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Therapeutic implications of *B-RAF* mutations in colo-rectal cancer

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Summary

Colorectal cancers (CRC) with *B-RAF* mutation carry a particularly poor prognosis. In this context, the value of first-line intensified chemotherapy associated with an anti-VEGF (Vascular endothelial growth factor) to treat metastatic CRC has recently been called into question.

In patients with mutated *B-RAF*, the efficacy of first-line anti-EGFR (Epidermal Growth Factor Receptor) associated with chemotherapy for treatment of metastatic CRC is uncertain while that of anti-VEGF has been shown to be effective.

The therapeutic pathways involving inhibition of B-RAF activity, although ineffective as monotherapy, have received marketing authorization when used in association with anti-EGFR for second-line treatment of metastatic CRC.

Immunotherapy has provided very encouraging results in a recent phase III study in patients with microsatellite instability, irrespective of their *B-RAF* status.

Finally, new therapies, targeting other RAF proteins and other specific receptors are currently under development.

Surgery for liver metastases in patients with the B-RAF mutation should be considered whenever possible, after a complete search for peritoneal carcinomatosis and distant metastases, similarly to workup for patients without the B-RAF mutation.

Key words

Colorectal cancer, B-RAF, immunotherapy, triple inhibition

List of abbreviations

ASCO: American Society of Clinical Oncology

B-RAFi: *B-RAF* inhibitors

B-RAFmt: *B-RAF*-mutant

B-RAFwt: wild type -*RAF*

CEA: carcino-embryonic antigen

CRC: colorectal cancer

CRCm: metastatic CRC

CTCAE: Common Terminology Criteria for Adverse Events

EGFR: Epidermal Growth Factor Receptor

ERK: Extracellular Receptor Kinase

ETS: early tumor shrinkage

HR: hazard ratio

IC: interval of confidence

LM: liver metastases

MAPK: mitogen-activated protein kinase

MEK: MAPK-ERK-Kinase

MEKi: MEK inhibitor

MMR: mismatch repair

MSI: microsatellite instability

MSI-H: Microsatellite instability high

MSI-H/MMRd: microsatellite-instability high/mismatch repair deficiency

MMS: microsatellite stability

OR: Odds ratio

OS: Overall survival

PFS: progression-free survival

PI3K / AKT: PhosphoInositidyl-3-Kinase / Protéine kinase B

RAF (stands for the early experimental origin “rapidly accelerated fibrosarcoma”): family of oncoproteins that intervene in cancerogenetic pathways

RAS protein (stands for Reticular Activating System; named after experiments with the rat sarcoma virus): family of (gene) proteins that regulate various cell behaviors

RAFmt: RAF-mutant

RASmt: RAS-mutant

RBD: Ras-Binding Domaine

RFS: Recurrence-free survival

VEGF: Vascular Endothelial Growth Factor

Introduction

Colorectal cancer (CRC) remains a serious health problem in France: 43336 new cases with 17117 related deaths were recorded in 2018 [1].

The identification of oncogenic mutations in tumor cells has clarified the prognosis and refined the choice of treatment for an increasing number of cancers. In CRC, the identification of mutation of the *B-RAF* (*B-Rapidly Accelerated Fibrosarcoma*) gene has enabled the definition of a subgroup of patients with a particularly bleak prognosis. The proportion of *B-RAF*-mutant (*B-RAFmt*) CRC remains low, involving 10 to 15% of non-metastatic tumor and 5 to 10% of metastatic CRC (CRCm). Such mutations are far more frequently observed in other types of cancer: 30% of ovarian tumors, 40% of papillary thyroid cancers, 50 to 70% of melanomas and nearly 100% of hairy cell leukemia [2].

The *B-RAF* ^{V600E} mutation is the most commonly observed subgroup. This mutation is characterized by substitution of valine by glutamic acid in the codon 600 of exon 15 in chromosome 7. Usually, *B-RAF* mutations do not occur in the presence of *RAS* (*Reticular Activating System*) mutations, and only 0.3% have a mutated *RAS/RAF* phenotype (*RASmt* / *RAFmt*). The *B-RAF* mutation is most often associated with right-sided colonic cancer, female sex, T4 tumors, poorly differentiated tumors, with mucinous histology, a microsatellite-instability high / mismatch repair deficiency (MSI-H / MMRd) profile, and secondary peritoneal or lymph node involvement (3).

B-RAF $^{non\text{-}V600E}$ mutations are rare, identified in 20% of patients with *B-RAFmt* and in only 2% with CRCm. The most frequently observed *B-RAF* $^{non\text{-}V600E}$ mutation (45% of cases) is located on the codon 594. Compared to *B-RAF* ^{V600E} mutations, *B-RAF* $^{non\text{-}V600E}$ mutations involve younger patients (58 *vs.* 68 years), more often men (54% *vs.* 35%), with earlier tumor stage (13% *vs.* 64 %), occur less often in the right colon (37% *vs.* 81%), with fewer peritoneal metastases (15% *vs.* 59%) and less microsatellite instability (6% *vs.* 30%). Prognosis is better with a median survival of 61 months *vs.* 11 months for the *B-RAF* ^{V600E} mutations and 43 months for patients with wild *B-RAF* (*B-RAFwt)* [4]. There does not seem to be any difference in prognosis between the different *B-RAF* $^{non\text{-}V600E}$ mutations[5].

The goal of this work was to outline the therapeutic implications of *B-RAF* mutations in colo-rectal cancer based on a review of the literature.

