplinary teams have better prognosis if they are subjected to metastases resections [22, 23].

BRAF V600E mutation is also included into more complex prognostic models. In the latest molecular classification of CRC, it is one of the determinants of the subtype CMS1 — an immunological subtype of CRC, associated with the activation of the immune system. As compared to other CRC subtypes, CMS1 is associated with the worst prognosis in metastatic disease (although not with the highest recurrence rate in early disease), but it is potentially the most sensitive to immunotherapy with immune checkpoints inhibitors [24].

In summary, the aggressive dynamics of *BRAF V600E* mutated cancer makes the median survival in this subgroup half as long as in the general population, and due to aggressive progression, less than half of patients undergo systemic treatment ≥ 2 line [18].

The relationship between BRAF and MSI

Deficiency of mismatch repair (dMMR) leads to microsatellite instability (MSI), e.g. accumulation of mutations including inclusions or deletions of short nucleotide sequences (microsatellites) characterised by reduced strength of DNA polymerase binding. This process may be associated with hereditary deficiency in activity of one of the genes responsible for this type of repair (*MLH1*, *MSH2*, *MSH6*, *PMS2*), which is the essence of Lynch syndrome pathogenesis (HNPCC — hereditary non-polyposis colorectal cancer). However, a more frequent cause of MSI is sporadically occurring loss of function of one of aforementioned genes (most often in the epigenetic mechanism).

It was noticed that *BRAF V600E* mutation is several times more frequent in MSI cancers caused by sporadically occurring dysfunction of MMR (Table 3) than in MSS (microsatellite stable) cancers [10, 25–28]; whereas *BRAF V600E* mutation almost never occurs in cancers developed in the course of Lynch syndrome [29]. The data shown in table 3 demonstrate that up to 42% of all *BRAF V600E* mutations in colon cancer coexist with microsatellite instability, although when limited only to disseminated cancers this rate decreases to around 20% (Table 3).

The nature of the relationship between *BRAF* mutation and MSI occurrence is the subject of controversy. The genomic instability accompanying MMR deficiency seems to be a simple explanation of increased mutations frequency in general, including those in *BRAF*. This hypothesis, however, does not explain lower frequency of *RAS* mutations in this population, as well as the aforementioned absence of *BRAF* mutations in HNPCC cancers.

Several authors have noticed that the negative prognostic effect of *BRAF V600E* mutation on overall survival is lower or completely absent in the case of coexistent microsatellite instability [25, 28, 30, 31]. This is consistent with the previously reported, less frequent coexistence of *BRAF V600E* mutation with MSI in disseminated disease, as compared to the general population (this would indicate that the negative effect of *BRAF V600E* mutation on survival at disseminated stage is mainly caused by cancers without microsatellite instability). The reason for this association is still not clear, but it is worth noting that in research exploring this subject the patients diagnosed in stage IV were underrepresented, and the status of biomarkers (RAS, BRAF, MSI) was most commonly assessed in primary tumours, thereby not taking into account the heterogeneity of tumour phenotype in space (metastasis *vs.* primary tumour) and in time (patients untreated *vs.* resistant to systemic treatment).

The relationship between *BRAF* mutation and MSI is complex and not fully understood. Studies on multiparameter molecular classification of CRC indicate that these disorders seem to have different consequences depending on whether they occur together or separately, and whether they occur in local or metastatic disease [24, 31]. This issue is becoming increasingly important, especially in the context of the recently described relationship between MSI and sensitivity of mCRC to treatment with immune checkpoint inhibitors and subsequent registration of pembrolizumab and nivolumab in this indication.

Table 3. Incidence of *BRAF V600E* mutations and relative risk (RR) of its occurrence in MSI cancers compared to MSS cancers

Publication Clinical stage (CS)	*BRAF V600Emut* among MSS	*BRAF V600Emut* among MSI	RR	MSI among *BRAF V600Emut*
Lochhead et al. (2013) [25]; CS I–IV	7.6% (81/1060)	52.3% (101/193)	6.88	55.5% (101/182)
Venderbosch et al. (2014) [10]; CS IV	6.8% (197/2910)	34.6% (53/153)	5.09	21.2% (53/250)
Seppälä et al. (2015) [26]; CS I–IV	5.4% (34/634)	57.7% (60/104)	10.69	63.8% (60/94)
Nakaji et al. (2017) [27]; CS I–III	5.4% (23/428)	40.9% (18/44)	7.57	43.9% (18/41)
Taieb et al. (2017) [28]; CS III	7.1% (279/3934)	42.1% (201/477)	5.93	41.9% (201/480)
In total	**6.8% (614/8966)**	**44.6% (433/971)**	**6.56**	**41.4% (433/1047)**

