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BRAF Mutation and Its Importance in Colorectal Cancer

Lee-Jen Luu and Timothy J. Price

Abstract

BRAF mutation is seen in nearly one in ten patients with advanced colorectal cancer. Despite major improvements in survival for advanced colorectal cancer overall, patients with BRAF mutation continue to have a very poor prognosis often with median survival of less than 12 months. It is important for clinicians to be aware of this subgroup as the treatment approach should be different. Treatment options beyond standard chemotherapy are crucial to achieve better outcomes and the role of anti-EGFR therapy alone remains controversial. Current trials assessing combinations of molecular targeted agents have seen some promise. This chapter explores the background of BRAF mutation and current treatment strategies.

Keywords: BRAF, colorectal cancer, V600E mutation

1. Introduction

The RAS/RAF/MEK/ERK signalling cascade, also known as the MAPK (mitogen-activated protein kinase) pathway, is involved in cell proliferation, differentiation, survival and apoptosis [1]. It receives input from multiple sources including internal metabolic stress and DNA damage pathways and altered protein concentrations as well as through signalling from external growth factors, cell-matrix interactions and communication from other cells [2]. This allows for a nodal point for therapeutic targeting, however, dysregulation of this pathway can also increase malignant behaviour [3].

Multiple signals activate RAS (KRAS, NRAS and HRAS), a family of GTPases. This, in turn, activates downstream RAF protein kinases (ARAF, BRAF and CRAF). The dominant substrates of RAF kinases are the MAPK/ERK kinases, MEK1 and MEK2. ERKs phosphorylate a variety of substrates, including multiple transcription factors that regulate several key cellular activities (**Figure 1**).

Mutations in RAS and RAF are the most common oncogenes in human cancer [4]. The focus of this chapter will be on BRAF mutations in colorectal cancer, in particular the V600E mutation, the clinical significance, molecular and clinical pathogenesis as well as treatment, now and into the future.
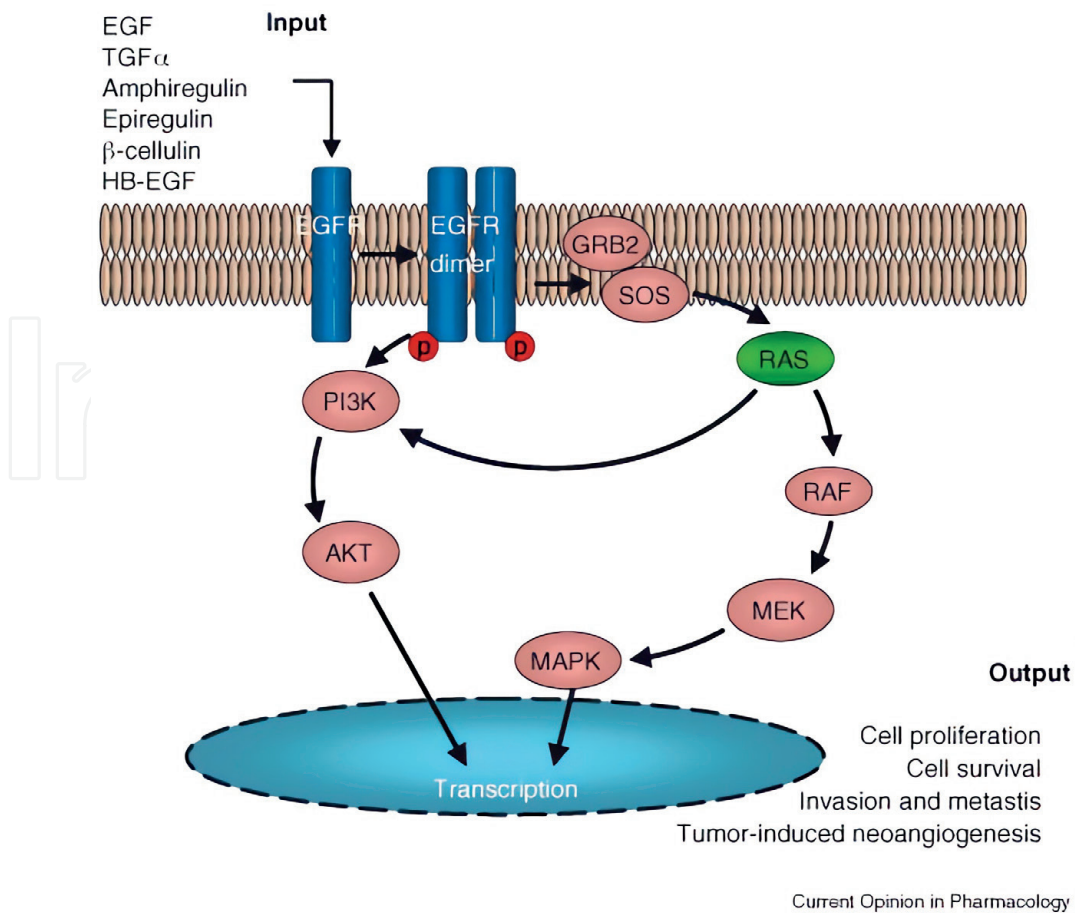
Figure 1.
The MAPK pathway [80].

