Expert Review of Molecular Diagnostics

ISSN: 1473-7159 (Print) 1744-8352 (Online) Journal homepage: http://www.tandfonline.com/loi/iero20

BRAF-mutant colorectal cancer, a different breed evolving

E Lai, A Pretta, V Impera, S Mariani, R Giampieri, L Casula, V Pusceddu, P Coni, D Fanni, M Puzzoni, L Demurtas, P Ziranu, G Faa & M Scartozzi

To cite this article: E Lai, A Pretta, V Impera, S Mariani, R Giampieri, L Casula, V Pusceddu, P Coni, D Fanni, M Puzzoni, L Demurtas, P Ziranu, G Faa & M Scartozzi (2018): BRAF-mutant colorectal cancer, a different breed evolving, Expert Review of Molecular Diagnostics, DOI: 10.1080/14737159.2018.1470928

To link to this article: https://doi.org/10.1080/14737159.2018.1470928

Accepted author version posted online: 30 Apr 2018.

Publisher: Taylor & Francis

Journal: *Expert Review of Molecular Diagnostics*

DOI: 10.1080/14737159.2018.1470928

Review

Title: BRAF-mutant colorectal cancer, a different breed evolving

Authors: Lai E[1,2], Pretta A[1,2], Impera V[1,2], Mariani S[2], Giampieri R[3], Casula L[2], Pusceddu V[2], Coni P[4], Fanni D[4], Puzzoni M[2], Demurtas L[2], Ziranu P[2], Faa G[4], Scartozzi M[2]

Affiliations:

[1] Medical Oncology, Sapienza-University of Rome, Italy;

[2] Medical Oncology Unit, University Hospital and University of Cagliari, Italy;

[3] Medical Oncology Unit, University Hospital and Università Politecnica delle Marche, Ancona, Italy;

[4] Department of Surgical Sciences, Division of Pathology, University of Cagliari, Italy

Abstract:

Introduction: BRAF mutant colorectal cancer (BRAF MT CRC) is a unique category of colorectal tumour with peculiar molecular, pathological and clinical features and poor prognosis; despite recent research, BRAF mutation predictive value and standard treatment of BRAF MT CRC still have to be defined. In this review, we focused on this challenging topic.

Areas Covered: The potential use of BRAF mutational status among recent additional prognostic and predictive indicators and current treatment strategy in use in these patients is discussed. Moreover, implications and characteristics of new BRAF mutations other than BRAFV600E are analyzed. An in-deep outlook on the immediate future for clinical and translational research in this subgroup of patients is also presented, such as combination therapy with agents targeting the RAS/RAF/MEK/ERK pathway and standard chemotherapy in order to overcome resistance. We performed a research on Pubmed typing "BRAF mutation", "colorectal cancer", "predictive and prognostic value", "targeted therapy", "BRAF inhibition".

Expert Commentary: BRAFV600E mutation represents a strong, independent negative prognostic factor in II-III stage MSS CRC and mCRC. The best treatment still has to be identified; currently, in good performance status patients, an intensive-chemotherapy-combination remains the standard of care. Further investigations are warranted to explore new horizons to change BRAF MT mCRC outcomes.

Keywords: BRAF, Colorectal cancer, Prognostic value, Predictive value, BRAF inhibitors

1. Introduction

The mutational status of the genes involved in the receptor tyrosine kinase (RTK) signal transductional pathways has a crucial role in patients with metastatic colorectal cancer (mCRC). In particular, the V600E mutation in v-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene identifies a subgroup of patients with different clinical, prognostic and predictive features (1).

The signal transduction from RTK (Fig.1), and the two signaling pathways that are downstream to the rat sarcoma (RAS) gene, the mitogen–activated protein kinase (MAPK) pathway and the phosphoinositidyl-3-kinase (PI3K/AKT) pathway, are extremely important in promoting cancer cell growth, regulating cancer cell metabolism and cell survival (2). The serine/threonine kinase BRAF gene, located immediately downstream to the RAS gene in the MAPK signalling, appears to be mutated in about 5-10% (3, 4) of patients with advanced disease and in the 10-15% of patients with stage II-III tumours (5, 6).

These mutations are almost mutually exclusive with RAS ones. The most frequent BRAF mutation is V600E ($BRAF^{V600E}$) and it is defined by single aminoacid substitution at codon 600 of exon 15 in chromosome 7, with replacement of valine for glutamic acid (7-10).

V600 mutations enable BRAF to adopt an active kinase conformation without dimerization, allowing these mutant proteins to act as RAS-independent monomers. Most other oncogenic BRAF proteins, including non-V600 mutants and BRAF altered proteins, need dimerisation for their transforming activity through RAS-independent self-dimerisation or RAS-dependent heterodimerisation with C-RAF.

$BRAF^{V600E}$ mutation is found in 5-10% of colorectal cancer (CRC) and, as shown in several retrospective studies and meta-analysis, it is frequently associated with right-sided CRC, T4 stage, poor differentiation and mucinous histology. The prevalence of this mutation is also higher in elderly female patients (> 70 years) with an Eastern Cooperative Oncology Group Performance Status (ECOG PS) 2.

Loupakis et al. elaborated a nomogram for predicting BRAF status in metastatic CRC (mCRC) based on two retrospective series of patients from two Italian institutions as training-set (TS) and validation-set (VS). In the TS RAS wild type (RAS WT) patients, right-sided primary (odds ratio (OR): 7.80, 95% confidence interval (CI) 3.05–19.92), female gender (OR: 2.90, 95% CI 1.14–7.37) and mucinous histology (OR: 4.95, 95% CI 1.90–12.90) were independent predictors of $BRAF^{V600E}$ mutation. In the nomogram, patients with the highest score (right-sided primary, female and mucinous) had 81% of probability to have $BRAF^{V600E}$ mutant (BRAF MT) CRC. In the VS, the same three features were confirmed as independent predictors of $BRAF^{V600E}$ mutation with high accuracy. Authors concluded that this nomogram showed high sensitivity and specificity in predicting BRAF mutational status (11).

Furthermore, BRAF MT tumours show often microsatellite instability (MSI) and multiple metastatic sites, with higher rate of peritoneal and distant lymphnode metastases and a lower rate of lung localizations (12-17).

$BRAF^{V600E}$ mutation has a widely accepted prognostic value in advanced setting. Poorer overall survival

(OS) was also demonstrated for patients with stage II and III CRC, though this observation seemed limited to microsatellite stable (MSS) tumours. As a consequence, microsatellite (MS) status should also be considered relevant in defining BRAF MT tumours prognosis (17).

As many as 2.2% of the US population diagnosed with CRC has a BRAF non-V600E ($BRAF^{non\text{-}V600E}$) mutation (Table1). This group of patients represents a distinct category with a different outcome profile. Patients with $BRAF^{V600E}$ MT CRC usually show a median OS around 11 months, while patients with $BRAF^{non\text{-}V600E}$ mutations have a longer median OS, approaching 60 months. Notably patients with wild type BRAF (BRAF WT) status have a median OS of 43 months.

In a recent multicenter retrospective cohort study, *Jones et al.* compared multiple clinical features of $BRAF^{non\text{-}V600E}$ MT versus $BRAF^{V600E}$ MT CRC, combining data from next-generation sequencing (NGS) databases. Patients harbouring a non-V600E mutation were significantly younger (median age 58 vs. 68 years respectively). They were less likely female (46% vs 65%) and they had less frequently high-grade tumours (13% vs 64%) or right-sided primary tumours (37% vs 81%). In this patient subset also peritoneal metastases were less frequent (15% vs 59%).

Patients with $BRAF^{non\text{-}V600E}$ mutation were more likely to have concomitant RAS mutations compared to patients with $BRAF^{V600E}$ (26% vs 2%). In this cluster of patients left-sided primary tumours were more common (54% vs 31%) and MSI-high (MSI-H) status was less frequent than in $BRAF^{V600E}$ MT patients (6% vs. 30%). Globally, patients with CRC harbouring a $BRAF^{non\text{-}V600E}$ mutation seemed to have a less aggressive clinical phenotype and they might not require a more aggressive treatment strategy. Currently it is unclear whether colorectal tumours with $BRAF^{non\text{-}V600E}$ mutations share the anti-epidermal growth factor (EGFR) therapy resistant profile observed in $BRAF^{V600E}$ MT CRC. Furthermore, $BRAF^{non\text{-}V600E}$ mutations seem to escape the paradoxical extracellular signal-regulated kinase (ERK) reactivation that occurs with BRAF inhibitors (BRAFi) therapy. Even mutations with impaired kinase activity are able to phosphorylate ERK twofold more than BRAF WT (18). More particularly, as shown by *Yao et al.*, mutations with impaired kinase activity or kinase-dead identify the third class of BRAF MT tumours (class 1: active monomers; class 2: constitutively active dimers). Class 3 BRAF MTs are sensitive to ERK-mediated feedback, have a RAS-dependent signalling activation and lead to increased ERK signalling, thanks to enhancement of CRAF WT activation. The model suggests that coexisting RAS activation maintenance - despite ERK-dependent feedback – is necessary for class 3 mutants to induce signalling dysregulation; indeed, in melanomas with these class 3 BRAF mutations, also RAS mutations or NF1 deletions are found. On the contrary, in class 3 MT lung cancer and CRC, RTK signal is responsible for RAS activation, and this explains susceptibility to RAS activation inhibition through RTK inhibitors (RTKis) (19).

2. Prognostic role of BRAF mutation

The molecular mechanism explaining the striking prognostic impact of BRAF mutational status is poorly understood. It has been investigated both on adjuvant and metastatic setting.

Farina-Sarasqueta et al. showed that, in stage II-III CRC, BRAF seems to be an independent prognostic factor for OS (HR= 0.45, 95% CI 0.25–0.8) and cancer-specific survival [HR = 0.47, 95% CI 0.22–0.99]. In this Dutch study, the presence of a BRAF MT tumour accounts for a significantly higher risk of dying of cancer-related causes, independently of other factors like age, sex, location of the tumour, MSI status, Kirsten RAS oncogene homolog (KRAS) mutational status, differentiation grade, tumour (T) and node (N) stage (20).

MSI is the result of a deficient DNA mismatch repair (dMMR) system, which is caused by epigenetic silencing of the mismatch repair (MMR) gene in sporadic CRC or germline mutations in the MMR genes (Lynch syndrome). Tumours with a dMMR status have distinct features, such as proximal origin in the colon, prominent lymphocytic infiltrate, poorly differentiated morphology, mucinous or signet ring differentiation and association with favourable prognosis in early stage CRC (21).

The increased frequency of BRAF mutations observed in the MSI-H population might seem a biological paradox, considering the good prognostic effect of MSI-H in early stages. Different studies showed in fact that BRAF mutation was a negative prognostic factor in stage II-III MSS CRC (20, 22-24). BRAF mutation was prognostic for OS in patients with MSI-low (MSI-L) and MSS tumours, whereas this was not the case for relapse free survival (22). A recent analysis from the PETACC-8 (Oxaliplatin, Fluorouracil, and Leucovorin With or Without Cetuximab in Patients With Resected Stage III Colon Cancer Randomized Phase III) trial suggested that MMR, BRAF, and KRAS were all relevant for patients prognostic stratification in the adjuvant setting. $BRAF^{V600E}$ and KRAS mutations were associated with shorter disease-free survival (DFS) and OS in patients with MSS CRC but not in those with MSI-H tumours (25).