MSS — microsatellite stable; MSI — microsatellite instability

Predictive value

The predictive value of *BRAF V600E* mutation for cytotoxic chemotherapy remains controversial. A retrospective study showed equivalence of oxaliplatin and irinotecan administered in a first-line setting [32]. Moreover, it seems that the parameters of response to first-line systemic treatment (PFS, DCA, or objective response rate — ORR) do not differ significantly in cancers with this mutation as compared to the general population [10, 18].

The impact of *BRAF V600E* mutation on efficacy of anti-EGFR therapy (cetuximab, panitumumab) also remains a matter of controversy. Activating mutations in *KRAS* and *NRAS* genes are known negative predictive factors of response to drugs in this group. It was suggested that activating mutation of *BRAF* gene — a protein that is the next signal transducer directly downstream of RAS in this pathway — will also be associated with resistance to anti-EGFR antibodies. In previously treated patients, this effect was observed for both antibodies retrospectively [33–35], and then for panitumumab in a prospective PICCOLO study [9]. Interestingly, a statistically significant predictive value of *BRAF* mutation was not observed in studies on the role of anti-EGFR antibodies in first-line combined chemotherapy regimens: FIRE-3; AGITG CO.17; NORDIC-VII, CRYSTAL, OPUS [4, 36–38].

In a meta-analysis published in 2015, Pietrantonio et al. analysed data from 10 clinical trials evaluating the effect of anti-EGFR drugs on prognosis: six studies in first line, two in the second line, and two in subsequent lines. There was no benefit adding cetuximab or panitumumab to standard treatment (chemotherapy or supportive care) in patients with *BRAF* mutation as compared to the mutation-negative population in terms of PFS (HR 0.88; 95% CI 0.67–1.14, P = 0.33) as well as OS (HR 0.91, 95% CI 0.62–1.34, P = 0.63).

However, in a meta-analysis published in the same year by Rowland et al. data from eight clinical trials assessing the impact of anti-EGFR drugs on prognosis were analysed: four first-line studies, one in the second line, and three in subsequent lines — all of them were also analysed in the meta-analysis mentioned above. The authors of this publication excluded from analysis the studies in which the comparator contained bevacizumab and used a different methodology of statistical analysis. They concluded that it is impossible to prove the lack of benefits from anti-EGFR therapy in patients with *BRAF* mutation compared to mutation-negative patients because they found only statistically insignificant differences between them: for PFS, HR 0.86 (95% CI 0.61–1.21) *vs.* 0.81 (95% CI 0.70–0.95), respectively; for OS, HR 0.97 (95% CI 0.67–1.41) *vs.* 0.62 (95% CI 0.50–0.77), respectively.

Ddistinct features of *BRAF* mutations other than *V600E*

Until recently, clinical knowledge about the role of *BRAF* activation in mCRC biology was limited to the best-known mutation — *V600E*. The first descriptions of less frequent mutations were published in 2015 [12]; however, better characteristics were provided in the recent the recent publication by Jones et al. of their analysis of genome sequences from almost 10,000 cancers from American databases [13]. The incidence of *BRAF non-V600E* mutations is shown in Table 1.

In this study 112 out of 208 cases of this type of mutation were associated with a decrease and 44 with an increase in kinase activity. The biological significance of the remaining 52 mutations has not been determined. Based on analysis of 101 cases with available clinical data, it was revealed that the aforementioned clinical and pathological features of the *BRAF V600E* mutations were less frequent in the group of patients with mutations other than *V600*.

In particular, *BRAF* non-*V600E* mutations were associated with a better prognosis, not only compared to patients with *V600E* mutation, but also compared to the general population (median OS 60.7 *vs*. 11.4 *vs.* 43.0 months, respectively, P < 0.001). This fact is easily explained by reduced kinase activity; however, the authors' review of preclinical studies shows the greater complexity of the biological mechanism and also the potential for new therapeutic targets.

Intensification of first-line chemotherapy

It has been shown that the exposure to all drugs active in mCRC (5-FU, oxaliplatin, irinotecan) is associated with a longer survival [39]. Since patients with the *BRAF* mutation have a significantly lower chance of receiving the second and subsequent treatment lines [18], the intensification of the first-line regimen may be the strategy that gives hope for improvement in their prognosis.