2. BRAF

The RAF protein is made of three conserved regions: CR1, CR2 and CR3. CR1 and CR2 are situated in the N terminus. CR1 acts as the main binding domain for RAS; CR2 is the regulatory domain. CR3 is situated in the C terminus and functions as the catalytic kinase domain. CR3 contains two regions important for RAF activation: the activation segment and the regulatory region [5]. Of the RAF family of protein kinases, BRAF is the most frequently mutated and remains the most potent activator of MEK.

The BRAF protooncogene, which encodes for the BRAF protein kinase, is located on chromosome 7 (q34) and is composed of 18 exons. There have been more than thirty BRAF mutations identified to date, occurring in various frequencies. The most common is BRAF V600E mutation (MT), which corresponds to a thymine to adenine transversion at position 1799, resulting in the substitution of valine by glutamate at position 600 of the protein [5]. This lies within the activating segment of the kinase domain. It renders BRAF constitutively active, increasing kinase activity relative to BRAF wild-type (WT) by 10 times [6]. Because of this, co-mutations in the MAPK signalling cascade offers no selective advantage for developing tumours and therefore BRAF mutations are mutually exclusive with KRAS or NRAS mutations [7].

The V600E mutation accounts for more than 85% of BRAF mutations in melanoma, more than 50% of the mutations in non-small cell lung cancer and more than 95% of mutations in cholangiocarcinoma and hairy cell leukaemia. It accounts for more than 90% of BRAF mutations in colorectal cancer (CRC) [8]. Other BRAF mutations include R461I, I462S, G463E, G463V, G465A, G465E, G465V, G468A, G468E, N580S, E585K, D593V, F594L, G595R, L596V, T598I, V599D, V599E (V600E), V599K, V599R, V600K, and A727V [9].

3. Prevalence and clinical features of BRAF MT CRC

BRAF mutations have been found in 7–10% of patients with metastatic CRC [7, 10]. BRAF MT CRC has been associated with a particular phenotype in multiple studies and meta-analysis and specifically pertaining to the BRAF V600E mutation. BRAF tumours are more prevalent in women and in patients >70 years of age. BRAF is not associated with age at diagnosis of less than 60 years [11]. BRAF mutation is more prevalent in proximal colon tumours and is rarely found in the left colon [7]. Histopathology also differs, with 60% of BRAF MT tumours being poorly differentiated and a higher rate of mucinous pathology [12]. There is an association with larger primary tumours. BRAF MT CRC is also associated with a high rate of peritoneal metastases and less lung and liver-limited disease [13–15]. In contrast, most non-V600 mutations were more likely to be lower grade and left-sided tumours with a greater overall survival [16, 17], except for codon 601/597 mutations which behave similarly to V600E MT CRC [18].

4. The serrated neoplastic pathway

The pathogenesis of CRC is a heterogeneous and complex process. The classic model of adenoma-carcinoma sequence was initially described by Vogelstein and accounts for approximately 80% of sporadic CRC [19]. Mutation of the tumour suppressor gene, APC, occurs early in the process and additional mutations and chromosomal instability leads to neoplastic progression [20].

The serrated neoplastic pathway is an alternative model of CRC pathogenesis with distinct morphologic and molecular characteristics. It is estimated about 20% of CRC develop via this pathway. These lesions develop from aberrant crypt foci and hyperplastic polyps (HP) into traditional serrated adenoma (TSA) and sessile serrated adenoma (SSA), with malignant potential. BRAF mutation occurs early in the pathway, shown to be present in HP, hyperplastic adenomas and SSA [21].

