# *BRAF* mutation in colorectal cancer: an update

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Arch Oncol 2023; 29(1):16-9

Published Online
May 13th, 2022
https://doi.org/10.2298/AOO220130004C

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Received **2022-01-30**
Received in revised form **2022-01-30**
Accepted **2022-03-23**

## SUMMARY

*Colon cancer is a leading cause of cancer-related deaths worldwide. About 10% of all colon cancer patients are found to have a mutation in BRAF proto-oncogene that arise as a result of a substitution of amino acid valine with glutamate at position 600 (V600E). This specific mutation is also found in melanomas, but at even higher percent – in up to 60% of patients. A particular category of drugs called* BRAF *inhibitors, have been developed in order to increase survival. But, while in patients with melanoma this class of drugs work well especially when combined with mitogen-activated protein kinase inhibitors, they have low efficacy in patients with metastatic colorectal cancer suggesting different mechanism of action and development of drug resistance. This review summarise recent findings aimed to highlight events in BRAF mutations in metastatic colorectal cancer.*

**KEY WORDS:** BRAF, *colorectal cancer, biomarker, oncogene*

## INTRODUCTION

Metastatic colorectal cancer (mCRC) is the third leading cause of cancer related death in the United States (1). mCRC is a molecularly heterogeneous type of cancer, characterized by profound molecular alterations that influence its resistance to therapies and progression of the disease with different clinical behaviours.

For this reason molecular classification, that was based on molecular subtypes of the disease was proposed, where progression of the disease arise through multistep genetic mutations that follow one another in a chain through the activation of oncogenes and the inactivation of oncogen-suppressors (2).

Today, this model (named *the big bang model*) has been upgraded with the addition of subtypes with new profiles of mutations that arise along the initial mutation contributing to the heterogeneity of the tumor (3).

Mutations in *BRAF* proto-oncogene are examples of this type of mutation and in mCRC they are present in 10% of patients (4). The *BRAF* mutated mCRC are more frequently located in the right colon, and these patients have a worse outcome (5, 6).

In melanomas, where *BRAF* mutations are present in 60% of cases response rates obtained after treatment with BRAF inhibitors are from 60-80%, while in colorectal cancers, this treatment gives a response rate of only 5% (7, 8, 9). Thus, one of the important challenges in oncology is to overcome this resistance (10, 11). This review aims to highlight recent knowledge on the therapeutic strategies and forms of drug resistance in patients with *BRAF* mutated mCRC.

## *BRAF* MUTATIONS

The BRAF protein plays a crucial role in epidermal growth factor receptor (EGFR) mediated mitogen-activated protein kinase (MAPK) pathway (12). BRAF and its isoforms (ARAF and CRAF), affect cell growth, differentiation and proliferation, cell migration through Ras homologous protein (RHO) and small GTPase by regulating B-cell lymphoma 2 (BCL2), and cell survival by interacting with the HIPPO pathway (13). For this reason, it is found constitutively activated in 15% of tumors (14). The *BRAF* proto-oncogene can be mutated at various points, but the best known mutation is V600E (1799T>A, nucleotide change), which constitutes up to 80% of all *BRAF* mutations (14). This mutation involves the substitution of a single amino acid which determines constitutive kinase activation.

Most *BRAF* mutations occur after either the acquirement of new phosphomimic residues or due to auto-inhibitory conformation imposed by the N-terminal region that stimulates dimerization of the kinase domain which is known to be a crucial in *BRAF* activity.

BRAF inhibitors have been distributed by different pharmaceutical companies, as vemurafenib, dabrafenib, LGX818, XL 281 and CEP 32496 (15).

Colon polyps can be classified into several groups: adenomatous polyps that involve 10% of all polyps and hyperplastic polyps that are present in about 90% of cases. Hyperplastic polyps do not progress to CRC. Some polyps are also referred to as serrated polyps due to their saw-toothed morphology. For a long time, these polyps were considered incapable of a malignant transformation, a concept which was later changed. Serrated polyps are sub-classified into two categories: traditional serrated adenomas (TSAs), and sessile serrated adenomas (SSAs) (16). TSAs and SSAs are now considered to be pre-cancerous lesions.