Among 2686 stage III CRC patients enrolled in the North Central Cancer Treatment Group (NCCTG) N0147 adjuvant study, BRAF mutational status was prognostic for OS and DFS. These differences in survival were particularly clear in MSS CRC, whereas in the MSI-H subgroup (although admittedly smaller) the interaction test was not significant (26).

However, suggestive additional confirmatory studies pooling specimens and data from multiple trials will be required to clarify the impact of BRAF mutation in MSI-H early stage CRC patients.

In mCRC, BRAF mutational status is a strong negative prognostic factor. Indeed BRAF MT mCRC is associated with significantly poorer prognosis, with a median OS ranging from 9 to 14 months independently from the chemotherapy and the biological agent used (27-31).

A pooled analysis of four phase III studies in first-line treatment of mCRC (CAIRO, CAIRO2, COIN and FOCUS) assessed the relationship between dMMR and BRAF status in respect to prevalence and outcomes. Authors showed that dMMR and BRAF mutation prevalence in mCRC patients is low and both biomarkers confer a poor prognosis. In particular, median progression-free survival (PFS) and OS were significantly worse for patients with BRAF MT compared to BRAF WT tumours (PFS: 6.2 versus 7.7 months, respectively, HR 1.34, 95% CI 1.17-1.54, $p<0.001$; OS: 11.4 versus 17.2 months, respectively, HR 1.91, 95% CI 1.66-2.19, $p<0.001$). The median PFS and OS were significantly worse for patients with dMMR

compared to proficient MMR (pMMR) tumours (PFS: 6.2 versus 7.6 months, respectively, HR 1.33, 95% CI 1.12-1.57, p=0.001; OS: 13.6 versus 16.8 months, respectively, HR 1.35, 95% CI 1.13-1.61, p=0.001).

Furthermore, in BRAF WT tumours stratified by MMR status, there was no significant survival difference for patients with MSS compared to MSI-H tumours (PFS: 6.3 versus 7.8 months, respectively, HR 1.32, 95% CI 1.00-1.75, p=0.051; OS: 15.0 versus 17.3 months, respectively, HR 1.22, 95% CI 0.91-1.65, p=0.463). Similarly, no significant survival differences were observed in BRAF MT tumours stratified by MMR status (PFS; 6.1 versus 6.2 months, respectively, HR 0.95, 95% CI 0.62-1.46, p=1.000; OS; 11.7 versus 11.3 months, respectively, HR 1.05, 95% CI 0.68-1.63, p=1.000).

Conversely, in MSS tumours stratified by BRAF status, significantly decreased median PFS and OS were observed for patients with BRAF MT compared to BRAF WT tumours (PFS: 6.2 versus 7.8 months, respectively, HR 1.34, 95% CI 1.10-1.64, p<0.001; OS: 11.3 versus 17.3 months, respectively, HR 1.94, 95% CI 1.57-2.40, p<0.001).

Despite these pooled data set should be interpreted attentively, the Authors suggested that the poor prognostic value of dMMR is driven by the BRAF MT status (32).

The recently published pooled analysis from three randomized trials (FOCUS, PICCOLO and COIN), showed that BRAF MT mCRC patients, treated with chemotherapy alone, had a worse OS; this observation was independent of associated clinical-pathological features. Surprisingly, BRAF MT patients, treated with first-line oxaliplatin/fluorouracile, had a similar disease control rate (DCR) compared to BRAF WT (59.2% versus 72%; adjusted HR = 0.76, P = 0.24) and PFS (5.7 versus 6.3 months; adjusted HR = 1.14, P = 0.26). In second line, there were no significant differences between BRAF MT and WT patients in terms of PFS and response rate (RR) (RR adjusted OR = 0.56, P = 0.45; PFS adjusted HR = 1.01, P = 0.93). These data are limited by the relatively small numbers of BRAF MT patients [FOCUS 61/787 (7.8%), COIN 130/1284 (10.1%) and PICCOLO 40/459 (8.7%)] compared to BRAF WT and therefore findings should be interpreted with caution (33).

The role of BRAF mutation in mCRC patients who were candidate for potentially radical surgery of liver or lung metastases, has been evaluated in fewer studies.

Renaud et al. published a retrospective analysis, conducted in 180 mCRC patients who underwent radical resection of lung metastases. BRAF MT patients (10,6% of total population) had a significantly worse 5-year OS if compared to KRAS mutant (KRAS MT) or KRAS wild type (KRAS WT) tumours (0 % BRAF MT, 44% KRAS MT and 100% WT, respectively; p < 0.0001). Median OS was 15 months, 55 months and 98 months respectively (p < 0.0001) (34).

Yaeger et al. reported data from 1941 consecutive mCRC patients attending the Memorial Sloan-Kettering Cancer Center (MSKCC). At a median follow-up of 25 months, median OS from the time of metastatic disease diagnosis was 20 months for patients with BRAF MT mCRC versus 47 months for those with BRAF WT tumours (P <.001). Complete resection of metastatic disease was possible in 201 patients (23 with BRAF MT and 178 with BRAF WT tumors). Median OS after metastasectomy was found to be significantly

shorter in patients with BRAF MT mCRC with 61% of these patients alive at 2 years (95% CI, 34%–80%) compared to 86% of patients with BRAF WT mCRC (95% CI, 78%–91%) (P =.003) (35).

Finally, a recent meta-analysis of over 11 retrospective or prospective trials, was conducted to assess the outcome of KRAS and BRAF mutational status on 1833 patients receiving radical surgical resection of liver metastases. Only 3 out of 11 studies included patients outcome according to BRAF mutation. These studies confirmed that BRAF mutation was negatively associated with OS (HR, 3.055; 95% CI, 1.794-5.204; P < .001) (36).

Recent advances in mutational testing allowed the identification of new mutations with different clinical-pathological phenotype and prognostic implication. In fact, few reports described mCRC with other BRAF mutations besides $BRAF^{V600E}$ ($BRAF^{non\text{-}V600E}$ mutations), with higher incidence in Asian (5,1%) than in Caucasian patients (1,6-2,2 %). $BRAF^{non\text{-}V600E}$ MT CRC patients are younger compared with $BRAF^{V600E}$ MT CRC patients. Moreover, they are more frequently male and present with left-sided MSS tumours. In addition, $BRAF^{non\text{-}V600E}$ MT tumours are mostly low grade and do not often metastasize to the peritoneum (18, 37-39).

Interesting data from the retrospective analysis conducted by *Jones et a.l,* demonstrated that $BRAF^{non\text{-}V600E}$ MT patients have a far better OS compared with patients with $BRAF^{V600E}$ status (60.7 v 11.4 months, respectively). Surprisingly, in this study OS of $BRAF^{non\text{-}V600E}$ MT CRC patients seems to be even more favourable compared with BRAF WT patients (60.7 v 43.0 months, respectively), but this latter finding needs further confirmation (18). *Cremolini et al.* reported 10 cases with BRAF mutations in codons 594 or 596, showing favourable prognosis from first line treatment (39) .

3. Predictive role of BRAF mutational status in colorectal tumors

While the role of BRAF mutation as a strong negative prognostic factor is well established, its predictive value still remains controversial. It was investigated in the contest of targeted treatments with anti-EGFR monoclonal antibodies (mAbs), in particular with regard to relation with tumour sidedness, anti-Vascular Endothelium Growth Factor (anti-VEGF) mAbs and in chemotherapy.

3.1 Anti-EGFR and role of tumour sidedness

The predictive role of BRAF mutation in determining the clinical benefit from anti-EGFR mAbs was evaluated in several retrospective studies (Table 2). Some of them seemed to suggest that BRAF mutation predicts primary resistance to anti-EGFR mAbs or a less significant benefit compared to that of patients with BRAF WT tumors, but others did not identify this relationship (40-44). *Di Nicolantonio et al.* retrospectively analysed objective tumour responses, time to progression, OS and the mutational status of KRAS and BRAF in 113 patients with mCRC treated with cetuximab or panitumumab and the effect of the $BRAF^{V600E}$ mutation on cetuximab or panitumumab response. $BRAF^{V600E}$ mutation was identified in 11 of 79 (13,9%) of KRAS WT patients; none of them obtained OR, conversely none of the responders carried BRAF mutations

(P= .029). BRAF MT patients had significantly shorter PFS (P= .011) and OS (P < .0001) than BRAF WT patients. According to the Authors, BRAF WT is required for response to panitumumab or cetuximab and could be used to select eligible patients for treatment with anti-EGFR mAbs (31).

The PICCOLO phase III study, in which investigators evaluated addition of panitumumab to irinotecan in KRAS WT fluorouracil-resistant mCRC, showed a detrimental effect of panitumumab on OS (HR 1,84, 95% CI 1,10–3,0) in BRAF MT patients (45). *Loupakis et al.* investigated the role of KRAS codons 61 and 146 and $BRAF^{V600E}$ mutations in predicting resistance to cetuximab plus irinotecan in a cohort of 87 KRAS codons 12 and 13 WT patients. 15% had $BRAF^{V600E}$ mutation; none of them responded to the treatment, compared to 32% of BRAF WT disease patients (P= 0.016). BRAF mutation was associated with unfavorable outcome in terms of both PFS and OS, with a trend towards shorter PFS (median PFS: 2.6 vs 4.4 months in BRAF WT; HR: 0.59 (0.24–1.07), p=0.073) and with significantly shorter OS (median OS: 4.1 vs 13.9 months in BRAF WT; HR 0.51 (0.18–0.95), p=0.037) (46). *De Roock et al*. conducted a retrospective consortium analysis to investigate the relationship between mutations in BRAF, neuroblastoma RAS viral oncogene homolog (NRAS), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), KRAS and treatment response and survival in mCRC patients treated with cetuximab combined with chemotherapy. The Authors collected tumour samples and clinical data on cetuximab-treated mCRC patients. 761 tumour samples were screened for BRAF mutations p.D594G, p.V600E, p.V600M, and p.K601E; 36 patients carried one of these mutations (mostly p.V600E (n535) and one p.D594G). BRAF MT, compared with BRAF WT had a significantly lower RR (8,3% [2/24] vs 38,0% [124/326]; OR 0,15, 95% CI 0,02–0,51; p=0,0012) and DCR (37,5% [9/24] vs 77,3% [252/326]; OR 0,176, 0,071–0,41; p<0,0001), shorter PFS (median 8 vs 26 weeks; HR 3,74, 95% CI 2,44–5,75; p<0,0001) and OS (median 26 vs 54 weeks in WT, HR 3,03, 1,98–4,63; p<0,0001). 2 out of 24 patients responded to treatment despite BRAF mutations: one had a p.D594G mutation that leads to weaker activation of the MAPK pathway compared with p.V600E mutations and the other one had a low copy number of $BRAF^{V600E}$ MT genes (47,48). The same Authors underline how $BRAF^{V600E}$ mutation confers resistance to anti-EGFR mAbs in patients with chemotherapy-refractory KRAS WT mCRC (49). In a retrospective matched case-control study by Kayhanian, real-world data on treatment and survival for BRAF MT patients compared with a matched BRAF WT control group were analysed. Response to anti-EGFR mAbs was poor for BRAF MT patients, so anti-EGFR mAbs did not appear to benefit BRAF MT mCRC in this study (50). *Karapetis et al*. examined the predictive and prognostic significance of BRAF, PIK3CA, and phosphatase and tensin homolog (PTEN). In the KRAS WT subgroup, the OS adjusted HR according to BRAF mutation status was 1.39 (interaction P = 0.69). Authors concluded that evaluation of predictive significance of BRAF mutations required a larger sample size. The study was not sufficiently powered to conclude whether or not the $BRAF^{V600E}$ mutation is a biomarker of primary resistance to anti-EGFR agents in CRC (51).