The first study by the Greek HORG group evaluating the low-intensity FOLFOXIRI regimen (irinotecan 150 mg/m^2 d1; oxaliplatin 60 mg/m^2 d1; leucovorin 200 mg/m^2 d1, fluorouracil 400 mg/m^2 bolus and 600 mg/m^2 in 22 h infusion d1–2; all repeated every two weeks) did not show its advantage as compared to the FOLFIRI regimen [40].

On the contrary, the phase III clinical trial carried out almost simultaneously by the Itatilan GONO research group proved the effectiveness of more aggressive (classic) version of the FOLFOXIRI scheme (irinotecan 165 mg/m^2 d1; oxaliplatin 85 mg/m^2 d1; leucovorin

200 mg/m^2 d1, fluorouracil 3,200 mg/m^2 in 48-h infusion from d1; all repeated every two weeks), compared to the standard FOLFIRI scheme in patients with unresectable CRC, regardless of *RAS* and *BRAF* gene status. In the experimental group, there were significantly more neurotoxicity and neutropaenic episodes of grade 3–4 according to the CTCAE (Common Toxicity Criteria — Adverse Events) although frequency of febrile neutropaenia was < 10% and did not significantly differ between the arms. ORR (66% *vs*. 31%, P = 0.0002); PFS (median 9.8 *vs*. 6.9 months, P = 0.0006) and OS (median 22.6 *vs.* 16.7 months, P = 0.032) were significantly better [41]. This version of regimen is the best studied so far.

A single-arm phase II study evaluating the classic FOLFOXIRI scheme in combination with bevacizumab (BEV) in mCRC patients demonstrated activity of this regimen regardless of *BRAF* gene status [42]. The authors conducted thereafter a phase II study evaluating the response to this chemotherapy regimen only in the population of patients with *BRAF* mutation. Polled analysis of both studies showed the following effectiveness parameters of FOLFOXIRI regimen in patients (n = 25) with *BRAF V600E* mutation: ORR 72%; DCR 88%; medians PFS and OS: 11.8 months and 24.1 months, respectively [43]. Although this comparison is associated with obvious limitations, confronting the results of this analysis with previously reported survival parameters of patients with *BRAF V600E* mutation treated in other studies in a similar period (median PFS of seven months and median OS of 12 months) indicates clear activity of the FOLFOXIRI scheme in this subpopulation.

The GONO group also conducted a phase III study (TRIBE) comparing the effectiveness of FOLFOXIRI *vs.* FOLFIRI, both regimens with addition of bevacizumab, in 508 previously untreated mCRC patients. Bevacizumab was administered at a dose of 5 mg/kg bw, using the FOLFOXIRI scheme as in the previous studies of this group, while the FOLFIRI scheme was used in a more aggressive form (irinotecan 180 mg/m^2 dl; leucovorin 200 mg/m^2 d1; fluorouracil 400 mg/m^2 — bolus and 2400 mg/m^2 — in 46-h infusion). After 12 cycles all patients received maintenance treatment with fluorouracil and bevacizumab. Patients in the active arm significantly more frequently experienced neutropaenia, diarrhoea, and neurotoxicity. The incidence of febrile neutropaenia was below 10% and did not differ significantly between the arms. The study showed a significant advantage of FOLFOXIRI + BEV as regards to ORR (65% *vs.* 53%, P = 0.013), OS (29.8 *vs.* 25.8 months, P = 0.03), and PFS (12.3 *vs.* 9.7 months, P = 0.006) in the general population. Multivariate subgroup analysis showed no significant differences between the studied regimens in patients with *RAS* or *BRAF* mutation, with a trend towards higher activity of four-drug regimen in the population with *BRAF* mutation [44].

Unfortunately, no prospective clinical trial comparing FOLFOXIRI with the same regimen in combination with bevacizumab has been performed so far. The best available study exploring this topic is a pooled analysis of the GONO group's trials, showing the benefit of adding bevacizumab, both in univariate and multivariate analysis, with better OS (29.8 *vs.* 23.6 months, P = 0.014) and PFS (12.3 *vs.* 10.0 months, P = 0.013), but not ORR (65.1% *vs.* 55.7%, P = 0.280) [45].

Overall, the combination of FOLFOXIRI + bevacizumab appears to be an active regimen in mCRC patients with *BRAF* mutation (Table 4); however, it should be remembered that all studies on this regimen recruited only relatively young patients with good performance status. Although there is no confirmation of these findings in prospective studies, a combination FOLFOXIRI + bevacizumab is mentioned as a therapeutic option in mCRC patients with *BRAF* mutation in international guidelines, and many experts consider it as a therapeutic standard for mCRC with this molecular profile.