It is thought that the early transformation of healthy epithelia into TSA and SSA polyps is induced by the *BRAF* mutation considering this mutagenic step an early phase of progression in colorectal cancer, while the activation of the Wingless/Integrated (WNT) pathway together with the inactivation of p53 and p16, seem to intervene in the late stages of CRC development (17).

*BRAF* mutated tumors are characterized by being located in the right colon, arising in old age, being more present in women, having a high grading, poor prognosis and being associated with microsatellite instability MSI (18, 19).

MSI is a particular genetic form of colorectal cancer, characterized by instability of microsatellites, consequent to the deficiency in mismatch repair. MSI colorectal cancers are characterized by having a good prognosis. In addition, it was also noted that patients with the MSI-associated *BRAF* mutation have a better prognosis than MSS (microsatellite stable) *BRAF* mutated colorectal cancer, although this advantage has not been shown to be statistically significant (18).

As previously mentioned, *BRAF* mutations are mostly found in the right colon, but the reason for this is still unknown (20, 21).

## *BRAF* AS PROGNOSTIC AND PREDICTIVE FACTOR FOR mCRC

While the predictive role of *RAS* mutations in response to anti-EGFR drugs is well known, the real predictive value of *BRAF* mutation in

colorectal cancer was not clear until few years ago. After obtaining data from the large, phase III BEACON study in patients with *BRAF* mutated mCRC in second and third line, it was clear that strongly predictive response to the combination of binimetinib, encorafenib and cetuximab according to the type of *BRAF* mutation was obtained (22).

In patients with wild-type *RAS* and mutated *BRAF* the use of anti-EGFR therapy has been debated due to discordance in different retrospective studies.

Based on one of the studies patients with mutated *RAS* and *BRAF* respond to anti-EGFRs (23). In the second one it was demonstrated that the *BRAF* mutation in patients with *RAS* mutations affect the response of anti-EGFR therapy (24).

On the other hand, *BRAF* prognostic effect is well known and as previously mentioned - mutated *BRAF* mCRC has a worse prognosis (5, 6).

In the adjuvant setting *BRAF* mutations were evaluated in a retrospective analysis on 1200 mCRC patients in stages II and III underlining that confirmed *BRAF* mutation predicted bad outcome, unlike *KRAS* that does not influence the prognosis of patients with mCRC at this stage of disease (6).

## RESISTANCE TO BRAF INHIBITOR

Since *BRAF* mutation has been found to be a bad predictive factor of response to BRAF inhibitor therapy in mCRC, this underlined the fact that the mechanism by which it acts on the MAPK pathway is quite complex.

In recent years, the functioning of mutated *BRAF* and its drug resistance mechanisms has been studied extensively. Clearly, the first tumor type in which BRAF inhibitor drugs found application was melanoma, due to the high percentage of *BRAF* V600E mutation in this aggressive skin tumor.

In the last two years, number of studies have been carried out on patients with *BRAF* mutatations (including mCRC) in first, second and third line of treatment (25).

Number of mechanisms of resistance to BRAF inhibitors have been identified that include: *MEK1, MEK2,* and *NRAS* activating mutations, *BRAF* amplification, *COT* overexpression, platelet - derived growth factor receptor and *EGFR* overexpression, secondary *RAF* related mutations and the expression of constitutively active splicing variants of *BRAF* (25, 26) that all influence hyper-activation of *MAPK*. The researchers are trying to overcome this problem with the association of anti BRAF drugs with MEK inhibitors, leading the FDA to authorize dabrafenib in combination with trametinib for the treatment of V600E *BRAF* mutated, non-resectable metastatic melanoma (27).