An updated analysis of OS according to tumour KRAS and BRAF mutation status in patients receiving cetuximab in addition to FOLFIRI was conducted by Van Cutsem. Statistical significance of improvement in PFS (median 8.0 v 5.6 months; HR, 0.934; P = .87) and OS (median, 14.1 v 10.3 months; HR, 0.908; P= .74)

associated with the addition of cetuximab to FOLFIRI in BRAF MT patients was not reached. In this case, BRAF mutation status was not predictive of treatment effects of anti-EGFR plus FOLFIRI (52-55). In the pooled analysis of CRYSTAL and OPUS clinical trials, BRAF mutation confirmed its role of negative prognostic biomarker, but was not a predictive one in this setting (56).

To date, four meta-analysis have been conducted. Pietrantonio et al. analysed the impact of cetuximab and panitumumab on overall response rate (ORR), PFS, OS in advanced RAS WT/BRAF MT CRC. Nine phase III trials and one phase II trial (six first-line and two second-line trials and two trials involving chemorefractory patients), that included 463 RAS WT/BRAF MT mCRC, were examined. The addition of anti-EGFR mAbs was not associated with a significant OS (HR, 0.91; 95% CI, 0.62–1.34; p = 0.63), PFS (HR, 0.88; 95% CI,0.67–1.14; p = 0.33), or ORR (relative risk, 1.31; 95% CI 0.83–2.08, p = 0.25) benefit in BRAF MT patients, particularly in first-line setting, compared with standard chemotherapy or best supportive care. According to the Authors, in front of these clinically not meaningful, and statistically not significant increase of outcome endpoints, treatment with cetuximab and panitumumab in BRAF MT mCRC is not justified considering their toxicities and socio-sanitary costs. This is the first meta-analysis suggesting a negative predictive value of BRAF mutation for anti-EGFR mAbs; limitations of this work are the lack of comparison with BRAF WT mCRC and the small number of BRAF MT patients. Furthermore, only $BRAF^{V600E}$ mutation data were available and rarer mutations were not examined (57).

Wang's meta-analysis was in line with these findings. Its aim was to investigate the association between $BRAF^{V600E}$ mutation and clinical outcome in mCRC patients treated with anti-EGFR mAbs; seven studies were examined. $BRAF^{V600E}$ mutation was related to lack of response (pooled odds ratio for ORR of BRAF MT over BRAF WT was 0.27 95% CI=0.10-0.70) and worse survival (HR for PFS 2.78, 95% CI=1.62-4.76; HR for OS 2.54, 95% CI=1.93-3.32) in KRAS WT mCRC treated with anti-EGFR (58).

Rowland's meta-analysis has different inclusion criteria and statistical methods of analysis. Eight randomized clinical trials (RCTs) published in seven study reports evaluating the effect of BRAF mutation on the treatment benefit (OS, PFS) from anti-EGFR mAbs in mCRC were reviewed. Of the 3168 participants with RAS WT tumours across the eight RCTs, 2817 were BRAF WT and 351 (11.1%) were BRAF MT. All studies compared the addition of an anti-EGFR agent (four cetuximab and four panitumumab) with background therapy. Five studies restricted the analysis to KRAS WT tumours and two restricted analyses to KRAS WT and NRAS WT tumours. Eight RCTs met the inclusion criteria for assessment of PFS and seven RCTs met the inclusion criteria for assessment of OS. The Authors reported outcomes for the BRAF MT subgroup but also compared BRAF MT patients with BRAF WT subgroup. BRAF MT patients had no significant benefit with anti-EGFR mAbs in PFS (HR PFS benefit 0.86 (95% CI; 0.61–1.21) as compared with RAS WT/BRAF WT patients (HR PFS benefit 0.62 95% CI; 0.50–0.77). No benefit in OS was shown in RAS WT/BRAF MT, with HR for OS benefit with anti-EGFR mAb therapy 0.97 (95% CI; 0.67–1.41), whereas HR was 0.81 (95% CI; 0.70–0.95) for RAS WT/BRAF WT tumours. The difference between RAS WT/BRAF MT and RAS WT/BRAF WT tumours with respect to benefit from cetuximab and panitumumab was not statistically significant either in PFS (test of interaction; P=0.07) or in OS (test of interaction;

P=0.43). An exploratory analysis on impact of line of therapy showed very little difference in first-line setting with anti-EGFR mAb therapy between RAS WT/BRAF WT and RAS WT/BRAF MT tumours in OS efficacy (hazard ratio 0.87 vs 0.89, P=0.96) or PFS efficacy (hazard ratio 0.75 vs 0.83, P=0.45). For non-first-line anti-EGFR mAb therapy there was a nonstatistically significant trend towards difference in efficacy between RAS WT/BRAF WT and RAS WT/BRAF MT tumours both in OS (HR 0.74 vs 1.06, p=0.38) and PFS (HR 0.53 vs 0.84, p=0.05). Authors conclude that there is insufficient evidence to definitively consider BRAF mutation a negative predictive biomarker of survival benefit from anti-EGFR mAb therapy for mCRC, so further studies are needed. Moreover, they underline that there are insufficient data to justify mandatory clinical application of BRAF MT status of RAS WT mCRC to determine eligibility for anti-EGFR mAb therapy, as well as the exclusion of anti-EGFR mAb for patients with RAS WT/BRAF mCRC (59).

The fourth meta-analysis was conducted by Therkildsen and evaluated the potential predictive value of additional biomarkers in the RAS–RAF–MAPK and PI3K– protein kinase B (Akt)–mammalian target of rapamicine (mTOR) pathways, including BRAF, in determining the clinical benefit from anti-EGFR treatment. The Authors investigated the correlation between alterations in KRAS (exons 3 and 4), BRAF, PIK3CA and PTEN and clinical outcome during anti-EGFR treatment in 2395 patients from 22 studies (21 RCTs and one non randomized trial). Seventeen studies investigated the predictive role of BRAF mutations in KRAS exon 2 WT tumors; BRAF mutations were detected in 123 patients. KRAS (exons 3 and 4), BRAF, PIK3CA and PTEN mutations all significantly predicted poor RR (OR = 0.26, OR = 0.29, OR = 0.39 and OR = 0.41, respectively), shorter PFS (HR = 2.19, HR = 2.95, HR = 2.30 HR = 1.88, respectively) and shorter OS (HR = 1.78, HR = 1.85, HR = 2.52 and HR = 1.43, respectively). The Authors conclude that this meta-analysis reveals an independent predictive value from KRAS, NRAS, BRAF, PIK3CA and PTEN and that they should be tested in mCRC in order to optimize identification of patients who will benefit from anti-EGFR treatment [60].

BRAF MT CRC are more commonly found in right sided colon and BRAF mutations may be responsible for less sensitivity of right CRC (RCRC) to anti-EGFR therapy. Right sided colon and left sided colon have a different embriologic origin (right colon originates from midgut, while left colon originates from hindgut) and a different molecular pattern which probably explains different clinical behaviour and response to therapy (61-64). *Shimada et al.* investigated tumours of 201 patients with either primary RCRC or left CRC (LCRC) for genetic alterations using a 415-gene panel including alterations associated with resistance to anti-EGFR therapy: RTK (ERBB2, MET, EGFR, FGFR1, and PDGFRA), RAS pathway (KRAS, NRAS, HRAS, BRAF, and MAPK2K1), and PI3K pathway (PTEN and PIK3CA). 6 of 56 patients (11%) with RCRC were all WT compared with 41 of 145 patients (28%) with all WT LCRC (p= 0.009). Among the 49 patients who received anti-EGFR therapy, RCRC had significantly worse PFS than LCRC (p= 0.022), and MT RCRC showed significantly worse PFS compared with all WT LCRC (P = 0.004). According to the Authors, RCRC is more likely to have genetic alterations associated with resistance to anti-EGFR therapy

compared with LCRC and that primary tumour sidedness is a surrogate for the non-random distribution of genetic alterations in CRC, including BRAF mutations (65).

It's not clear if CRCs with $BRAF^{non\text{-}V600E}$ mutations hold the same resistance to anti-EGFR therapy than cancer with $BRAF^{V600E}$ mutations (18). Certain $BRAF^{non\text{-}V600E}$ mutations might contribute to lesser benefit derived from anti-EGFR drugs. (66). A possible explanation could be related with the different kinase activity (high, intermediate, impaired) of BRAF mutations in the kinase domain (67). The $BRAF^{non\text{-}V600E}$ mutations are heterogeneous, spanning different codons of the gene, with important variability in biologic effects. The Biomarker Research for anti-EGFR mAbs by Comprehensive Cancer Genomics (BREAC) study showed that overall clinical outcomes of $BRAF^{non\text{-}V600E}$ mutations in the kinase domain appeared to be significantly worse compared to RAS/BRAF WT tumours. $BRAF^{V600E}$ mutation produces high kinase activity, whereas $BRAF^{G469A}$, $BRAF^{L485F}$ and $BRAF^{V600R}$ mutations belong to the intermediate subtype and the $BRAF^{D594G}$ mutation belongs to the impaired subtype. Patients with $BRAF^{D594G}$MT tumours did not achieve objective response (OR) to anti-EGFR mAbs treatment. Moreover, enhanced kinase activity was observed in $BRAF^{L525R}$ MT and its enhanced downstream signal may contribute to primary resistance to cetuximab. The $BRAF^{Q524L}$ MT had intermediate kinase activity and did not induce resistance to cetuximab in vitro cell model (66). *De Roock et al.* reported that two patients harbouring $BRAF^{D594G}$ MT mCRC achieved a partial response (PR) to cetuximab monotherapy (47).

3.2 Anti-VEGF

Data on predictive role of BRAF mutation for anti-VEGF efficacy are even poorer and most concern bevacizumab. In a recent review *Lech et al.* underline how to date a predictive biomarker for bevacizumab has not yet been identified, despite several studies investigated this aspect (68). In the phase III AGITG MAX Trial of Capecitabine Alone or in Combination With Bevacizumab and Mitomycin in Advanced Colorectal Cancer, BRAF gene mutation status was not predictive of the effectiveness of bevacizumab for PFS or OS (test for interaction p=0.46 and 0.32, respectively) (69). *Nakayama et al.* conducted a retrospective study to assess if RAS/PIK3CA/BRAF mutational status could be used to select patients who would obtain the greatest clinical benefit from bevacizumab plus chemotherapy as first-line treatment for mCRC. Multivariate analysis revealed a negative predictive value of RAS and BRAF tumour mutations with respect to first-line bevacizumab treatment, but there were no statistically significant differences in ORR and PFS according to BRAF mutations in patients receiving bevacizumab plus chemotherapy, probably because of the rarity of BRAF mutations and the small number of patients. So, the Authors conclude that there are insufficient data to justify the exclusion of patients with a RAS, PIK3CA, or BRAF tumour mutation from bevacizumab treatment regimens and to restrict this therapy to patients affected by wild type EGFR pathway genes tumours (70).