From a practitioner's point of view, an important issue is selection of second-line treatment after progression upon a FOLFOXIRI ± BEV scheme. The obvious choice may be rechallenge with the first-line regimen; however, this strategy, although widely used, seems to be justified in patients with a long time to progression, which in mCRC harboring *BRAF* mutation it is an exception rather than the rule. For this reason, new strategies for combined systemic treatment are being developed, based on the use of drugs targeting the key molecular mechanism – MAPK pathway activated by mutant BRAF kinase.

Strategies for blocking BRAF in mCRC

Since the discovery of the role of BRAF as a driver of tumour growth, numerous inhibitors of this kinase have been studied. Two currently commercialised drugs are vemurafenib (Zelboraf™, Roche) and dabrafenib (Tafinlar™, Novartis) — both have been approved for the treatment of advanced melanoma with the *BRAF V600* mutation. Further BRAF inhibitors have also been developed: LGX818 (encorafenib; Novartis), CEP-32496 (Ambit Biosciences Corporation), XL281 (Exelixis), HM95573 (Hanmi), ASN003 (BRAF + PI3K, Asana Biosciences), LXH254 (pan-RAF, Novartis). Due to the very high activity of BRAF inhibitors in melanoma therapy, numerous studies have been initiated to assess the effectiveness of these drugs in the treatment of other cancers with the same molecular alteration. In 2015, Kopetz et al. presented data from the phase II study on anti-BRAF monotherapy in previously treated

Table 4. Comparison of activity parameters of FOLFOXIRI scheme in the GONO group studies

Study name Intervention *vs.* comparator	Response rate		Median PFS (months)		Median OS (months)	
	Intervention	Comparator	Intervention	Comparator	Intervention	Comparator
GONO Falcone et al. [41] (n = 244) FOLFOXIRI *vs.* FOLFIRI	66% (P = 0.0002)	41%	9.8 (P = 0.0006)	6.9	22.6 (P = 0.032)	16.7
TRIBE (n = 508) [44] FOLFOXIRI + bevacizumab *vs.* FOLFIRI + bevacizumab	65% (P = 0.013)	53%	12.3 (P = 0.006)	9.7	29.8 (P = 0.03)	25.8
GONO Loupakis et al. [43] BRAFmut (n = 25) Pooled analysis of phase II studies FOLFOXIRI + bevacizumab	72% ND	ND	11.8 ND	ND	24.1 ND	ND
TRIBE BRAFmut (n = 28) [44] FOLFOXIRI + bevacizumab *vs.* FOLFIRI + bevacizumab	56% ND	42%	7.5 ND	5.5	19.0 ND	10.7

PFS — progression-free survival; OS — overall survival

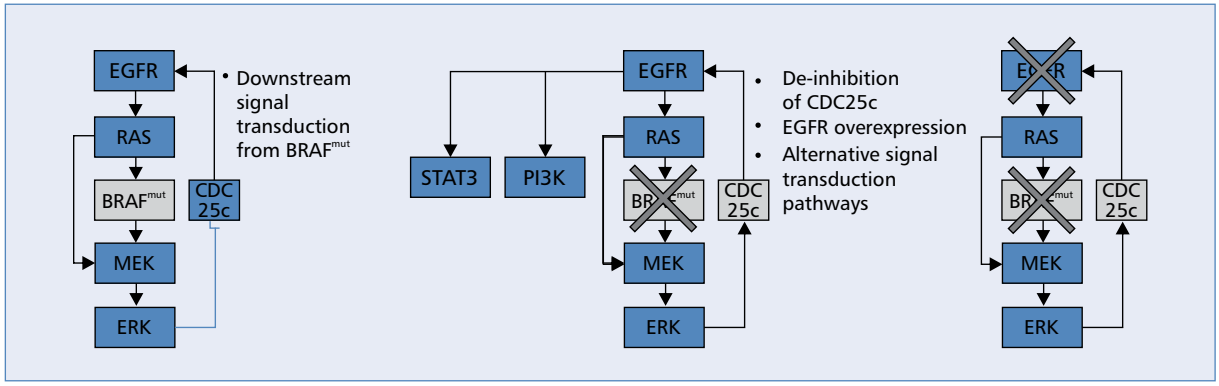

Figure 2. Resistance to BRAF blocking by feedback activation of EGFR signalling

patients with mCRC with the *V600E* mutation [46]. The activity of this strategy was not very encouraging, with an ORR of 5% and a median PFS of 2.1 months. As the results of similar studies published by other authors were comparable [47, 48], further research focused on finding resistance mechanisms.