SSA are also characterised by the CpG island methylator phenotype (CIMP) [22]. A cytosine nucleotide followed by a guanine nucleotide (CpG dinucleotide) can be found in dense clusters (CpG islands) in the promoter regions of approximately half of all genes [23]. Aberrant hypermethylation of these CpG islands can lead to silencing of tumour suppressor genes that, in turn, lead to carcinogenesis. CIMP can be described as high, low or negative. Hypermethylation of the mismatch repair gene *MLH1* results in microsatellite instability (MSI) in sporadic CRC [24].

MSI is implicated in 15% of sporadic CRC and >95% of Hereditary Non Polyposis Colorectal Cancer (HNPCC), also known as Lynch syndrome. It is caused by deficiency of the DNA mismatch repair (MMR) system, composed of multiple interacting proteins including MSH2, MLH1. The majority of sporadic MSI high CRC is due to the hypermethylation of the mismatch repair gene *MLH1* [25]. Sporadic MSI high CRC is also associated with BRAF mutation. BRAF mutations have been observed in 30–50% of MSI high CRC compared with 10% in microsatellite stable tumours [26, 27]. Germline mutations in 1 of 4 mismatch repair genes (MLH1, MSH2, MSH6 and PMS2) account for the majority of cases of HNPCC. BRAF mutations rarely occur in patients with germline mutations in MMR genes [28].

5. Prognostic significance of BRAF mutation

BRAF MT CRC is strongly associated with inferior survival compared with BRAF WT disease. Randomised control trials of first line treatment of metastatic CRC demonstrate differences in OS of up to 12 months, shown in **Table 1**.

Study	Treatment regimen	Key outcomes in BRAF MT disease	Prognostic finding
MRC FOCUS [76]	5-FU/irinotecan or 5-FU/oxaliplatin	HR for OS 1.82 (P = 0.0002)	BRAF predicts poor OS but no difference in PFS
MRC COIN [43]	Fluoropyrimidine/oxaliplatin ± cetuximab	OS 8.8 vs. 20.1 months	Median OS was significantly shorter in patients with BRAF, KRAS or NRAS mutations than in patients with WT KRAS, NRAS, and BRAF tumours, irrespective of treatment (P < 0.0001)
OPUS [77]	FOLFOX ± cetuximab	Median OS, 20.7 months with cetuximab + FOLFOX	Small numbers precluded definitive conclusions
CRYSTAL [78]	FOLFIRI ± cetuximab	Median PFS (cetuximab + FOLFIRI vs. FOLFIRI), 8.0 vs. 5.6 months (HR, 0.934; P = 0.87) Median OS, 14.1 vs. 10.3 months (HR 0.908; P = 0.74)	BRAF MT was strong indicator of poor prognosis
NORDIC-VII [48]	FLOX ± cetuximab	BRAF MT had lower ORRs than BRAF WT (20 vs. 50%; P < 0.001)	BRAF mutations was a strong negative prognostic factor
PRIME [42]	FOLFOX ± panitumumab	Median PFS: Panitumumab + FOLFOX vs. FOLFOX, 6.1 vs. 5.4 months Median OS: Panitumumab + FOLFOX vs. FOLFOX, 10.5 vs. 9.2 months	BRAF mutation was a negative prognostic factor
CAIRO2 [10]	Capecitabine + oxaliplatin + bevacizumab vs. CAPOX + bevacizumab + cetuximab	Lower median PFS, 5.9 and 6.6 months in BRAF-MT vs. 12.2 and 10.4 months in BRAF WT tumours with CAPOX + bevacizumab and CAPOX + bevacizumab + cetuximab, respectively Lower median OS, 15.0 and 15.2 months in BRAF MT vs. 24.6 and 21.5 months in BRAF WT with CB and CBC, respectively	BRAF mutation was a negative prognostic marker
AGITG MAX [79]	Capecitabine ± bevacizumab	Median OS, 20.8 months in BRAF WT vs. 8.6 months in BRAF MT tumours	BRAF mutation was a marker of poor prognosis irrespective of treatment

Table 1.
BRAF mutation as a prognostic factor in clinical studies of first-line treatment of metastatic CRC.