A prospective observational study of 55 patients with first-time diagnosed mCRC was designed to evaluate the incidence of KRAS, NRAS, BRAF and PIK3CA mutations in mCRC patients receiving first-line oxaliplatin based chemotherapy with or without bevacizumab and to evaluate their prognostic and predictive significance, but a very low incidence of BRAF mutant patients was found. 29.1% of patients had KRAS,

NRAS, BRAF and PIK3CA WT tumours and in the bevacizumab group they all obtained a partial or complete response. The difference in RR in FOLFOX4 plus bevacizumab compared to the FOLFOX4 group was significant (p = 0.03). So, in the view of the Authors these results suggest the potential predictive value of the extended mutation analysis of KRAS, NRAS, BRAF and PIK3CA genes, with the knowledge that this hypothesis should be tested on larger patient population (71). Recently, primary results were presented of a follow-up study (NCT01754272) of phase III VELOUR trial to acquire tumour samples for biomarker analyses to assess efficacy according to RAS, BRAF status and sidedness. Interestingly, BRAF MT (all RAS WT) showed a trend for better outcome for PFS and RR. Sidedness did not affect efficacy (HR: 0.83 (0.63-1.1) for left and (HR: 0.83 (0.54-1.3) for right. The hazard ratios of treatment favored RAS WT, but none of the mutations subgroups results showed significant interaction (72).

Considering this lack of evidence, further studies are needed to define BRAF predictive value for treatment with antiangiogenic drugs.

3.3 Anti-EGFR VS Anti-VEGF

Evidence for comparison of BRAF predictive role between anti-EGFR and anti-VEGF therapy is even more lacking. The only available data derive from a retrospective analysis by *Stintzing et al* of FIRE-3 (AIO KRK-0306) study. The Authors evaluated the efficacy of FOLFIRI plus cetuximab or bevacizumab in RAS MT and BRAF MT subgroups. In BRAF MT patients, no significative difference in tumour response, PFS and OS in both treatment arms was observed. Median treatment duration was longer in the bevacizumab than in the cetuximab arm (4.3 months versus 2.4 months), but this was only a non-statistically significant trend because of low patient number (Wilcoxon rank-sum test p = 0.28). Median deepness of response was comparable (-20.3% in cetuximab arm and -15.5% in bevacizumab arm; Wilcoxon test p=0.64). In BRAF MT patients treated with cetuximab, explorative analysis showed no significant differences between patients reaching early tumour shrinkage (ETS) and patients with no ETS with regard to tumour location, AREG and EREG levels or PIK3CA mutations. No statistical difference in secondary resection rate was shown between both arms (chi-square test p value 0.13). In FIRE-3, BRAF MT patients had a poor prognosis and significantly shorter PFS (6.6 versus 6.6 months) and OS (12.3 versus 13.7 months) than the intent to treat (ITT) population, regardless of treatment with cetuximab or bevacizumab, but no firm conclusions on survival can be made because of the retrospective nature of analyses and the small number of patients. The Authors highlight that comparable survival times were observed in BRAF MT patients irrespective of the treatment with FOLFIRI plus cetuximab or FOLFIRI plus bevacizumab and that optimal first-line treatment of these prognostic unfavourable subgroups has not been defined yet (73,74).

3.4 Chemotherapy

Despite new advances in BRAF MT mCRC molecular aspects and characterization, the best treatment still has to be identified. To date, there is insufficient evidence to definitively consider BRAF mutation as a predictive factor of intrinsec resistance to standard chemotherapy. Surely, prognosis of BRAF MT patients remains poor, with a median OS of 12 months, regardless of combination chemotherapy or targeted drugs

administrated. Furthermore, considering the very aggressive nature of this particular kind of disease, a very low number of patiens can get more than one line of therapy (about 60% will not receive subsequent chemotherapy) (53). The major efforts have necessarily to be made in first line. Intensive treatment was first evaluated in a phase II trial assessing the safety and activity of the combination of FOLFOXIRI plus bevacizumab in mCRC patients. In an exploratory post-hoc subgroup analysis, the Authors focused on the role of BRAF mutational status. In BRAF MT patients, mPFS reached 12.8 months, whereas in BRAF WT patients mPFS was 13.1 months; the mOS was 23.8 in BRAF MT and 30.9 months in BRAF WT, respectively. Based on these results, FOLFOXIRI plus bevacizumab appeared to be a possible option for BRAF MT mCRC (75). In a prospective cohort of 15 BRAF MT CRC patients, mPFS was 9.2 months (95% CI: 5.1–13.3), mOS was 24.1 months (95% CI: 3.3–45.0) and RR was 60%, respectively (76). Similar findings came from the pooled population of 24 prospectively and retrospectively treated patients: RR was 72%, mPFS and mOS were 11.8 months and 24.1 months, respectively. These results lead to phase III TRIBE study, in which the Authors compared the benefit of the triplet FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab. PFS was the primary endpoint and the superiority of the association FOLFOXIRI-bevacizumab was confirmed indipendently from BRAF status. Indeed, a preplanned subgroup analysis defined its effectiveness in the BRAF MT subgroup (28 patients - 7.5%): patients receiving FOLFOXIRI-bevacizumab reached a median PFS of 7.5 months versus 5.5 months for patients receiving FOLFIRI-bevacizumab (HR: 0.55; 95% CI: 0.26–1.18). Moreover, BRAF MT patients in the triplet plus bevacizumab arm gained an 8-month improvement in median OS (19.1 months vs 10.8 months; HR: 0.55; 95% CI: 0.24–1.23), even if not statistically significant. These data must be considered with prudence since they come from a subgroup analysis of a small number of patients, but surely they are encouraging and show intensive treatment as a reasonable choice for this cathegory of mCRC, if ECOG PS, age and organ function are permissive (75-78). The CHARTA trial was a phase II study developed by *Schmoll et al.* in Germany, parallel to TRIBE study, comparing FOLFOXIRI-bevacizumab versus FOLFOX-bevacizumab. Treatment was administered for a maximum of 6 months followed by maintenance with fluoropyrimidine and bevacizumab. The primary endpoint was met: PFS at 9 months was 56% versus 68% (p= 0.086); PFS was 9.8 in the triplet plus bevacizumab arm and 12.0 months in FOLFOX plus bevacizumab arm. However, even if CHARTA supports the superiority of FOLFOXIRI-bevacizumab, differences in RR and PFS between subgroups were not strong enough to safely identify patients with high potential benefit from the triplet plus bevacizumab combination (79). *Cremolini et al.* conducted a multicentre, single-arm, phase II trial evaluating the activity and safety of oral and intravenous vinorelbine (VNR), a spindle poison belonging to vinca alcaloids, in pre-treated patients with $BRAF^{V600E}$ MT mCRC. The rational of the study was based on the fact that suppression of RANBP2 gene, which is needed for the progression of the mitotic spindle, caused death in BRAF-like cell lines; the susceptibility to mitotic spindle poisons in CRC models was investigated, and VNR was the most active drug in BRAF-like models, without activity in non-BRAF-like ones. Furthermore, in a retrospective analysis of a study investigating vinca alkaloids in patients with mCRC, the

only patient with a prolonged complete response was found to have a BRAF-like gene signature. Unfortunately, this phase II trial showed no activity of VNR in pretreated BRAF MT patients (80).

4. CURRENT PERSPECTIVES AND INVESTIGATIONAL REGIMENS

4.1 BRAF inhibitors

In mCRC single agent BRAF-inhibition activity was disappointing, in contrast to BRAF MT melanoma. Vemurafenib (PLX4032) is a small oral BRAFi which showed dose-dependent inhibition of ERK and MEK phosphorylation in cell lines and xenograft models harboring $BRAF^{V600E}$ mutation, with arrest of cell proliferation and inhibition of cancer growth. Unfortunately, clinical activity was limited in BRAF MT mCRC, so BRAF mutation could not be considered a predictive factor for vemurafenib efficacy (81). In a Phase I extension trial of 21 BRAF MT mCRC pretreated patients, only one PR and four minor responses were observed (82). In the phase II of the same study, one patient had a PR (5%; 95% CI, 1% to 24%) and seven had stable disease (SD); median PFS was 2,1 months. Concurrent mutations, MSI status, CpG island methylation status, PTEN loss, EGFR expression, and copy number alterations were not associated with clinical benefit. These results confirmed that single-agent vemurafenib did not show meaningful clinical activity (83). Encorafenib (LGX818) is a highly selective ATP-competitive RAF kinase inhibitor suppressing MAPK pathway signaling in BRAF MT cells, but it showed no responses in monotherapy in mCRC patients in a phase I study (53).

Mechanism of poor response and resistance to single agent BRAFi can be categorized according to their ERK signaling dependence. ERK dependent resistance mechanisms include activating MEK1 mutations, COT overexpression, activating NRAS mutations, high CRAS activity and $BRAF^{V600E}$ alternative splicing or expression; ERK independent mechanisms consist of IGF1R activation, high amplification of hepatocyte growth factor, PI3K pathway and PDGFRβ overexpression. In the same patient, different mechanisms can co-exist (84). Surely, EGFR signaling plays a crucial role in bypassing BRAF inhibition (85). In vitro studies showed that BRAF inhibition induces a rapid ERK dependent negative feedback on EGFR, resulting in EGFR activation, formation of RAF protein dimers, and CRAF-mediated reactivation of MAPK signaling. Moreover, EGFR was over-expressed in $BRAF^{V600E}$ mutant CRC cells, while melanoma cells expressed low levels of EGFR and consequently were less sensitive to EGFR-mediated reactivation. *Corcoran et al.* showed that CRC cell lines harbored more phospho-EGFR compared to $BRAF^{V600E}$ melanomas and had a reactivated MAPK signaling through EGFR (84-85).

Furthermore, in agreement, *Ahronian et al.* identified that other molecular alterations within MAPK pathway, including RAS alterations (KRAS mutation, KRAS amplification), BRAF amplification and MEK1

mutation, that lead to reactivation of MAPK signalling and may be responsible of acquired resistance to simultaneous RAF/EGFR and RAF/MEK inhibition in BRAF MT CRC cells (86).

In summary, single targeted therapy may not be effective because of the cross-talk between different RTK signaling cascades, and when CRC cells are blocked with a single agent they often activate alternative pathways as escape strategies to overpass the blockade (84).

Based on these findings, combination therapy with agents targeting different members of RAS/RAF/MEK/ERK pathway and standard chemotherapy has been investigated to overcome resistance (Table 3.)