It has been demonstrated that BRAF blocking results in reactivation of MAPK pathway in the EGFR-dependent mechanism (Figure 2) [49, 50] and that the blockage of the latter may potentially overcome this resistance.

Several studies have explored the combination of BRAF inhibitor with EGFR blocking, showing promising activity [51, 52]. Subsequent studies have evaluated the addition of MEK inhibitor, PI3K inhibitor, or a cytotoxic drug to double blockade. In the phase II SWOG 1406 trial with previously treated mCRC patients with *BRAF V600* mutation, the benefit of adding BRAF inhibitor to the combination of cetuximab and irinotecan was evaluated. In a population of 106 randomised patients a significant reduction in the relative risk of progression by 58% (HR 0.42, 95% CI 0.26–0.66) was shown with median PFS of 4.4 months (IRI + CET + WEM) *vs.* 2.0 months (IRI + CET). The ORR in the experimental arm increased from 4% to 16% and DCR from 22% to 67%. Adverse reactions of grade 3–4 according to the CTCAE scale were more frequent in the experimental arm — neutropaenia (28% *vs.* 7%), anaemia (13% *vs.* 0%), and nausea (15% *vs.* 0%) [53–55]. With the cumulating of data on multi-level BRAF interaction with parallel signalling pathways, new clinical trials are exploring the combination of BRAF inhibition with other drugs (Table 5).

Table 5. Ongoing clinical trials in BRAFmut mCRC, with no published results

Evaluated regimens	Phase	NIH number
encorafenib (BRAF antagonist) + binimetinib (MEK antagonist)	I/II	NCT01543698
encorafenib + cetuximab ± binimetinib	III	NCT02928224
vemurafenib + cetuximab + irinotecan	I	NCT01787500
dabrafenib, trametinib, panitumumab — various combinations	II	NCT01750918
vemurafenib + cetuximab + 5-fluorouracil	II	NCT02291289
dactolisib (PI3K antagonist) + binimetinib	I	NCT01337765
buparlisib (PI3K antagonist) + binimetinib	I	NCT01363232
LGK974 (beta-catenin antagonist) ± PDR001 (anti-PD-1 antibody)	I	NCT01351103
irinotecan + AZD1775 (Wee1 antagonist)	I	NCT02906059

Summary

BRAF V600E mutation is an independent negative prognostic factor in colorectal cancer, although the presence of other molecular changes, in particular MSI, seems to modulate this mechanism. The negative effect of this disorder is observed consistently in both locally advanced and metastatic disease. It seems to result primarily from aggressive dynamics of the disease and rapidly progressing recurrences after first-line treatment, rather than from resistance to cytotoxic treatment. The recently described, less frequent *BRAF* mutations (outside of codon 600) are not associated with worse prognosis.

The predictive value of *BRAF V600E* mutation is a subject of controversy. Mutation may be associated with resistance to treatment with EGFR inhibitors; however, data on this subject are not consistent, suggesting potential heterogeneity in this area. Intensification of first-line chemotherapy to FOLFOXIRI + BEV regimen seems to be an effective therapeutic strategy in patients with *BRAF V600E* mutation, but the evidence suporting such strategy does not come from prospective studies. Identification of the importance of *BRAF* mutations in tumour biology, including colorectal cancer, have allowed the design of molecular targeted therapies of causative nature, suppressing the negative effect of excessive BRAF kinase activity and MAPK pathway.

Declaration of conflict of interest

Paweł Potocki: received honoraria and travel grants from Amgen, Merck, Roche.