EGFR receptor and the MAPK pathway

The activation of the *EGFR* (Epidermal Growth Factor Receptor) receptor triggers two main, distinct but interconnected, signalization pathways that play an important role in angiogenesis, cell transcription and proliferation: these include the B-RAF / MAP-kinase (mitogen-activated protein kinase or MAPK) and PI3K / ATK (phospho-inositidyl-3-kinase/ B protein kinase) pathways (**Figure 1**).

After binding to its ligand and dimerization of the EGFR receptor, the RAS protein initiates the phosphorylation cascade of the MAPK pathway. RAS activation recruits the three RAF (A-RAF, B-RAF, and C-RAF) proteins at the level of the plasmatic membrane and then activates them.

The RAF proteins possess a Ras-Binding Domaine (RBD), which, when it interacts with the RAS protein, spurs the formation of RAF dimers. The RAF proteins form hetero-dimers; the association B-RAF/C-RAF is the most frequently encountered [8,9].

These newly formed hetero-dimers become active kinases that promote the phosphorylation of MEK (MAPK-ERK-Kinase), which in turn provokes the phosphorylation and activation of the Extracellular signal-Regulated Kinase (ERK). The activated ERK then allows the expression of early coding genes for the transcription factors (c-FOS, c-MYN, c-JUN) leading to the expression of several of the cell proliferation genes.

ERK exercises a negative retro-control and once activated, leads to the phosphorylation of several of the components of the MAPK pathway, including the RAF protein, resulting in the cleaving of the hetero-dimer. This retro-control is indispensable to attenuate the signal and its suppression constitutes an escape mechanism against B-RAF inhibitors that are used in monotherapy [10].

The consequences of EGFR, RAS or RAF mutations are the long-lasting activation of the RAS/MAPK pathway, which is not influenced by anti-EGFR because the sequence takes place downstream from the receptor.

When the mutation occurs in the coding gene for the EGFR receptor or RAS protein, the B-*RAFwt* proteins form most of the B-RAF/C-RAF hetero-dimers. When the mutation is located in B-RAF, B-RAF/B-RAF dimers are generated (in the case of *BRAF* $^{non\text{-}V600E}$ mutation), or the B-RAF monomer takes on an active kinase configuration without the need for dimerization (in the case of *BRAF* ^{V600E} mutation).

Prognostic value of *B*-RAF mutations

a) Non-metastatic CRC (stage II/III):

A *B-RAF* mutation is a negative prognostic factor for overall survival (OS) in stage II and III CRC, independent of age, sex, tumor location, *K-RAS* status, tumor differentiation or the T or N stage [11].

The frequent association (20% of cases) between the micro-satellite instability (MSI) phenotype (favorable prognostic factor) and the *B-RAFmt* status is recognized as a biological paradox.

The mismatch repair (MMR) status seems to outweigh the B-RAF status in terms of survival. In 2580 patients with stage III CRC, survival was analyzed according to MMR status [12]. Three hundred and fourteen patients (12%) had the *B-RAF* ^{V600E} mutation. Recurrence-free survival (RFS) was not statistically significantly different between patients with the *B-RAF* ^{V600E} mutation and *B-RAFwt* when they were MSI (HR 1.33, 95% CI [0.87-2.04]) whereas RFS was shorter when they were micro-satellite stable (MSS) (HR 1.51, 95% CI [1.19-1.92]).

A recent *post-hoc* analysis confirmed these results [13]. OS and RFS were shorter in patients who were *B-RAF* ^{V600E} - MSS compared to those who were *B-RAFwt* - MSS (OS: HR 1.84; p = 0.046 and PFS at 3 years: HR 1.74; p = 0.01). In multivariable analysis, patients who were *B-RAF* ^{V600E} - MSI had a longer RFS compared to pa-

tients without the mutation, but there was no statistically significant difference in OS (OS: HR 0.19, p = 0.08 and PFS: HR 0.23, p = 0.04).

As *B-RAF* status is rarely determined in localized CRC, there are no specific recommendations in terms of adjuvant therapy or surveillance in contrast to patients with CRC who do not have the *B-RAF* mutation. The efficacy of adjuvant FOLFOX in stage II / III CRC is not influenced by *B-RAF* status[14,15].

Finally, the MMR status influences the prognosis in patients with localized CRC and the MSI status « protects » patients with the *B-RAF* mutation. Moreover, adjuvant chemotherapy does not seem to be less effective in patients with mutant *B-RAF*.

b) Metastatic CRC (stage IV):

Several studies have suggested that the *B-RAF* ^{V600E} mutation is a negative prognostic factor.

A 2014 meta-analysis that included four prospective randomized trials (CAIRO, CAIRO2, COIN, FOCUS) [16] for a total of 3063 patients showed that patients with mutated *B-RAF* ^{V600E} had shorter OS and PFS compared to patients who were carriers of *B-RAFwt* (OS: 11.4 *vs.* 17.2 months, p < 0.001; PFS: 6.2 *vs.* 7.7 months, p < 0.001). In contrast to non-metastatic CRC, OS and PFS were shorter in patients with MSI compared to patients with MSS (PFS: 6.2 *vs.* 7.6 months; OS: 13.6 *vs.* 16.8 months). Moreover, the MMR status did not influence survival in the same *B-RAF* group. Effectively, there was no statistically significant difference between the patients who were *B-RAFwt* or *B-RAF* ^{V600E} with respect to their MSI or MSS status.