Corcoran et al. evaluated in a Phase I/II trial the combination of dabrafenib, a BRAFi, with trametinib, a MEK inhibitor (MEKi). This association showed only a limited improvement of activity in BRAF MT mCRC: among the 43 patients enrolled, 12% achieved PR and one patient achieved a durable complete response (CR); 51% of patients had SD. The most common adverse events were fatigue, pyrexia, and anemia (53,87).

Yaeger et al. conducted a pilot trial assessing the response rate and safety of vemurafenib combined with panitumumab in 15 patients with BRAF MT CRC; results were encouraging with tumour regressions in 10 out of 12 evaluable patients and PR in 2 patients (88).

A phase 2 "basket" study of vemurafenib in $BRAF^{V600E}$ MT non-melanoma cancers included 2 cohorts of mCRC heavily pretreated patients (one to six lines of previous therapy, median 2 lines). In the cohort of patients who received vemurafenib monotherapy, no responses were observed; median PFS and OS were 4.5 months (95% CI, 1.0 to 5.5) and 9.3 months (95% CI, 5.6 to not reached). In the cohort treated with vemurafenib and cetuximab, one patient achieved a response and almost 50% of the patients had tumour regression but did not reach the standard criteria for a PR; median PFS and OS were 3.7 months (95% CI, 1.8 to 5.1) and 7.1 months (95% CI, 4.4 to not reached), respectively (89).

A phase I/II trial (MEK116833; NCT01750918) evaluated combinations of panitumumab, dabrafenib and trametinib with integrated biomarker analyses. 134 eligible patients (120 pretreated, 14 enrolled in the first line) with BRAF MT mCRC received dabrafenib plus panitumumab (n = 20), trametinib plus panitumumab (n = 31), or dabrafenib, trametinib and panitumumab (n = 83). Pretreatment and on-treatment tumour biopsies were assessed for phosphorylated ERK (pERK) by immunohistochemistry and serial circulating tumor DNA (ctDNA) samples were screened for mutations in BRAF, KRAS, NRAS, and PIK3CA. Patients receiving combination of dabrafenib and panitumumab showed a 10% RR (one CR and one PR) with 80% rate of SD; in this arm median duration of response was 6.9 months and PFS was 3.5 months. The most common adverse events were dermatitis acneiform (Grade1/2 55%) and fatigue (G1/2 45%). In the trametinib plus panitumumab arm rates of confirmed CR/PR and SD were respectively 0% and 53%, median PFS was 2.8 months. In the triplet arm a RR of 18% and stable disease rate of 67% were reached, while data on median PFS were not mature at the time of analysis; the most frequent toxicities were diarrhea (G1/2 60%, G3 9%) and dermatitis acneiform (G1/2 47%; G3 9%). Median reduction in pERK during treatment vs baseline biopsies was 23% for dabrafenib-panitumumab arm, 50% for trametinib-panitumumab and 54% for

the triplet combination. Serial ctDNA analysis in patients receiving three drugs demonstrated >70% reduction in $BRAF^{V600E}$ mutant fraction (MF) in 12 of 14 pts (86%) by week 4, with 6 of these 12 pts achieving PR at week 6; conversely $BRAF^{V600E}$ MF increased in 10 patients upon progression (90-94).

Based on preclinical data reported by *Yang et al*. from $BRAF^{V600E}$ MT CRC cell lines and xenografts evaluating combinations of vemurafenib with different therapies (81,93), a 3+3 phase I study was conducted in patients with $BRAF^{V600E}$ advanced solid cancers receiving cetuximab and irinotecan with escalating doses of vemurafenib. 18 patients with mCRC were enrolled and treated at three dose levels. The RR was 35%, with one CR; median duration of response was 25 weeks and PFS was 7.7 months. $BRAF^{V600E}$ circulating cell-free DNA (cfDNA) trends correlated with radiographic changes; moreover, at progression, acquired mutations from cfDNA in genes reactivating MAPK signaling were detected (93-95). The phase II (SWOG 1406) trial on 106 patients was presented at ASCO GI 2017 and showed that the addition of vemurafenib to cetuximab and irinotecan induced a prolongation of PFS and a higher DCR. PFS (primary endpoint) was improved by the addition of vemurafenib (HR = 0.42, 95%, CI: 0.26 - 0.66, $P < 0.001$) with median PFS of 4.4 (95% CI: 3.6 – 5.7) months vs 2.0 (95% CI: 1.8 – 2.1) compared to median PFS of 2 months in the control arm (HR of 0.42 $P<0.001$). In the vemurafenib-cetuximab-irinotecan arm RR was 16% versus 4% in control arm and DCR was 67% versus 22%. No increase in skin adverse events or fatigue was reported; grade 3/4 adverse events more common in the triplet arm included neutropenia (28% vs 7%), anemia (13% vs 0%), and nausea (15% vs 0%). Approximately 50% of patients in the control aim crossed over at the time of progression, so OS and efficacy at cross-over data remain immature. The Authors conclude that simultaneous EGFR and BRAF inhibition is an effective combination therapy in ^{BRAFV600E} MT CRC (93,96).

Another mechanism of resistance to BRAF inhibition may be represented by the activation of the PI3K pathway, with consequent MAPK pathway inhibition bypass and contribution to cell proliferation and survival (85). Several evidences showed that CRC cell lines have higher levels of PI3K/Akt signalling compared to melanoma cells and in vitro experiments showed that the concurrent presence of PTEN or PI3K mutations in BRAF MT CRC cells caused reduced sensitivity to vemurafenib because of activation of PI3K/AKT pathway signalling (97). In BRAF and PI3K double MT CRC cells (such as RKO, HT-29, NCI-H508 or WiDr), the inhibition of both pathways seemed to be synergistic. A Phase Ib/II study investigated in $BRAF^{V600}$ MT mCRC the simultaneous inhibition of BRAF, EGFR and PI3K with encorafenib, cetuximab and alpelisib (BYL719). Alpelisib is a class I α-specific PI3K inhibitor which showed antitumour activity in various cancer cell lines, especially those harboring PIK3CA mutations, and in tumour xenograft models with mutated or amplified PIK3CA. In the phase II trial, 102 pretreated patients with $BRAF^{V600E}$ MT CRC were randomized to receive encorafenib plus cetuximab (n = 50) or encorafenib plus cetuximab and alpelisib, a PI3Kα-specific inhibitor (n = 52). Patients treated with the three drugs achieved an ORR of 27% versus 22% in patients receiving encorafenib plus cetuximab. Median PFS was 5.4 months in the triplet arm and 4.2 months in the doublet arm (HR = 0.69; 95% CI 0.43–1.11; P = 0.064). The most common grade 3 and 4 adverse events were anemia (17 versus 6%), hyperglycemia (13 versus 2%), and increased lipase (8 versus 18%) for experimental and control arms respectively [53,84,85,98-103].

Several trials investigating combination therapy to overcome drug resistance are ongoing and hopely will provide new strategies to treat this cathegory of patients. A promising study with still active recrutiment is the BEACON CRC, a multicenter, randomized, Open-label, 3-Arm Phase III trial of Encorafenib + Cetuximab Plus or Minus Binimetinib (a MEK inhibitor) versus Irinotecan/Cetuximab or Infusional 5-Fluorouracil (5-FU)/Folinic Acid (FA)/Irinotecan (FOLFIRI)/Cetuximab With a Safety Lead-in of Encorafenib + Binimetinib + Cetuximab in Patients with $BRAF^{V600E}$ MT mCRC (NCT02928224). At European Society for Medical Oncology (ESMO) annual meeting in Madrid, results of safety lead-in (SLI) in 30 patients to determine the safety of the combination of binimetinib, encorafenib and Cetuximab were presented. The triplet at the full planned dose of each drug was generally well tolerated; diarrhea, nausea, dermatitis acneiform and fatigue were reported as the most frequent adverse events and between them no grade 4 toxicities were observed. Surely, longer follow up is required to have more mature safety data (104). In these SLI patients, the association of tumour markers Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen 19.9 (Ca 19.9) changes during treatment with clinical outcomes was evaluated. The ORR reported was 41%; in patients receiving the combination treatment for at least 5.6 mo, CEA and CA19-9 decreased markedly in responders and with SD. These data suggest additional evidence of the clinical activity of this regimen (105).

4.2 BRAF mutations, MMR deficiency, immunotherapy

BRAF MT tumours are also associated with the CpG island methylator phenotype (hypermethylated phenotype) which can induce the epigenetic inactivation of MLH1. This phenomenon results in dMMR and consequently MSI. 20% of patients affected by BRAF MT mCRC have also dMMR (103,106,107). Recently a classification was developed to identify four consensus molecular subtypes (CMS) of colorectal cancer: CMS1 (MSI Immune), 14% hypermutated, CIMP high, microsatellite unstable, BRAF MT, with strong immune infiltration and activation; CMS2 (Canonical), 37%, epithelial, chromosomally unstable, with marked WNT and MYC signaling activation; CMS3 (Metabolic), 13%, epithelial, with metabolic dysregulation; and CMS4 (Mesenchymal), 23%, prominent TGF-β activation, stromal invasion and angiogenesis (108-109). $BRAF^{V600E}$ MT and dMMR CRC represent a unique molecular subtype (CMS1) in which immunitary sistem plays a crucial role, so immunotherapy seems a challenging research field in these tumours. Immune checkpoint inhibitors (programmed death 1 [PD-1] and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) inhibitors) have shown clinical activity in patients with MSI-H mCRC (Table 4). Pembrolizumab, an anti-PD1 antibody, was evaluated in a phase II trial of heavily pretreated mCRC. Patients with dMMR CRC reached an ORR of 40% and an immune-related PFS at 20 weeks of 78%, whereas no OR was observed for pMMR mCRC. Median PFS and OS have not yet been reached. The progression-free rate at 24 months was 61%. However, the clinical activity of pembrolizumab in patients with BRAF MT MSI-H mCRC was not well studied and no analysis of clinical activity of pembrolizumab according to the BRAF mutational status was conducted (110). The CHECKMATE 142 phase II trial demonstrated that treatment with Nivolumab (anti-PD1 antibody) monotherapy and in combination with ipilimumab (anti-CTLA4

antibody) resulted in encouraging clinical activity in MSI-H mCRC. 20 patients had a pMMR tumour and 100 patients were affected by dMMR mCRC, of which 17% harbored $BRAF^{V600E}$ mutation. Among MSI patients, the ORR was 25.5% for Nivolumab monotherapy and 33.3% for Nivolumab plus ipilimimab; all were durable responses, whereas no antitumour efficacy was observed in patients with pMMR tumors. Among dMMR patients, responses were reached regardless of BRAF mutational status and the specifical effect of immunotherapy in patients with BRAF MT MSI-H mCRC was not investigated (111). These trials suggest a potential role of immunotherapy for BRAF MT mCRC treatment, but surely further research is needed with more attention focused on relationship with BRAF mutational status.