Piotr Wysocki: received honoraria, travel grants from Roche, Merck, Amgen, Novartis.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136(5): E359–E386, doi: 10.1002/ijc.29210, indexed in Pubmed: 25220842.
2. Didkowska J, Wojciechowska U, Olasek P. Nowotwory złośliwe w Polsce w 2015 roku Cancer in Poland in 2015. Cancer, 2017. [Online]. Available: http://onkologia.org.pl/raporty/. [Accessed: 02-Jan. 2018].
3. Seymour MT, Maughan TS, Ledermann JA, et al. FOCUS Trial Investigators, National Cancer Research Institute Colorectal Clinical Studies Group. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet. 2007; 370(9582): 143–152, doi: 10.1016/S0140-6736(07)61087-3, indexed in Pubmed: 17630037.
4. Stintzing S, Modest D, Rossius L, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. The Lancet Oncology. 2016; 17(10): 1426–1434, doi: 10.1016/s1470-2045(16)30269-8.
5. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature. 2002; 417(6892): 949–954, doi: 10.1038/nature00766, indexed in Pubmed: 12068308.
6. Fearon ER. Molecular genetics of colorectal cancer. Annu Rev Pathol. 2011; 6: 479–507, doi: 10.1146/annurev-pathol-011110-130235, indexed in Pubmed: 21090969.
7. Rad R, Cadiñanos J, Rad L, et al. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. Cancer Cell. 2013; 24(1): 15–29, doi: 10.1016/j.ccr.2013.05.014, indexed in Pubmed: 23845441.
8. Matallanas D, Birtwistle M, Romano D, et al. Raf family kinases: old dogs have learned new tricks. Genes Cancer. 2011; 2(3): 232–260, doi: 10.1177/1947601911407323, indexed in Pubmed: 21779496.
9. Seymour MT, Brown SR, Middleton G, et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. Lancet Oncol. 2013; 14(8): 749–759, doi: 1016/S1470-2045(13)70163-3, indexed in Pubmed: 23725851.
10. Venderbosch S, Nagtegaal ID, Maughan TS, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. Clin Cancer Res. 2014; 20(20): 5322–5330, doi: 10.1158/1078-0432. CCR-14-0332, indexed in Pubmed: 25139339.
11. Modest DP, Ricard I, Heinemann V, et al. Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group. Ann Oncol. 2016; 27(9): 1746–1753, doi: 10.1093/annonc/mdw261, indexed in Pubmed: 27358379.
12. Cremolini C, Di Bartolomeo M, Amatu A, et al. BRAF codons 594 and 596 mutations identify a new molecular subtype of metastatic colorectal cancer at favorable prognosis. Ann Oncol. 2015; 26(10): 2092–2097, doi: 10.1093/annonc/mdv290, indexed in Pubmed: 26153495.
13. Jones JC, Renfro LA, Al-Shamsi HO, et al. BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. J Clin Oncol. 2017; 35(23): 2624–2630, doi: 10.1200/JCO.2016.71.4394, indexed in Pubmed: 28486044.
14. Clancy C, Burke JP, Kalady MF, et al. BRAF mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. Colorectal Dis. 2013; 15(12): e711–e718, doi: 10.1111/codi.12427, indexed in Pubmed: 24112392.