Unfortunately, the discoveries made on mutated *BRAF* melanomas cannot be translated into colorectal cancers; first because the high response rate seen in melanomas is obviously different from that observed in colorectal cancers and then, the different percentage of *BRAF* mutation in melanomas compared to colorectal cancers, may underlie the difference in BRAF signalling between the two neoplasms.

In CRC, resistance to BRAF inhibitors was shown to be driven by feedback reactivation of *EGFR* that activates in turn *MAPK* via *CRAF* and *RAS* (28, 29).

Furthermore, in colorectal cancers, resistance to BRAF inhibitors has been repeatedly demonstrated that depends on feedback reactivation of *EGFR* which consequently activates *MAPK* through *CRAF* and *RAS* (28, 29).

This EGFR dependent feedback mechanism is more expressed in colon tumors while it is not very active in melanomas and many studies have shown that this feedback could be avoided by combining an anti EGFR with a BRAF and MEK inhibitors (28).

Experiments in mouse xenograft models have shown that the combination of the three drugs dramatically reduce tumor growth, which is not evident with single agent therapy (29). However, even with these combinations of drugs, resistances are created in the long time (30).

Another mechanism capable of giving resistance is expressed through the hepatocyte growth factor – mesenchymal – epithelial transition (MET) pathway also involved in EGFR inhibitory resistance caused by MET stimulation (31).

In one study, it was also seen that in patients resistant to the combination of BRAF, MEK and EGFR inhibitors, through the study of *exons* of tumor cells, it was noted that these acquire the ability to amplify *KRAS*, *BRAF* and *MEK1* with consequent activation of *MAPK* (30, 32).

Cells that become resistant to this drug mix retain *BRAF* mutations, but KRAS mutations such as G12D or G13D appear, giving a selective advantage to the combination of BRAF, MEK and EGFR inhibitors.

Furthermore, it must be remembered that it is not easy to find the double mutation of *BRAF* and *KRAS* in patients with colorectal cancer, because the cells with the double mutation are more easily susceptible to senescence (35).

Besides that it has been noted that in melanomas developing resistance to vemurafenib, after its suspension, a further reduction in tumor mass was seen (34).

Still, another mechanism that could explain the different effects of drugs in melanomas and colorectal cancers is activation level of *PI3K*. In cell cultures, activation levels have been observed to be much higher in colorectal cancers than in melanomas (35). It should be mentioned that resistance to BRAF inhibitors can be bypassed with the use of drugs that act on *PI3K*. A study performed on engineered mice with mutated *BRAF* colorectal tumor has demonstrated an important block of tumor growth with the use of anti BRAF in combination with drugs that block *Pi3K/mTOR* (36). This result, together with recent data from the BEACON study, which showed an increase in progression free survival (PFS) in patients in II and III line *BRAF* mutant mCRC, paved the way for FDA approval of the doublet encorafenib plus cetiximab in this kind of *BRAF* V600E mutated tumor (37).

## FUTURE ADDRESSES AND DISCUSSION

Although until a few years ago, treatment of *BRAF* mutated mCRC presented a huge problem for oncologists, today, thanks to the new data including large, phase III Beacon study we can say that second and third line in *BRAF* mutated mCRC treated with BRAF inhibitor encorafenib and EGFR inhibitor cetuximab have been coded - with marked and statistically significant improvement in PFS when compared to treatment with chemotherapy of the investigator's choice (37).

Thanks to the enormous effort of numerous investigators, great strides have been made on this subtype of mCRC. But still, there are many uncertainties in this small but complex group of patients with *BRAF*

mutated mCRC. First of all, it is necessary to understand, also on the basis of the BEACON study, when to use the doublet (encorafenib and cetuximab) or triplet therapy (encorafenib, cetuximab and binimetinib (37). Finally, it will be of the most importance to understand what role has the coupling of the *BRAF* mutation with the unstable phenotype of the microsatellites (MSI-H).

### Declaration of Interests

Authors declare no conflicts of interest.

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