One recent study looked at the influence of *B-RAF* status on survival according to the number of lines of chemotherapy [17]. Patients with *B-RAF*-mutant CRC who were administered first-line chemotherapy had poorer OS than patients with *B-RAFwt* while PFS and control of disease did not differ. The *B-RAF* status did not influence PFS or the response rate after second-line chemotherapy.

The largest retrospective cohort of patients with *B-RAF* CRCm tumors included 287 patients [18]. Among these patients, two thirds had synchronous metastases; more than half (52%) had liver metastases and 37% had peritoneal involvement. Median

OS was 20.8 months, and the factors associated with better survival were the metachronous character of metastases and curative resection of metastases as well as of the primary. The median OS of patients undergoing surgery for their metastases (essentially liver) was 47.4 months *versus* 19.5 months for patients who did not undergo surgery. The PFS was 4.3 months and the factors associated with better PFS were a low number of metastatic sites and curative surgery for metastases and primary tumor.

In summary, in patients with CRCm and mutant *B-RAF*, MSI status does not confer any better prognosis compared to patients who are MSS. OS is shorter in metastatic patients with mutant *B-RAF* treated with first-line chemotherapy. Conversely, PFS does not seem to be influenced by *B-RAF* status after second-line treatment. OS of patients with *B-RAF*-mutant CRCm undergoing curative surgery for metastases can be as long as 47 months.

The main studies are found in **Table 1**.

Influence of *B-RAF* mutations on the response to medical treatment

a) Need for intensification chemotherapy?

In 2015, a phase III study compared two treatment regimens in 508 patients of which 28 had mutant *B-RAF* CRCm: 16 patients were treated with FOLFIRINOX + Bevacizumab while 12 patients were administered FOLFIRI + Bevacizumab [(6)]. Increased OS and RFS was observed in the FOLFIRINOX + Bevacizumab arm, although the difference did not attain statistical significance (19 *vs.* 10.7 months and 7.5 *vs.* 5.5 months, respectively). This underscores that intensification chemotherapy such as FOLFIRINOX + Bevacizumab in patients with mutant *B-RAF* tumors warrants consideration.

However, these results were called into question during the 2020 ASCO (American Society of Clinical Oncology) meeting with the publication of a meta-analysis of five trials (TRIBE, OLIVIA, CHARTA, STEAM, TRIBE2) [(7)] that compared first-line bi-chemotherapy + Bevacizumab to tri-chemotherapy with FOLFIRINOX + Bevacizumab for patients with non-resectable CRC metastases. In all, 1697 patients were

included, 851 in the bi-chemotherapy (FOLFOX + Bevacizumab) group and 846 in the tri-chemotherapy (FOLFIRINOX + Bevacizumab) group. Analysis of OS and PFS showed that tri-chemotherapy was superior. Notwithstanding, subgroup analysis, and in particular that of 115 patients with mutant *B-RAF* showed contradictory outcomes. Effectively, after 40 months of follow-up, 43 of 54 bi-chemotherapy patients (79.6%) had died compared to 53 of 61 tri-chemotherapy patients (86.9%) (HR = 1.119; 95% CI (0.75 – 1.73). The authors concluded that FOLFIRINOX + Bevacizumab should not be proposed as first-line therapy for patients with mutant *B-RAF*; bi-chemotherapy with FOLFOX + Bevacizumab seems preferable in this specific population.

b) Anti-EGFR:

Cetuximab (ERBITUX®) [19] and Panitumumab (VECTIBIX®) [20] were granted marketing authorization as first-line treatment of patients harboring CRCm and wild-type *RAS* status, in 2004 and 2007, respectively.

These drugs are monoclonal antibodies that target the EGFR receptor (Figure 1) and for which the affinity is 5 to 10 times greater than that of endogenic ligands [21]. They are contra-indicated in patients with mutant *RAS* because, in this situation, the signal cascade is activated downstream from the receptor, rendering the treatment ineffective. The question of resistance of *B-RAFmt* tumors to anti-EGFR treatment is still under debate.

A 2014 meta-analysis including 463 patients with mutant *B-RAF* CRCm was unable to demonstrate an increased response rate or better OS or PFS when an anti-EGFR was added as compared to chemotherapy alone[22]. In 2017, another study on 592 patients with CRCm, 48 of whom displayed a mutant *B-RAF* profile, compared two treatment regimens: 23 patients received FOLFIRI – Cetuximab and 25 received FOLFIRI – Bevacizumab [23]. OS and PFS did not differ statistically significantly between the two groups, thereby confirming that anti-EGFR was not superior to anti-VEGF. However, early tumor shrinkage (ETS), defined as at least a 20% reduction in tumor size when re-imaging was compared to initial imaging, was assessed in 38 patients; 53% of patients receiving Cetuximab *vs.* 33% receiving Bevacizumab. Patients who attained the 20% ETS threshold showed longer OS and PFS rates compared to those who did not (OS: 29.8 *vs.* 5.9 months; PFS: 9 *vs.* 1.9 months). More-

over, resection of liver metastases (LM) was possible in two patients in the Cetuximab group (8.7%) compared to none in the Bevacizumab group.