4.3 Fighting against BRAF inhibitors resistance: future directions

All first-generation Raf inhibitors (vemurafenib, dabrafenib) are ATP-competitive inhibitors highly specific towards $BRAF^{V600E}$. Their limited activity is determined by effect on dimerisation of RAF and paradoxical activation of ERK signaling. When RAS is active, dimerizing potential is increased by these drugs, as they interact with the Raf catalytic domain and impact on dimer formation and dimer activity, increasing BRAF/CRAF dimer formation. Moreover, these agents exhibit negative cooperativity in binding to the second protomer of a Raf dimer, so they cannot achieve an effective inhibition of dimeric Raf complexes. Different approaches have been proposed to detect new generation of RAF inhibitors. The first approach is represented by agents able to inhibit both monomers and dimers, so that when RAF dimerizes, the drug can bind also to RAF dimers and inhibit RAF signaling. Several pan-RAF inhibitors, such as LY3009120 and BGB-283 have been investigated (112). *Hong et al.* discovered a potent and selective pan-RAF inhibitor, INU-152, which suppressed the growth of melanoma and CRC BRAF MT cells in vitro and in vivo and significantly reduced tumor volumes in xenograft mouse models of human melanoma and CRC, with an acceptable safety margin (113). Another approach to overcome resistance consists of inhibition of dimerization in presence of RAS activation by engagement of the ATP binding complex (114). PLX7904 belongs to this category of new generation of BRAFi, as well as PLX8394, which demonstrated to avoid paradoxical activation of MAPK signalling by preventing dimers formation and it is being evaluated in a ongoing clinical trial in solid unresectable tumors (NCT02428712) (112,114). Further research will provide new information on clinical efficacy and safety of these compounds.

5. Expert commentary

$BRAF^{V600E}$ mutation represents a strong prognostic factor with a worse survival than BRAF WT in II-III stage MSS CRC and mCRC patients, independently of other clinic-pathological features and treatment received. Considering small patient's number of each report as well as heterogeneity of population, it is difficult to conclude predictive and prognostic impact of $BRAF^{non\text{-}V600E}$ mutation.

In BRAF MT mCRC subset, the best treatment still has to be identified. Considering the very aggressive nature of this particular kind of disease, in good performance status patients, an intensive-chemotherapy-

combination remains the standard of care. Therefore, further investigations are warranted to explore combination therapies and immune checkpoint inhibitors to change BRAF MT mCRC outcomes.

6. Five-year view

We can hypothesize that the clinical management of BRAF MT CRC will change radically in the next few years. On the basis of the currently studies, we will hopefully use new therapeutic options for these patients. It is well known that single targeted therapy may not be effective because of the cross-talk between different RTK signaling cascades. As a consequence, CRC cells, blocked with a single agent, often will activate alternative pathways as escape strategies to overpass the blockade. Based on these findings, it appears promising the role of combination therapy with agents targeting EGFR, different members of MAPK signalling pathway and standard chemotherapy to overcome BRAF inhibition resistance.
Furthermore, given the strong association between MSI-H mCRC and BRAF mutations, immunotherapy may also have a role in future therapeutic approaches.

Key issues

- $BRAF^{V600E}$ mutation is found in 5-10% of CRC.
- The V600E mutation in BRAF gene identifies a subgroup of patients with different clinical, prognostic and predictive features.
- $BRAF^{V600E}$ mutation is frequently associated with right-sided CRC, T4 stage, poor differentiation and mucinous histology and the prevalence of this mutation is also higher in elderly female patients (> 70 years).
- $BRAF^{V600E}$ mutation has a negative widely accepted prognostic value in advanced setting. In patients with stage II and III CRC, this observation seemed limited to microsatellite stable tumours.
- As many as 2.2% of the population diagnosed with CRC has a $BRAF^{non\text{-}V600E}$ mutation. $BRAF^{non\text{-}V600E}$ mutation have different clinical-pathological phenotype and probably prognostic implication, compared with $BRAF^{V600E}$ MT CRC patients.
- The predictive role of BRAF mutation still remains controversial.
- Given the frequent association between $BRAF^{V600E}$ mutation and dMMR, immunotherapy might have. a potential role as BRAF MT mCRC treatment
- Single agent BRAF-inhibition activity was disappointing, by the early occurrence of resistance mechanism involving the activation of the EGFR, PI3K and MEK–driven molecular pathways.
- Combination treatment of BRAFi therapy with agents targeting different members of RAS/RAF/MEK/ERK pathway or standard chemotherapy might overcome resistance.

Funding
This paper was not funded.

Declaration of Interest
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Reference annotations

** Of interest*
*** Of considerable interest*

1. Scartozzi M, Giampieri R, Aprile G et al. The distinctive molecular, pathological and clinical characteristics of BRAF-mutant colorectal tumors. Expert Rev Mol Diagn. 2015;15(8):979-87. doi: 10.1586/14737159.2015.1047346. Epub 2015 May 15. Review. PubMed PMID: 25975986. **** A global and exhaustive analysis of BRAF mutant colorectal cancer.**

2. Scartozzi M, Giampieri R, Maccaroni E et al. Phosphorylated AKT and MAPK expression in primary tumours and in corresponding metastases and clinical outcome in colorectal cancer patients receiving irinotecan-cetuximab. J Transl Med. 2012 Apr 10;10:71. doi:10.1186/1479-5876-10-71. PubMed PMID: 22490361; PubMed Central PMCID: PMC3433313.

3. Xu Q, Xu AT, Zhu MM, et al: Predictive and prognostic roles of BRAF mutation in patients with metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: A metaanalysis. J Dig Dis 14:409-416, 2013

4. Yuan ZX, Wang XY, Qin QY, et al: The prognostic role of BRAF mutation in metastatic colorectal cancer receiving anti-EGFR monoclonal antibodies: A meta-analysis. PLoS One 8:e65995, 2013

5. Gavin PG, Colangelo LH, Fumagalli D, et al: Mutation profiling and microsatellite instability in stage II and III colon cancer: An assessment of their prognostic and oxaliplatin predictive value. Clin Cancer Res 18:6531-6541, 2012

6. Forbes SA, Bhamra G, Bamford S, et al: The catalogue of somatic mutations in cancer (COSMIC). Curr Protoc Hum Genet. 10:10.11, 2008

7. Erhardt P, Schremser EJ, Cooper GM. BRAF inhibit programmed cell death downstream of cytochrome c release from mitochondria by activating the MEK/Erk pathway. Mol Cell Biol 1999;19:5308-15

8. Preto A, Figueirido J, Velho S, et al. BRAF provides proliferation and survival signals in MSI colorectal carcinoma cells displaying BRAF (V600E) but not KRAS mutations. J Pathol 2008;214:320-7

9. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54

10. The Cancer Genome Network Atlas. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487(7407):330-337. doi:10.1038/nature11252.

11. Loupakis F, Moretto R, Aprile G, et al. Clinico-pathological nomogram for predicting BRAF mutational status of metastatic colorectal cancer Br J Cancer. 2016 Jan 12;114(1):30-6. doi: 10.1038/bjc.2015.399

12. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. Int J Cancer 2011;128:2075-84

13. Saridaki Z, Tzardi M, Sfakianaki M, et al. BRAF V600E mutation analysis in patients with metastatic colorectal cancer (mCRC) in daily clinical practice: correlation with clinical characteristic, its impact on patients' outcome. PloS One 2013;8:e84604

14. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAFV600E mutation. Int J Cancer. 2011;128(9):2075-2084. doi:10.1002/ijc.25555.

15. Clarke CN, Kopetz ES. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies. J Gastrointest Oncol. 2015;6(6):660-667. doi:10.3978/j.issn.2078-6891.2015.077.

16. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer. 2011;117(20):4623-4632. doi:10.1002/cncr.26086.

17. SeppÄlÄ TT, BÖhm JP, Friman M, et al: Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. BJC 2015

18. Jones JC, Renfro LA, Al-Shamsi HO et al. Non-V600BRAF-mutations define a clinically distinct molecular subtype of metastatic colorectal cancer. J Clin Oncol. 35:2624-2630, 2017

19. Yao Z, Yaeger R, Rodrik-Outmezguine VS, et al. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. Nature. 2017 Aug 10;548(7666):234-238

20. Farina-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: 2396–2402.

21. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005; 23:609–18. [PubMed: 15659508] *** In this systematic review, the role of microsatellite instability in colorectal cancer prognosis is analyzed.**

22. 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: 466–474.

23. 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: 6063–6069.

24. Lochhead P, Kuchiba A, Imamura Y et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst 2013; 105: 1151–1156.

25. Taieb J, Zaanan A, Le Malicot K, et al: Prognostic effect of BRAF and KRAS mutations in patients with stage III colon cancer treated with leucovorin, fluorouracil, and oxaliplatin with or without cetuximab: A post hoc analysis of the PETACC-8 trial. JAMA Oncol, 2016.

26. Sinicrope FA, Mahoney MR, Smyrk TC et al. Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. J Clin Oncol 2013; 31: 3664-3672.

27. Richman SD, Seymour MT, Chambers P et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol. 2009; 27:5931–7.

28. Heinemann V, von Weikersthal LF, Decker T et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol. 2014;15:1065–75.

29. Douillard JY, Oliner KS, Siena S et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013;369:1023–34.

30. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. N Engl J Med 2009;361:98-9

31. 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: 5705-5712 [PMID: 19001320 DOI: 10.1200/ JCO.2008.18.0786]

32. 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: 5322–5330.

33. 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. Ann Oncol 2017; 28: 562–568.

34. 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: 720–728.

35. 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: 2316–2324.

36. Tosi F, Magni E, Amatu A et al. Effect of KRAS and BRAF Mutations on Survival of Metastatic Colorectal Cancer After Liver Resection: A Systematic Review and Meta-Analysis. Clinical Colorectal Cancer, Vol. 16, No. 3, e153-63. 2017

37. Shen Y, Wang J, Han X et al. Effectors of epidermal growth factor receptor pathway: the genetic profiling of KRAS, BRAF, PIK3CA, NRAS mutations in colorectal cancer characteristics and personalized medicine. 2013 PLoS ONE 8: e81628.

38. Ciardiello F, Normanno N, Maiello E et al. Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRIGOIM trial. 2014 Ann Oncol 25: 1756–1761.

39. 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. 2015 Ann Oncol 26: 2092–2097.

40. Lo Nigro C, Ricci V, Vivenza D et al. Prognostic and predictive biomarkers in metastatic colorectal cancer anti-EGFR therapy. Journal of World J Gastroenterol 2016 August 14; 22(30): 6944-6954 ISSN 1007-9327 (print) ISSN 2219-2840 (online)

41. Giampieri R, Scartozzi M, Del Prete M et al. Molecular biomarkers of resistance to anti-EGFR treatment in metastatic colorectal cancer, from classical to innovation. Crit Rev Oncol Hematol. 2013 Nov;88(2):272-83. doi: 10.1016/j.critrevonc.2013.05.008. Epub 2013 Jun 24. Review. PubMed PMID: 23806981.

42. Scartozzi M, Bearzi I, Berardi R et al. Epidermal growth factor receptor (EGFR) downstream signalling pathway in primary colorectal tumours and related metastatic sites: optimising EGFR-targeted treatment options. Br J Cancer. 2007 Jul 2;97(1):92-7. Epub 2007 Jun 19. PubMed PMID: 17579627; PubMed Central PMCID: PMC2359660.