15. Fariña-Sarasqueta A, van Lijnschoten G, Moerland E, et al. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol. 2010; 21(12): 2396–2402, doi: 10.1093/annonc/mdq258, indexed in Pubmed: 20501503.
16. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010; 28(3): 466–474, doi: 10.1200/JCO.2009.23.3452, indexed in Pubmed: 20008640.
17. Kadowaki S, Kakuta M, Takahashi S, et al. Prognostic value of KRAS and BRAF mutations in curatively resected colorectal cancer. World J Gastroenterol. 2015; 21(4): 1275–1283, doi: 10.3748/wjg.v21.i4.1275, indexed in Pubmed: 25632202.
18. Seligmann JF, Fisher D, Smith CG, et al. Investigating the poor outcomes of BRAF -mutant advanced colorectal cancer: Analysis from 2530 patients in randomised clinical trials . Annals of Oncology. 2016: mdw645, doi: 10.1093/annonc/mdw645.
19. Yaeger R, Cercek A, Chou JF, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. Cancer. 2014; 120(15): 2316–2324, doi: 10.1002/cncr.28729, indexed in Pubmed: 24737664.
20. Renaud S, Romain B, Falcoz PE, et al. KRAS and BRAF mutations are prognostic biomarkers in patients undergoing lung metastasectomy of colorectal cancer. Br J Cancer. 2015; 112(4): 720–728, doi: 10.1038/bjc.2014.499, indexed in Pubmed: 25688918.
21. Schirripa M, Bergamo F, Cremolini C, et al. BRAF and RAS mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection. Br J Cancer. 2015; 112(12): 1921–1928, doi: 10.1038/bjc.2015.142, indexed in Pubmed: 25942399.
22. Mori Y, Nagasaka T, Tanioka H, et al. P-346Ovarian metastasectomy in colorectal cancer may improve the clinical outcomes of patients with metastatic colorectal cancer regardless of BRAF or KRAS mutational profiles. Annals of Oncology. 2017; 28(suppl_3), doi: 10.1093/annonc/mdx261.342.
23. Johnson B, Jin Z, Truty M, et al. Impact of Metastasectomy in the Multimodality Approach forBRAFV600E Metastatic Colorectal Cancer: The Mayo Clinic Experience. The Oncologist. 2017; 23(1): 128–134, doi: 10.1634/theoncologist.2017-0230.
24. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015; 21(11): 1350–1356, doi: 10.1038/nm.3967, indexed in Pubmed: 26457759.
25. Lochhead P, Kuchiba A, Imamura Yu, et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst. 2013; 105(15): 1151–1156, doi: 10.1093/jnci/djt173, indexed in Pubmed: 23878352.
26. Seppälä TT, Böhm JP, Friman M, et al. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. Br J Cancer. 2015; 112(12): 1966–1975, doi: 10.1038/bjc.2015.160, indexed in Pubmed: 25973534.
27. Nakaji Yu, Oki E, Nakanishi R, et al. Prognostic value of BRAF V600E mutation and microsatellite instability in Japanese patients with sporadic colorectal cancer. J Cancer Res Clin Oncol. 2017; 143(1): 151–160, doi: 10.1007/s00432-016-2275-4, indexed in Pubmed: 27672042.
28. Taieb J, Le Malicot K, Shi Q, et al. Prognostic Value of BRAF and KRAS Mutations in MSI and MSS Stage III Colon Cancer. J Natl Cancer Inst. 2017; 109(5), doi: 10.1093/jnci/djw272, indexed in Pubmed: 28040692.
29. Parsons MT, Buchanan DD, Thompson B, et al. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. J Med Genet. 2012; 49(3): 151–157, doi: 10.1136/jmedgenet-2011-100714, indexed in Pubmed: 22368298.
30. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. 2005; 65(14): 6063–6069, doi: 10.1158/0008-5472.CAN05-0404, indexed in Pubmed: 16024606.
31. Phipps AI, Limburg PJ, Baron JA, et al. Association between molecular subtypes of colorectal cancer and patient survival. Gastroenterology. 2015; 148(1): 77–87.e2, doi: 10.1053/j.gastro.2014.09.038, indexed in Pubmed: 25280443.
32. Morris V, Overman MJ, Jiang ZQ, et al. Progression-free survival remains poor over sequential lines of systemic therapy in patients with BRAF-mutated colorectal cancer. Clin Colorectal Cancer. 2014; 13(3): 164–171, doi: 10.1016/j.clcc.2014.06.001, indexed in Pubmed: 25069797.
33. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008; 26(35): 5705–5712, doi: 10.1200/JCO.2008.18.0786, indexed in Pubmed: 19001320.
34. Loupakis F, Ruzzo A, Cremolini C, et al. 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. 2009; 101(4): 715–721, doi: 10.1038/sj.bjc.6605177, indexed in Pubmed: 19603018.
35. Roock WDe, Claes B, Bernasconi D, et al. 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. The Lancet Oncology. 2010; 11(8): 753–762, doi: 10.1016/s1470-2045(10)70130-3.
36. Karapetis CS, Jonker D, Daneshmand M, et al. NCIC Clinical Trials Group and the Australasian Gastro-Intestinal Trials Group. PIK3CA, BRAF, and PTEN status and benefit from cetuximab in the treatment of advanced colorectal cancer — results from NCIC CTG/AGITG CO.17. Clin Cancer Res. 2014; 20(3): 744–753, doi: 10.1158/1078-0432.CCR13-0606, indexed in Pubmed: 24218517.
37. Tveit KM, Guren T, Glimelius B, et al. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. J Clin Oncol. 2012; 30(15): 1755–1762, doi: 10.1200/JCO.2011.38.0915, indexed in Pubmed: 22473155.
38. Bokemeyer C, Van Cutsem E, Rougier P, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer. 2012; 48(10): 1466–1475, doi: 10.1016/j.ejca.2012.02.057, indexed in Pubmed: 22446022.
39. Grothey A, Sargent D, Goldberg RM, et al. Survival of patients with advanced colorectal cancer improves with the availability of fluorouracil-leucovorin, irinotecan, and oxaliplatin in the course of treatment. J Clin Oncol. 2004; 22(7): 1209–1214, doi: 10.1200/JCO.2004.11.037, indexed in Pubmed: 15051767.
40. Souglakos J, Androulakis N, Syrigos K, et al. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). Br J Cancer. 2006; 94(6): 798–805, doi: 10.1038/sj.bjc.6603011, indexed in Pubmed: 16508637.
41. Falcone A, Ricci S, Brunetti I, et al. Gruppo Oncologico Nord Ovest. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. J Clin Oncol. 2007; 25(13): 1670–1676, doi: 10.1200/JCO.2006.09.0928, indexed in Pubmed: 17470860.
42. Masi G, Loupakis F, Salvatore L, et al. Bevacizumab with FOLFOXIRI (irinotecan, oxaliplatin, fluorouracil, and folinate) as first-line treatment for metastatic colorectal cancer: a phase 2 trial. The Lancet Oncology. 2010; 11(9): 845–852, doi: 10.1016/s1470-2045(10)70175-3.
43. Loupakis F, Cremolini C, Salvatore L, et al. FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer. Eur J Cancer. 2014; 50(1): 57–63, doi: 10.1016/j.ejca.2013.08.024, indexed in Pubmed: 24138831.
44. Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. 2015; 16: 1306–1315P. doi:10.1016/S14702045(15)00122-9.
45. Cremolini C, Loupakis F, Masi G, et al. FOLFOXIRI or FOLFOXIRI plus bevacizumab as first-line treatment of metastatic colorectal cancer: a propensity score-adjusted analysis from two randomized clinical trials. Ann Oncol. 2016; 27(5): 843–849, doi: 10.1093/annonc/mdw052, indexed in Pubmed: 26861604.
46. Kopetz S, Desai J, Chan E, et al. Phase II Pilot Study of Vemurafenib in Patients With Metastatic BRAF-Mutated Colorectal Cancer. J Clin Oncol. 2015; 33(34): 4032–4038, doi: 10.1200/JCO.2015.63.2497, indexed in Pubmed: 26460303.
47. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. N Engl J Med. 2015; 373(8): 726–736, doi: 10.1056/NEJMoa1502309, indexed in Pubmed: 26287849.
48. Gomez-Roca CA, Delord J, Robert C, et al. Encorafenib (lgx818), an oral braf inhibitor, in patients (PTS) with BRAF V600E metastatic colorectal cancer (mCRC): results of dose expansion in an open-label, phase 1 study. Ann Oncol. 2014; 25(suppl_4): iv182–iv183, doi: 10.1093/ /annonc/mdu333.38.
49. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR.