A 2019 phase II study evaluated the value of adding first-line anti-EGFR treatment in 96 patients with wild-type *RAS* CCRm, 16 of whom were also *B-RAF*-mutant (7 patients treated with FOLFIRINOX-Panitumumab (6 B-*RAF* ^{V600E}, 1 *B-RAF* $^{non\text{-}V600E}$) and 9 treated by FOLFIRINOX alone (8 B-RAF ^{V600E}, 1 B-RAF $^{non\text{-}V600E}$)). The objective response rate was 71% in the FOLFIRINOX-Panitumumab group *vs.* 22% in the FOLFIRINOX alone group, but without any statistically significant increase in OS or PFS [24]. Thus, the debate with respect to the efficacy of anti-EGFR in patients with mutant B-RAF is not over. In spite of these limitations, certain authors still consider using anti-EGFR in this setting [25].

c) Anti-VEGF:

Bevacizumab (AVASTIN®) is the most widely used anti-angiogenic treatment. After binding to circulating VEGF, it blocks the VEGF-to-receptor binding and thus inhibits tumor angiogenesis and growth [26] (**Figure 1**).

A phase III study published in 2011, including 471 patients with CRCm, 33 of whom had mutant *B-RAF,* reported an increased OS and PFS in patients treated by chemotherapy + Bevacizumab compared to patients treated with chemotherapy alone (9.2 *vs.* 6.3 months p = 0.34, and 5.5 *vs.* 2.5 months p = 0.71, respectively) [27].

A recent meta-analysis including 129 patients with *B-RAF*-mutant CRCm found an increased OS when anti-VEGF was associated with chemotherapy in second-line treatment (HR 0.5, 95% CI [0.29 – 0.85]) [28].

A phase II study published in 2010 evaluated first-line FOLFIRINOX + Bevacizumab in 57 patients with CRCm, of whom 10 had mutant *B-RAF* [29]. This intensified chemotherapy led to disease control (complete or partial response) in all patients. Median PFS for patients with the *B-RAF*-mutant was 12.8 months, not statistically significantly different from the group without mutated *B-RAF* (13.1 months). Two patients had liver surgery.

Thus, anti-VEGF in association with oxaliplatin-based chemotherapy seems justified in patients with the *B-RAF* mutation [30].

A clinical trial is currently underway comparing FOLFIRINOX + Cetuximab to FOLFIRINOX + Bevacizumab, as first-line treatment in patients with the *B-RAF* mutation (AIO-KRK-0116, NCT04034459) [31].

The outcomes of the main studies on this topic are summarized in **Table 2**.

New therapies and perspectives

1/ B-RAF inhibitors (B-RAFi)

Vemurafenib (ZELBORAF®) [32] and Dabrafenib (TAFINLAR®) [33] were the first B-RAFi to be granted marketing approval for the treatment of metastatic *B-RAFmt* melanoma; 90% of patients had tumor regression. The idea of specifically blocking the B-RAF kinase protein in patients with *B-RAF* mutated CRC seems enticing but all the phase I monotherapy studies evaluating the inhibition of B-RAF failed, either because of resistance due to paradoxical activation or over-expression of EGFR [34, 35], or by interconnections between various signal pathways (in particular with PI3K) [36].

Afterwards, progressively came the idea of developing a triple inhibition of the MAPK pathway using the association of an anti-EGFR, a B-RAFi and MEK inhibitor (MEKi). The BEACON study published in 2019 evaluated this strategy in 665 patients presenting a *B-RAF*-mutant CRCm [37], randomized into three groups: a) Encorafenib (B-RAFi) + Binimetinib (MEKi) + Cetuximab (anti-EGFR); b) Encorafenib + Cetuximab ; and c) the control group (Cetuximab + Irinotecan, or Cetuximab + FOLFIRI).

OS and PFS in the three-drug regimen (9 months and 4.3 months, respectively) and two-drug regimen (8.4 months and 4.2 months, respectively) groups were statistically significantly superior to these survival indexes in the control group (5.4 months and 1.5 months, respectively). The objective response rate was superior in the three-drug and two-drug groups compared to the control group (26% and 20% vs. 2%, respectively $p < 0.001$).

Moreover, tolerance was not statistically significantly different between the groups: 58% of patients in the three-drug group sustained a stage ≥ 3 adverse event according to the Common Terminology Criteria for Adverse Events (CTCAE) classification

[38] compared to 50% and 61% in the two-drug and control groups, respectively. Adverse events were essentially digestive symptoms (diarrhea, nausea, vomiting) or dermatological (acneiform dermatitis). In the three-drug group, central serous retinopathies or left ventricular dysfunctions, specific complications of iMEK, were observed. Seven percent of patients in the three-drug group had to discontinue their treatment because of adverse events compared to 8% in the two-drug group and 11% in the control groups.

The study was not designed to compare the two experimental (double and triple inhibition) groups. Moreover, this was an intermediary study, which limits the strength of the conclusions. Nonetheless, the analysis of OS (HR 0.79; 95% CI: 0.59-1.06) and the response rate was in favor of the three-drug regimen. An update of this study was reported at the 2020 ASCO meeting with six additional months of follow-up (39). Median OS was 9.3 months in both the three-drug (n = 224) and two-drug groups (n = 220) groups (95% CI (0.74-1.21). For certain patients, in particular those with more than three metastatic sites, the three-drug regimen was more effective than the two-drug regimen with respect to OS (8.5 *vs.* 6.7 months, HR: 0.69 95% CI (0.49-0.96)). The authors concluded that Encorafenib associated with Cetuximab with or without Binimetinib improved OS and the objective response rate compared to standard 2nd line chemotherapy in patients with *B-RAF* mutant CRCm. Thus, in December 2020, Encorafenib (BRAFTOVI®) associated with Cetuximab obtained the marketing approval for 2nd line (or later) chemotherapy for CRCm with BRAF V600E mutation that progressed after systemic therapy. The combined therapy with Encorafenib + Cetuximab led to an increased OS and PFS (3 and 2.7 months respectively). No benefit in terms of quality of life has yet been shown. The triple association Encorafenib + Cetuximab + Binimetinib has not received marketing approval by the transparency commission of the French High Health Authority because its efficacy is similar to the two-drug regimen and has higher toxicity.