43. Giampieri R, Mandolesi A, Abouelkhair KM et al. Prospective study of a molecular selection profile for RAS wild type colorectal cancer patients receiving irinotecan-cetuximab. J Transl Med. 2015 May 7;13:140. doi:10.1186/s12967-015-0501-5. PubMed PMID: 25943333; PubMed Central PMCID: PMC4424481.

44. Christine Koch, Joerg Trojan. Established and Potential Predictive Biomarkers in Gastrointestinal Cancer – c-Kit, Her2, Ras and Beyond. Digestion 2015;91:294–302 DOI: 10.1159/000376573

45. 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 14: 749–759.

46. 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: 715–721

47. De Roock W, 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. Lancet Oncol 2010, 11: 753–762. *** In this paper, the prognostic and predictive role of different biomarkers is discussed.**

48. Van Brummelen EMJ, De Boer A, Beijnen OH et al. BRAF Mutations as Predictive Biomarker for Response to Anti-EGFR Monoclonal Antibodies. Oncologist. 2017 Jul;22(7):864-872. doi: 10.1634/theoncologist.2017-0031. Epub 2017 Jun 2

49. De Roock W, De Vriendt V, Normanno N et al. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol. 2011 Jun;12(6):594-603. doi: 10.1016/S1470-2045(10)70209-6. Epub 2010 Dec 14.

50. Kayhanian H, Goode E, Sciafani F, et al. Treatment and Survival Outcome of BRAF-Mutated Metastatic Colorectal Cancer:A Retrospective Matched Case-Control Study Clin Colorectal Cancer. 2017 Oct 16. pii: S1533-0028(17)30069-5. doi: 10.1016/j.clcc.2017.10.006.

51. Karapetis CS, Jonker D, Daneshmand M, et al. 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;20:744–53.

52. Van Cutsem E, Kohne CH, Lang I et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 2011; 29:2011–9.; 29:2011-9; PMID:21502544; http://dx.doi.org/10.1200/ JCO.2010.33.5091

53. Cohen R, Cervera P, Svcek M, et al. BRAF-Mutated Colorectal Cancer: What Is the Optimal Strategy for Treatment? Curr Treat Options Oncol. 2017 Feb;18(2):9. doi: 10.1007/s11864-017-0453-5

54. Roma C, Rachiglio AM, Pasquale R. et al, BRAF V600E mutation in metastatic colorectal cancer: Methods of detection and correlation with clinical and pathologic features. Cancer Biol Ther. 2016 Aug 2;17(8):840-8. doi: 10.1080/15384047.2016.1195048

55. Modest DP, Jung A, Moosmann N et al. The influence of KRAS and BRAF mutations on the efficacy of cetuximab-based first-line therapy of metastatic colorectal cancer: an analysis of the AIO KRK-0104-trial. Int J Cancer 2012; 131:980–6.

56. 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 randomized clinical trials. Eur J Cancer. 2012 Jul;48(10):1466-75. doi: 10.1016/j.ejca.2012.02.057. Epub 2012 Mar 23.

57. Pietrantonio F, Petrelli F, Coinu A. et al, Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: A meta-analysis. Eur J Cancer. 2015 Mar;51(5):587-94. doi: 10.1016/j.ejca.2015.01.054. Epub 2015 Feb 9.

58. Wang Q, Hu WG, Song QB, et al. BRAF V600E mutation as a predictive factor of anti-EGFR monoclonal antibodies therapeutic effects in metastatic colorectal cancer: a meta-analysis. Chin Med Sci J. 2014 Dec;29(4):197-203.

59. Rowland A, Dias MM, Wiese MD, et al, Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. Br J Cancer. 2015 Jun 9;112(12):1888-94. doi: 10.1038/bjc.2015.173. Epub 2015 May 19. **** In this meta-analysis, BRAF mutation predictive role across different trials is evaluated.**

60. Therkildsen C, Bergmann TK, Henrichsen-Schnack T, et al. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and metaanalysis Acta Oncol. 2014 Jul;53(7):852-64. doi: 10.3109/0284186X.2014.895036. Epub 2014 Mar 25.

61. Sinicrope FA, Okamoto K, Kasi PM, et al. Molecular biomarkers in the personalized treatment of colorectal cancer. Clin Gastroenterol Hepatol. 2016 May; 14(5): 651-658.

62. Gao XH, Yu GY, Gong HF, et al. Differences of protein expression profiles, KRAS and BRAF mutation, and prognosis in right-sided colon, left-sided colon and rectal cancer Sci Rep. 2017 Aug 11;7(1):7882. doi: 10.1038/s41598-017-08413-z

63. Wang X, Wei Q, Gao J, et al. clinicopathological features of chinese patients with braf mutated metastatic colorectal cancer: a retrospective observational study Chin J Cancer 2017 36:81

64. Demurtas L, Puzzoni M, Giampieri R et al. The role of primary tumour sidedness, EGFR gene copy number and EGFR promoter methylation in RAS/BRAF wild-type colorectal cancer patients receiving irinotecan/cetuximab. British Journal of Cancer (2017), 1–7.

65. Shimada Y, Kameyama H, Nagahashi M, et al. Comprehensive genomic sequencing detects important genetic differences between right-sided and left-sided colorectal cancer Oncotarget. 2017 Aug 24;8(55):93567-93579. doi: 10.18632/oncotarget.20510. eCollection 2017 Nov 7.

66. Eiji Shinozaki, Takayuki Yoshino, Kentaro Yamazaki et al. Clinical significance of BRAF non-V600E mutations on the therapeutic effects of anti-EGFR monoclonal antibody treatment in patients with pretreated metastatic colorectal cancer: the Biomarker Research for anti-EGFR monoclonal Antibodies by Comprehensive Cancer genomics (BREAC) study. British Journal of Cancer (2017) 117, 1450–1458 | doi: 10.1038/bjc.2017.308

67. Wan PT, Garnett MJ, Roe SM et al. Mechanism of activation of the Raf-MEK signaling pathway by oncogenic mutations of B-Raf. 2004 Cell 116: 856–867.

68. Lech G, Robert Słotwiński, Maciej Słodkowski et al. Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances 2016 Colorectal Cancer: Global view World J Gastroenterol 2016 February 7; 22(5): 1745-1755 ISSN 1007-9327 (print) ISSN 2219-2840

69. Price TJ, Hardingham JE, Lee CK, et al. Impact of KRAS and BRAF Gene Mutation Status on Outcomes From the Phase III AGITG MAX Trial of Capecitabine Alone or in Combination With Bevacizumab and Mitomycin in Advanced Colorectal Cancer. J Clin Oncol 2011; 29: 2675-2682

70. Nakayama I, Shinozaki E, Matsushima T, et al. Retrospective study of RAS/PIK3CA/BRAF tumor mutations as predictors of response to first-line chemotherapy with bevacizumab in metastatic colorectal cancer patients. BMC Cancer. 2017 Jan 9;17(1):38. doi: 10.1186/s12885-016-2994-6.

71. Baltruškevičienė E, Mickys U, Žvirblis T, et al. Significance of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer patients receiving Bevacizumab: a single institution experience. Acta Med Litu. 2016;23(1):24-34. doi: 10.6001/actamedica.v23i1.3267.

72. PWirapati P, Pomella V, Vandenbosch B, et al. Velour trial biomarkers update: Impact of RAS, BRAF, and sidedness on aflibercept activity. DOI: 10.1200/JCO.2017.35.15_suppl.3538 Journal of Clinical Oncology 35, no. 15_suppl (May 2017) 3538-3538.

73. Stintzing S, Miller-Phillips L, Modest DP, et al. Impact of BRAF and RAS mutations on first-line efficacy of FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab: analysis of the FIRE-3 (AIO KRK-0306) study. Eur J Cancer. 2017 Jul;79:50-60. doi: 10.1016/j.ejca.2017.03.023. Epub 2017 Apr 29

74. Stintzing S, Jung A, Rossius L, et al. Analysis of KRAS/NRAS and BRAF mutations in FIRE-3: a randomized phase III study of FOLFIRI plus cetuximab or bevacizumab as first-line treatment for wild-type (WT) KRAS (exon 2) metastatic colorectal cancer (mCRC) patients. Eur J Cancer. 2013;49(suppl_3):S7–S19.

75. 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. Lancet Oncol 2010;11:845-52

76. Loupakis F, Cremolini C, Masi G, et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. N Engl J Med 2014;371:1609-18

77. 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 **** In this study, triplet chemotherapy plus bevacizumab for BRAF mutant colorectal cancer was evaluated.**

78. Strickler JH, Wu C, Bekaii-Saab T. Targeting BRAF in metastatic colorectal cancer: Maximizing molecular approaches. Cancer Treat Rev. 2017 Nov;60:109-119. doi: 10.1016/j.ctrv.2017.08.006. Epub 2017 Aug 31.

79. Schmoll HJ, Meinert FM, Cygon F, et al. "CHARTA": FOLFOX/Bevacizumab vs. FOLFOXIRI/Bevacizumab in advanced colorectal cancer—Final results, prognostic and potentially predictive factors from the randomized Phase II trial of the AIO. J Clin Oncol. 2017 , no. 15_suppl (May 2017) Abstract 3533. DOI: 10.1200/JCO.2017.35.15_suppl.3533

80. Cremolini C, Pietrantonio F, Tomasello G, et al. Vinorelbine in BRAF V600E mutated metastatic colorectal cancer: a prospective multicentre phase II clinical study. ESMO Open. 2017 Aug 10;2(3):e000241. doi: 10.1136/esmoopen-2017-000241. eCollection 2017.

81. Yang H, Higgins B, Kolinsky K et al. Antitumor activity of BRAF inhibitor vemurafenib in preclinical models of BRAF-mutant colorectal cancer. Cancer Res. 2012 Feb 1;72(3):779-89. doi: 10.1158/0008-5472.CAN-11-2941. Epub 2011 Dec 16.

82. Kopetz S, Desai J, Chan E, et al. PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. J Clin Oncol 2010;28:15s

83. 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 Dec 1;33(34):4032-8. doi: 10.1200/JCO.2015.63.2497. Epub 2015 Oct 12.

84. Bahrami A, Hesari A, Khazaei M, et al. The therapeutic potential of targeting the BRAF mutation in patients with colorectal cancer. J Cell Physiol. 2018 Mar;233(3):2162-2169. doi: 10.1002/jcp.25952. Epub 2017 May 23.

85. Strickler JH, Wu C, Bekaii-Saab T. Targeting BRAF in metastatic colorectal cancer: Maximizing molecular approaches. Cancer Treat Rev. 2017 Nov;60:109-119. doi: 10.1016/j.ctrv.2017.08.006. Epub 2017 Aug 31.

86. Ahronian LG, Sennott EM, VAN Allen EM, et al. Clinical acquired resistance to RAF inhibitor combinations in BRAF-mutant colorectal cancer through MAPK pathway alterations. Cancer Discov 2015. [Epub ahead of print]

87. Corcoran RB, Falchook GS, Infante JR, et al. Phase 1-2 trial of the BRAF inhibitor dabrafenib (D) plus MEK inhibitor trametinib (T) in BRAF V600 mutant colorectal cancer (CRC): Updated efficacy and biomarker analysis. J Clin Oncol 2014; 32(Suppl):abstr. 3517

88. 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;21:1313-20

89. Hyman DM1, Puzanov I, Subbiah V, et al. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. N Engl J Med. 2015 Aug 20;373(8):726-36. doi: 10.1056/NEJMoa1502309.