Nature. 2012; 483(7387): 100–103, doi: 10.1038/nature10868, indexed in Pubmed: 22281684.
50. Corcoran RB, Ebi H, Turke AB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer Discov. 2012; 2(3): 227–235, doi: 10.1158/2159-8290.CD-11-0341, indexed in Pubmed: 22448344.
51. Yaeger R, Cercek A, O'Reilly EM, et al. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. Clin Cancer Res. 2015; 21(6): 1313–1320, doi: 10.1158/1078-0432. CCR-14-2779, indexed in Pubmed: 25589621.
52. Desai J, Markman B, Ananda S, et al. A phase I/II trial of combined BRAF and EGFR inhibition in patients (pts) with BRAF V600E mutated (BRAFm) metastatic colorectal (mCRC): The EViCT (Erlotinib and Vemurafenib in Combination Trial) study. J. Clin Oncol. May 2017, vol. 35, no. 15_suppl, p. (suppl.3557): 3557P, doi: 10.1200/JCO.2017.35.15_.
53. van Geel RM, Tabernero J, Elez E, et al. A Phase Ib Dose-Escalation Study of Encorafenib and Cetuximab with or without Alpelisib in Metastatic-Mutant Colorectal Cancer. Cancer Discov. 2017; 7(6): 610–619, doi: 10.1158/2159-8290.CD-16-0795, indexed in Pubmed: 28363909.
54. Corcoran RB, André T, Yoshino T, et al. Efficacy and circulating tumor DNA (ctDNA) analysis of the BRAF inhibitor dabrafenib (D), MEK inhibitor trametinib (T), and anti-EGFR antibody panitumumab (P) in patients (pts) with BRAF V600E–mutated (BRAFm) metastatic colorectal cancer (mCRC). Annals of Oncology. 2016; 27(suppl_6), doi: 10.1093/annonc/mdw370.04.
55. Kopetz S, McDonough S, Morris V, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG 1406). Journal of Clinical Oncology. 2017; 35(4_suppl): 520–520, doi: 10.1200/jco.2017.35.4_suppl.520.