A clinical trial is currently underway to evaluate this three-drug therapy (Encorafenib, Binimetinib, Cetuximab) as first-line treatment of patients with mutant *B-RAF*^{V600E} CRCm (ANCHOR trial, NCT03693170) [40].

2/ Immunotherapy

Immunotherapy is based on monoclonal antibodies against the checkpoint inhibitors of the immune system, capable of reversing tumor-induced immunosuppression. Microsatellite instability high (MSI-H) is an important predictive factor of the efficacy of immunotherapy in CRCm. In a 2018 phase II study, 119 patients with CRCm received nivolumab and ipilimumab once every three weeks for four doses and then nivolumab once every two weeks, until disease progression or discontinuation because of toxicity, death or withdrawal of informed consent[41]. The objective response rate was 54.6%, of which 3.4% were complete and 51.3% were partial. The response was durable, lasting more than six months in 83% of patients. Median OS and PFS had not been attained at the date of publication. The OS and PFS at one year were 85% and 71%, respectively. Of the 29 patients with mutant B-RAF, 16 (55%) had an objective response, and control of disease for more than 12 weeks was obtained in 23 (79%) patients.

The results of the phase III Keynote 177 trial (NCT02563002) [42] were presented at the 2020 ASCO meeting validating the place of first-line Pembrolizumab in the treatment of CRCm with MSI. Two groups were compared: one group had Pembrolizumab immunotherapy, the other chemotherapy (FOLFOX6 or FOLFIRI ± Bevacizumab or Cetuximab). PFS was longer in the immunotherapy groups at 12 and 24 months (55 *vs.* 37% and 48 *vs.* 19%, respectively). The objective response rate was higher in the immunotherapy group (44%, of which 11% were complete *vs.* 33% of which 4% were complete in the chemotherapy group). Immunotherapy was better tolerated than chemotherapy, with fewer severe adverse events (22 *vs.* 66% stages ≥ 3 adverse events according to the CTCAE classification).

Of the 307 patients included in the study, 77 had the *B-RAF*^{V600E} mutant*;* 34 received Pembrolizumab while 43 underwent chemotherapy. PFS was longer in the Pembrolizumab group compared to the chemotherapy group (HR 0.48; 95% CI [0.27 – 0.86]).

Immunotherapy is therefore the first choice of treatment of patients with MSI CRCm, irrespective of *B-RAF* status.

3/ Future therapeutic modalities

Other treatments are currently under development that block different RAF proteins and not only *B-RAF,* in order to counter the escape phenomena due to C-RAF activation (LYS3009120, TAK-580, CCT196969, CCT241161).

Another approach consists of blocking the formation of hetero-dimers in the presence of activated *RAS* (PLX7904) [43].

Finally, the CXCR4 receptor could be a potentially interesting therapeutic target [44]. This receptor is found on the surface of tumor cells and regulates the MAPK pathway, driving phosphorylation of ERK and an increased phosphorylation of the EGFR receptor after activation.

Surgical management of liver metastases in patients with mutant *B-RAF*

Although several series have dealt with this question, they included only a limited number of patients having the *B-RAF* ^{V600E} mutation, fewer than 10 for most of them[45-52].

In a recent Japanese retrospective study [53], 31 of 33 patients with *B-RAF* ^{V600E} mutation undergoing surgery for LM developed local or systemic recurrence with a median RFS of 5.3 months, similar to that of patients with non-resectable LM treated with FOLFIRINOX – Bevacizumab [6].

A 2015 study evaluated the survival of 309 patients with CRCm undergoing surgery for LM, 12 of whom were mutant *B-RAF* [54]. Median OS and RFS were shorter in patients with *B-RAFmt* (22.6 months and 5.7 months, respectively) in comparison to patients who were *B-RAFwt* (63.3 months and 14.4 months, respectively). Thus, certain authors have questioned the usefulness of hepatic surgery in patients with mutated B-RAF considering that hepatic metastasectomy did not provide better results than chemotherapy alone.

However, two recent French studies reported interesting data in patients with the *B-RAF* mutation, undergoing surgery for LM.

The first, published by Bachet *et al.* in 2019 [55], is the largest study of patients with mutated *B-RAF* undergoing surgery for LM. This case-control study included 66 patients with mutant *B-RAF* who had CRC LM matched to 183 patients with LM without mutant *B-RAF*. The patients were matched according to initial resectability status (resectable *vs.* non-resectable), the synchronous or metachronous appearance of

LM, their unilobar or bilobar distribution, and the number of LM (< 4 *versus* ≥ 4). Nearly 80% of patients had synchronous LM, confirming once again the metastatic propensity of CRC with mutant *B-RAF.*

Most of the patients had received pre-operative FOLFOX. The histologic response to neo-adjuvant treatment was “major” in 19 patients, of whom six were complete in the *B-RAFmt* group, similar to the outcome in the matched *B-RAFwt* patients. Therefore, the *B-RAFmt* status did not influence the objective response to pre-operative chemotherapy. Likewise, the objective imaging response rate was 65% in the *B-RAFmt* group, similar to the rate in patients who were *B-RAFwt.*

There was no statistically significant difference found in terms of R1 resection rates between the patients who were *B-RAFmt* or not.