90. Van Cutsem E, Atreya C, Andre´ T et al. LBA-07 Updated Results of the MEK inhibitor trametinib (T), BRAF inhibitor dabrafenib (D), and anti- EGFR antibody panitumumab (P) in patients (pts) with BRAF V600E mutated (BRAFm) metastatic colorectal cancer (mCRC). Ann Oncol 2015; 26: iv119.

91. Atreya CE, Van Cutsem E, Bendell JC et al. Updated efficacy of the MEK inhibitor trametinib (T), BRAF inhibitor dabrafenib (D), and anti-EGFR antibody panitumumab (P) in patients (pts) with BRAF V600E mutated (BRAFm) metastatic colorectal cancer (mCRC). J Clin Oncol 2015; 33: 103.

92. Corcoran RB, Andre´ 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). Ann Oncol 2016; 27: 455O–455

93. Sanz-Garcia E, Argiles G, Elez E, et al. BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives Annals of Oncology, 28: 2648-2657, 2017

94. Vogel A, Hofheinz RD, Kubicka S, et al. Treatment decisions in metastatic colorectal cancer - Beyond first and second line combination therapies. Cancer Treat Rev. 2017 Sep;59:54-60. doi: 10.1016/j.ctrv.2017.04.007. Epub 2017 May 4.

95. Hong DS, Morris VK, El Osta B, et al. Phase IB Study of Vemurafenib in Combination with Irinotecan and Cetuximab in Patients with Metastatic Colorectal Cancer with BRAFV600E Mutation. Cancer Discov. 2016 Dec;6(12):1352-1365. Epub 2016 Oct 11.

96. Kopetz SMS, Morris VK, Lenz H. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG 1406). J Clin Oncol 2017; 35: a bstract 520.

97. Ahmed D, Eide PW, Eilersen DA, et al. Epigenetic and genetic features of 24 colon cancer cell lines. Oncogenesis 2013;2:e71

98. Van Geel R, Elez E, Bendell JC, et al. Phase I study of the selective BRAFV600 inhibitor encorafenib (LGX818) combined with cetuximab and with or without the a-specific PI3K inhibitor BYL719 in patients with advanced BRAF-mutant colorectal cancer. J Clin Oncol 2014;32:5s

99. Elez E, Schellens J, van Geel R et al. LBA-08 Results of a phase 1b study of the selective BRAF V600 inhibitor encorafenib in combination with cetuximab alone or cetuximab þ alpelisib for treatment of patients with advanced BRAF-mutant metastatic colorectal cancer. Ann Oncol 2015;26: iv120.

100. Van Geel R, Tabernero J, Elez E et al. A phase Ib dose-escalation study of encorafenib and cetuximab with or without alpelisib in metastatic BRAFmutant colorectal cancer. Cancer Discov 2017; 7: 610–619.

101. Tabernero J, Geel RV, Guren TK et al. Phase 2 results: encorafenib (ENCO) and cetuximab (CETUX) with or without alpelisib (ALP) in patients with advanced BRAF-mutant colorectal cancer (BRAFm CRC). J Clin Oncol 2016; 34: 3544.

102. Krittiya Korphaisarn and Scott Kopetz. BRAF-directed Therapy in Metastatic Colorectal Cancer Cancer J. 2016 May-Jun; 22(3): 175–178. doi: 10.1097/PPO.0000000000000189

103. Sinicrope FA, Okamoto K, Kasi PM, et al. Molecular biomarkers in the personalized treatment of colorectal cancer. Clin Gastroenterol Hepatol. 2016 May; 14(5): 651-658.

104. Huijberts S, Schellens JHM, Elez E et al. BEACON CRC: safety lead-in (SLI) for the combination of binimetinib (BINI), encorafenib (ENCO), and cetuximab (CTX) in patients (pts) with BRAF-V600E metastatic colorectal cancer (mCRC). Annals of Oncology (2017) 28 (suppl_5): v158-v208.

105. Van Cutsem E, Cuyle PJ, Huijberts S et al. BEACON CRC study safety lead-in (SLI) in patients with BRAFV600E metastatic colorectal cancer (mCRC): Efficacy and tumor markers. DOI: 10.1200/JCO.2018.36.4_suppl.627 J Clin Oncol 36, no. 4_suppl (February 1 2018) 627-627.

106. Gao XH, Yu GY, Gong HF, et al. Differences of protein expression profiles, KRAS and BRAF mutation, and prognosis in right-sided colon, left-sided colon and rectal cancer Sci Rep. 2017 Aug 11;7(1):7882. doi: 10.1038/s41598-017-08413-z

107. Wang X, Wei Q, Gao J, et al. clinicopathological features of chinese patients with braf mutated metastatic colorectal cancer: a retrospective observational study Chin J Cancer 2017 36:81

108. Shimada Y, Kameyama H, Nagahashi M, et al. Comprehensive genomic sequencing detects important genetic differences between right-sided and left-sided colorectal cancer Oncotarget. 2017 Aug 24;8(55):93567-93579. doi: 10.18632/oncotarget.20510. eCollection 2017 Nov 7.

109. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21:1350–6.
110. Le DT, Uram JN, Wang H, et al. Programmed death-1 blockade in mismatch repair deficient colorectal cancer. J Clin Oncol. 2016;34:1abstr 103. *** In this paper, the role of immunotherapy in mismatch repair deficient colorectal cancer is analyzed.**
111. Overman MJ, Kopetz S, Lonardi S, et al. Nivolumab ± ipilimumab treatment (Tx) efficacy, safety, and biomarkers in patients (pts) with metastatic colorectal cancer (mCRC) with and without high microsatellite instability (MSI-H): results from the CheckMate-142 study. Ann Oncol. 2016;27(supplement 6):abstract 479P.
112. Durrant DE, Morrison DK. Targeting the Raf kinases in human cancer: the Raf dimer dilemma. Br J Cancer. 2018 Jan;118(1):3-8. doi: 10.1038/bjc.2017.399. Epub 2017 Dec 14.
113. Hong SP, Ahn SK. Discovery of a novel pan-RAF inhibitor with potent anti-tumor activity in preclinical models of BRAFV600E mutant cancer Life Sci. 2017 Aug 15;183:37-44. doi: 10.1016/j.lfs.2017.06.021. Epub 2017 Jun 21.
114. Tutuka CSA, Andrews MC, Mariadason JM, et al. PLX8394, a new generation BRAF inhibitor, selectively inhibits BRAF in colonic adenocarcinoma cells and prevents paradoxical MAPK pathway activation Mol Cancer. 2017 Jun 28;16(1):112. doi: 10.1186/s12943-017-0684-x.

Some BRAF mutations and effects on kinase activity	
Activated	Impaired
K601E	D594G
G469A	D594N
G469V	G466V
L597R	K483M
K601N	D594A
G464V	Y472C
N581S	G496R
L597Q	S602A
L597R	T599A
A598V	T599I
G464E	D594Y
G466A	D594V
G469E	D594E

Table 1.Some BRAF mutations and effects on Kinase activity

Table 2

Retrospective studies regarding BRAF mutation predictive role of anti EGFR activity.

RR: Response Rate; PFS: Progression-free survival; OS: Overall survival

Author (year)	Total number of patients	BRAF mutant patients (%)	Treatment line	Drugs	RR%	PFS (months)	OS (months)	Ref.
Di Nicolantonio et al. (2008)	113	10	≥ second line	• Cetuximab • Cetuximab plus chemotherapy • Panitumumab	0 vs 332 (p=0.02)	1,6 vs 3,6 (p=0.001)	6 vs 12 (p<0.0001)	31
Seymour et al. (2014)	969	29	≥ second line	• Panitumumab plus chemotherapy • chemotherapy	Not reported	Not reported HR=1.4	Not reported HR=1.84	45
Loupakis et al. (2009)	87	15	≥ second line	• Cetuximab plus chemotherapy	0 vs 32 (p=0.016)	2.6 vs 4.4 (p=0.073)	4.1 vs 13.9 (p=0.037)	46
De Roock et al. (2011)	708	4.7	≥ second line	• Panitumumab • Cetuximab • Cetuximab plus chemotherapy • Missing	8,3 vs 38 (p=0.001)	2 vs 6.5 (p<0.0001)	6.5 vs 13.5 (p<0.0001)	49
Kayhanian et al. (2017)	503	12	First line, ≥ second line	• Anti-EGFR • Anti-EGFR plus chemotherapy Chemotherapy • Chemotherapy plus anti-VEGF	Not reported	8.1 vs 9.2 (p=0.571)	18.2 vs 41.1 (p<0.001)	50
Karapetis et al. (2011)	572	3.2	≥ second line	• Cetuximab • Best supportive care	0 vs 14 (p=1)	Not reported (HR=0.76, p=0.69)	1.77 vs 2.97 (p=0.81)	51
Van Cutsem et al. (2011)	1198	6	First line	• Cetuximab plus chemotherapy • Chemotherapy	Not reported	8.0 vs 10.9	14.1 vs 25.1	52
Bokemeyer et al. (2012)	708	9	First line	• Cetuximab plus chemotherapy • Chemotherapy	Not reported	8.0 vs 10.9	14.1 vs 25.1	56

Table 3

Recent and ongoing trials with BRAF inhibitors in BRAF mutant mCRC

Category	Drug names	Investigations, references
First generation BRAFi monotherapy	vemurafenib (PLX4032)	• Cell lines, xenograft models (81) • Phase I (82) • Phase II (83)
	encorafenib (LGX818)	Phase I (53)
New BRAFi monotherapy	LY3009120	Cell lines, xenograft models (112)
	BGB283	Cell lines (112)
	INU-152	Cell lines, xenograf models (113)
	PLX7904	Cell lines (112,114)
	PLX8394	Cell lines (112,114)
BRAFi + anti-MEK	dabrafenib + trametinib	Phase I/II (53,87)
BRAFi + anti-EGFR	vemurafenib + cetuximab	Phase II (89)
BRAFi + anti-MEK + anti-EGFR	dabrafenib + trametinib + panitumumab	Phase I/II (90-94)
	encorafenib + binimetinib + cetuximab	Phase III on going NCT02928224
BRAFi + anti-EGFR + chemotherapy	vemurafenib + cetuximab + irinotecan	• Phase I (93-95) • Phase II (93,96)
BRAFi + anti-EGFR + anti-PIK3CA	encorafenib + cetuximab + alpelisib (BYL719)	• Phase Ib/II • Phase II (53,84,85,98-103)

Table 4

Immunotherapy in BRAF MT mCRC

Author	Study	Drugs
Le et al. (2015)	Phase II study NCT01876511	Pembrolizumab
Overman et al. (2016)	Phase II CHECKMATE 142	Nivolumab Nivolumab + Ipilimumab

Figures 1 The signal transduction from RTK and the two signaling pathways: the mitogen–activated protein kinase (MAPK) pathway and the phosphoinositidyl-3-kinase (PI3K/AKT) pathway.