As for oncologic outcomes, there was no statistically significant difference found in RFS between the *B-RAFmt* and *B-RAFwt* groups (median RFS 10 *versus* 13 months in *B-RAFmt* and *B-RAFwt*, respectively). Notwithstanding, OS was shorter in patients with *B-RAFmt*, with a median of 52.7 months while the median was not attained for *B-RAFwt* patients at the time of publication. In this study, 158 patients developed recurrence, 44 with *B-RAFmt* compared to 114 with *B-RAFwt*. These recurrences were more often multi-site (21/44 *vs*. 34/114 in the *B-RAFmt* and *B-RAFwt* groups, respectively p = 0.034), and OS after recurrence was lower in patients with *B-RAFmt* in comparison with patients with *B-RAFwt* (median OS after recurrence: 23 months *vs.* 44.3 months in the *B-RAFmt* and *B-RAFwt* groups, respectively; p = 0.05).

The authors concluded that surgery for LM was not contraindicated in patients with the *B-RAF* mutation. OS was inferior mainly because of poor OS after recurrence, undoubtedly because the recurrence occurred in several sites. In case of recurrence in patients with the *B-RAF* mutation, a new operation was less often possible compared to patients without the mutation (12/44 *vs.* 48/144 in the *B-RAFmt* and *B-RAFwt* groups, respectively, p = 0.085).

The second study was published by *Gagnière et al.* in 2020 [56]. This data-base analysis compared survival and prognostic variables in 35 patients with CRCm and *B-RAF* mutation to those of 1497 patients with CRCm but without mutant *B-RAF*. The metastases in the *B-RAFmt* group were more often synchronous, smaller, multiple

and required more often a major hepatectomy compared to metastases in patients who were *B-RAFwt*. Median OS and RFS were statistically significantly lower in patients who were *B-RAFmt* compared to those who were *B-RAFwt* (40 *versus* 81 months, $p < 0.001$ and 10 *versus* 22 months, $p < 0.001$, respectively). The factors associated with poorer OS were lymph node positive primary tumors, CEA > 200 µg/L and a « clinical risk score » ≥ 4 (Score composed of five variables: lymph node involvement, synchronous metastases, > 1 metastasis, size of largest metastasis > 5 cm, CEA > 200 µg/L). Recurrence occurred in 91% of patients with mutant *B-RAF* compared to 54% in patients without the mutation ($p < 0.001$). For the authors, the selection of the best candidates for LM surgery in patients with CRCm and mutant *B-RAF* should be based on three criteria for good prognosis (absence of lymph node involvement in the primitive tumor, low CEA and clinical risk score < 4).

A 2018 cohort study [57] assessed the impact of *B-RAF* and *K-RAS* mutations on survival in 849 patients undergoing surgery for LM, 43 of whom had *B-RAF* mutation (33 with a *B-RAF V600E* mutation). The authors concluded that the impact of a *B-RAF V600E* mutation was more important than *K-RAS* mutation with respect to OS and RFS.

A study on the influence *B-RAF* status on the possibility of secondary resections of LM initially deemed to be unresectable after neo-adjuvant treatment was recently published [58]. Multivariable analysis found that the presence of lung metastases (OR 0.35, 95% CI 0.19–0.63; $p<0.001$), *B-RAF* mutation (OR 0.33, 95% CI 0.12–0.82; $p = 0.026$) and alkaline phosphatase > 300 U/L (OR 0.42, 95% CI 0.18–0.9; $p = 0.033$) were independent risk factors for not being able to perform a secondary resection. Similar results were reported in patients with *B-RAF* mutation in a series of 74 patients with liver and extra-hepatic metastases, six of whom had the *B-RAF ^{V600E}* mutation [59]. Eleven patients (15%) were able to undergo secondary R0 resection of their metastases but none of these patients were *B-RAFmt*.

In spite of the small samples in these series, related to the rarity of *B-RAF* mutation, it seems that patients with CRCm and *B-RAF* mutation can be candidates for LM surgery. Of note, RFS was identical for patients with or without *B-RAF* mutation [55]. Nonetheless, recurrence after surgery seems to occur more frequently and more aggressively in patients who are *B-RAFmt*. For the moment, none of the available stud-

ies showed that the mutational status alone is a good selection criterium for LM surgery and the presence of *B-RAFmt* does not contra-indicate LM surgery. When possible, surgery remains a viable option for patients with mutant *B-RAF* according to the same technical modalities as for patients without the mutation. FOLFOX neo-adjuvant chemotherapy acts similarly on tumor regression in patients with or without *B-RAF* mutation. Performing formal anatomic liver resections has not modified the rate of R1 resections in patients with a *B-RAF* mutation. Contrary to LM with *K-RAS* mutation for which formal anatomic resections are associated with a three-fold increase in RFS compared to non-anatomic resections [(60)], there are not enough data to recommend anatomical resections in patients with the *B-RAF* mutation. Taking into account the high incidence of peritoneal localizations, a pre-operative CT scan to eliminate peritoneal or distant metastases should be included in the assessment of operability for patients with *B-RAF* mutation potentially candidates for liver surgery. During surgical abdominal exploration, the search for peritoneal carcinomatosis must be meticulous and exploratory laparoscopy, whenever possible, should be proposed before liver resection. As for surveillance after liver surgery for metastases in patients with the *B-RAF* mutation, there is no consensus and the multi-site recurrence pattern often observed complicates any surveillance policy of a specific organ.

Figure 2 synthesizes the management of patients with CRC *B-RAF* mutation in view of the available data.

Conclusion

The prognosis of CRC with the *B-RAF* mutation is poor. The value of first-line intensification for CRCm with FOLFIRINOX-Bevacizumab is currently under debate and it seems that FOLFOX associated with an anti-VEGF (bichemotherapy) is preferable. The efficacy of first-line anti-EGFR is uncertain for CRCm with *B-RAF* mutation whereas it has been demonstrated for anti-VEGF.

First-line anti-checkpoint immunotherapies have been shown to be effective in patients who are CRCm MSI-H independently of their *B-RAF* status.

B-RAF inhibitors, although poorly effective when used in monotherapy, have obtained marketing approval for use in association with Cetuximab as second-line treatment of patients with CRCm and the *B-RAF V600E* mutation.

Finally, surgery for liver metastases in patients with the *B-RAF* mutation remains a viable option even though recurrence after surgery occurs more frequently and is more severe.

Essential points

- *B-RAF* mutation is a recognized poor prognostic factor for patients with CRC, in particular when CRC is metastatic.
- First-line bi-chemotherapy associating FOLFOX + Bevacizumab seems better than triple therapy FOLFIRINOX + Bevacizumab, which has recently been called into question.
- *B-RAF* inhibitors are not useful in monotherapy regimens. The French High Health Authority granted marketing authorization for the double association Encorafenib + Cetuximab in the second-line treatment of metastatic CRC but did not approve the triple association (Encorafenib + Cetuximab + Binimetinib) as a possible alternative because of more severe side-effects. First-line triple inhibition (EGFR, B-RAF, MEK) is currently under evaluation for metastatic CRC.
- Immunotherapy is effective in patients with the MSI phenotype, including patients who are mutant *B-RAF*.

- Surgery for liver metastases in patients with mutant *B-RAF* should be considered after thorough search for peritoneal or distant metastases, even if it is recognized that tumor recurrence occurs more often and prognosis is more severe in this setting.

Legends to Figures:

Figure 1: Signaling MAPK and PI3K pathways after EGFR receptor activation. The main therapeutic targets are marked in red
MAPK : Mitogen-activated protein kinase ; PI3K : Phosphoinositidyl-3-kinase ; EGFR : Epidermal Growth Factor Receptor

Figure 2 : Algorithm for management of mutant *BRAF* Colorectal cancer
CRC: colo-rectal cancer

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<u>Table 1</u>: Summary of main studies95% CIted

Author	B-RAF status for primary or metastasis	Type of study	Patients B-RAFmt / total	Metastatic (B-RAFmt, n)	Resection of LM, n	OS B-RAFmt *vs.* B-RAFwt (months)	PFS B-RAFmt *vs.* B-RAFwt (months)
Vender-bosch 2016 (16)	ND	Meta-analysis of 4 trials (CAIRO, CAIRO2, COIN, FOCUS)	**250** / 3063	NA	NA	**11.4** *vs.* 17.2 ; p < 0.001	**6.2** *vs.* 7.7 ; p < 0.001
Seligmann 2017 (17)	ND	Meta-analysis of 3 trials (COIN, FOCUS, PICCOLO)	**231** / 2299	Liver (159) Lungs (60) Peritoneum (58)	NA	1st line: **10.8** *vs.* 16.4 ; p < 0.001 2nd line: **6.7** *vs.* 10.2 ; NS	1st line: COIN: **5.7** *vs.* 6.3; NS FOCUS: **8.2** *vs.* 8.8; NS 2nd line: **3.5** *vs.* 4; NS
De La Fourchardière 2019 (18)	Primitive	Retrospective cohort	**287** / 287	Liver (149) Peritoneum (107) lymph nodes (89) lungs (74)	**41**	**20.8**	**4.3**

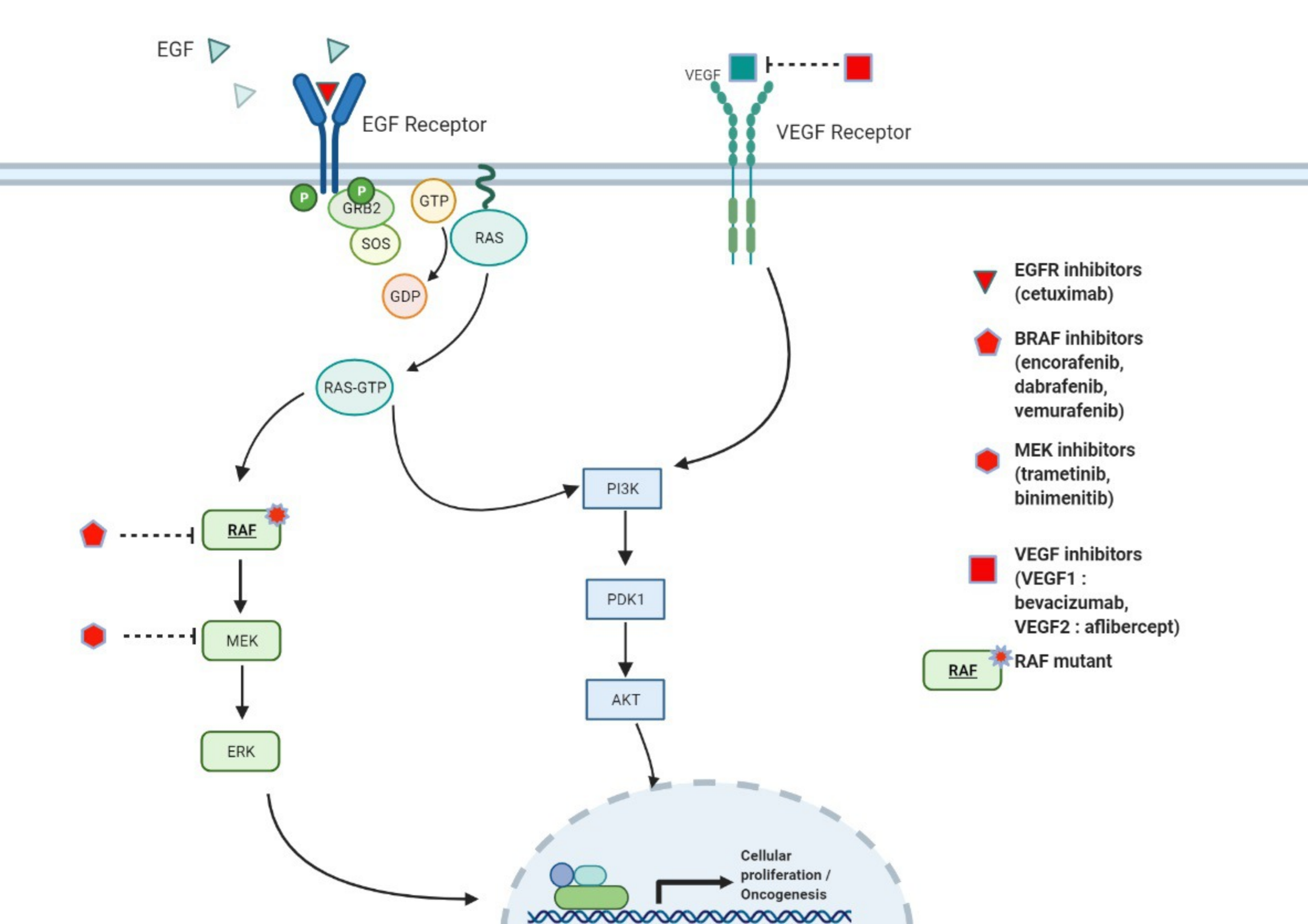
NA Not available; NS: Not statistically significant; LM: Liver metastases; OS: Overall survival; PFS: Progression-free survival; B-RAFmt : Mutant *B-RAF* ; B-RAFwt: no mutant (wild type) *B-RAF*

Table 2: Summary of main studies cited

Author	Type of study	B-RAF status of primitive (P) or metastasis (M)	Patients B-RAFmt / total	Metastatic site, n	Secondary resection of metastases	OS (months)	PFS (months)
Cremolini 2015 (6)	Phase III Folfirinox + Avastin vs Folfiri + Avastin Non resectable CRCm	P or M	**28** / 508	NA	NA	19 (Folfirinox) *vs.* 10.7 (Folfiri)	7.5 (Folfirinox) *vs.* 5.5 (Folfiri)
Cremolini 2020 (7)	Meta-analysis Bi-C + Avastin vs tri-C + Avastin Non resectable CRCm	P or M	**105** / 1697	NA	NA	NS	NA
Pietrantonio 2015 (22)	Meta-analysis C + anti-EGFR *vs.* C alone	NA	463 / 463	ND	ND	**NS**	**NS**
Stintzing 2017 (23)	Post-Hoc analysis (Folfiri + CET *vs.* Folfiri + BEV)	P	**48** / 400	Liver 32 ≥ 2 sites, 30	2 (CET group)	**12.3 (**CET) *vs.* 13.7 (BEV); NS	**6.6 (**CET) *vs.* 6.6 (BEV); NS
Modest 2019 (24)	Phase II Folfirinox + Panitumumab *vs.* Folfirinox alone	ND	**16** / 96	ND	ND	NS	NS
Price 2011 (27)	Phase III C + BEV *vs.* C alone	ND	**33** / 471	Liver, 20 lungs, 7 Peritoneum, 5	1	**9.2** (BEV) vs 6.3 (C alone); NS	**5.5** (BEV) vs 2.5 (C alone); NS
Masi 2010	Phase II Folfi-	P or M	**10** / 57	ND	2 (LM)	**23.8**	**12.8**

(30)	rinox + Avastin non-resectable CRCm						

LM: liver metastases, BEV: Bevacizumab, CET: Cetuximab, NA: Not available, NS: Not statistically significant, OS: Overall survival, PFS: Progression-free survival, C: Chemotherapy, B-RAFmt: Mutant *B-RAF*


EGF
EGF Receptor
VEGF
VEGF Receptor
P
P
GRB2
SOS
GTP
RAS
GDP
RAS-GTP
RAF
MEK
ERK
PI3K
PDK1
AKT
Cellular proliferation / Oncogenesis
EGFR inhibitors (cetuximab)
BRAF inhibitors (encorafenib, dabrafenib, vemurafenib)
MEK inhibitors (trametinib, binimenitib)
VEGF inhibitors (VEGF1 : bevacizumab, VEGF2 : aflibercept)
RAF
RAF mutant

Non-metastatic Colorectal Cancer

Standard treatment of Colorectal Cancer

Metastatic Colorectal Cancer

Unresectable

Resectable

Mismatch Repair (MMR) Status

dMMR

pMMR

Immune Checkpoint Inhibitors : PEMBROLIZUMAB

Chemotherapy : FOLFOX – AVASTIN (FOLFIRINOX – AVASTIN)

Secondary Resectable

Surgery of metastasis

Progression

Dual inhibition : ENCORAFENIB – CETUXIMAB