Venderbosch et al. reported a pooled analysis of the CAIRO, CAIRO2, COIN and FOCUS studies examining mismatch repair and BRAF status [29]. BRAF MT was associated with a poor prognosis with OS of 11.4 vs. 17.2 months, and PFS of 6.2 vs. 7.7 months compared with BRAF WT. This analysis also found dMMR to indicate poor prognosis, despite significant evidence to show that MSI-high tumours confer a better prognosis. However, it is concluded that as there is no interaction between BRAF MT and dMMR, the poor prognostic value of dMMR is likely driven by BRAF MT. There was no difference in OS or PFS between dMMR BRAF MT and pMMR BRAF MT tumours. In a study examining RAS and BRAF mutations, BRAF patients had the worst overall survival. The median OS for WT, KRAS, NRAS and BRAF patients were 49.2, 36.2, 30.1 and 22.5 months, respectively [30].

Similarly, BRAF MT has been shown to be a negative prognostic factor in stage II and III disease. Data from the PETACC-3 was extracted, with KRAS, BRAF and MSI status examined [31]. MSI-high tumours were associated with better prognosis. BRAF MT was not prognostic of PFS. The MSI-high status appeared to attenuate the negative prognostic effect of BRAF MT on OS; BRAF MT is a negative prognostic factor in MSS CRC. However, more recently, a meta-analysis of 1164 patients with MSI-high non-metastatic CRC has shown that BRAF V600E mutation does correlate with adverse overall survival, but not disease recurrence [32].

Survival following metastasectomy is also worse for BRAF MT mCRC as demonstrated in a meta-analysis of patients undergoing resection of liver metastases. It showed the BRAF mutation was negatively associated with OS (HR 3.055, $P = 0.00004$) [33].

In contrast, non-V600E BRAF mutations have a different prognosis. BRAF codons 594 and 596 mutations, when compared with V600E BRAF mutations, are more frequently rectal, non-mucinous with no peritoneal spread. In a study of 10 patients, all BRAF 594 and 596 tumours were microsatellite stable. OS was significantly longer (62 vs. 12.6 months, $P = 0.002$) [34]. Jones et al. identified 208 metastatic CRC patients out of 9643 with non-V600E mutations. When compared with V600E BRAF mutation patients, those with non-V600E mutations were found to be younger, more likely male, and had lower grade tumours. In addition, median OS was significantly longer compared with both V600E BRAF mutant and BRAF wild-type patients (60.7 vs. 11.4 vs. 43 months respectively) [35]. This has also been demonstrated in a retrospective study of 98 patients, 6 of whom had non-V600E BRAF mutations. Although only a small sample size, OS was significantly better compared with V600E BRAF MT patients ($P = 0.38$) [17].

6. Treatment of BRAF-mutation CRC

6.1 Standard treatment

Doublet chemotherapy remains the standard of care for metastatic BRAF MT CRC in patients with appropriate performance status [36, 37]. First-line chemotherapy options include 5 fluorouracil, leucovorin and oxaliplatin (FOLFOX), 5-fluorouracil, leucovorin and irinotecan (FOLFIRI) and capecitabine plus oxaliplatin. A retrospective study reported no difference in median PFS between irinotecan-based or oxaliplatin-based chemotherapy regimens in the first line for BRAF-MT CRC [38].

A more intensive triplet chemotherapy regime has been proposed based on 5 fluorouracil, leucovorin, oxaliplatin and irinotecan with bevacizumab (FOLFOXIRI+bev). A phase II trial of FOLFOXIRI and bevacizumab in the metastatic CRC population

showed a statistically significant benefit to progression free survival and trend towards improved overall survival at the expense of greater incidence of grade three toxicities [39]. An exploratory analysis of the BRAF-MT cohort (25 patients in a pooled population) reported a median PFS of 11.8 months, median OS of 24.1 months and an impressive response rate of 72%, including one patient with complete response [40]. This was followed up by the open label phase III TRIBE study comparing FOLFIRI plus bevacizumab with FOLFOXIRI with bevacizumab [41]. In the molecular subgroup analysis, 28 out of 391 cases were BRAF mutant. There was a trend towards benefit in overall survival (19.0 months in the FOLFOXIRI plus bevacizumab arm vs. 10.7 months in the FOLFIRI plus bevacizumab arm, HR 0.54); however, this was not statistically significant. This was also seen in median PFS (7.5 vs. 9.5 months, HR 0.57) and best overall response (56 vs. 42%). While not statistically significant, this regime has been proposed in the first line setting for BRAF-MT mCRC patients with good performance status given the overall survival data.

6.2 EGFR inhibitors

The epidermal growth factor receptor (EGFR) is involved in signalling upstream of the RAS-RAF-MEK-ERK pathway. Monoclonal antibodies directed against EGFR, cetuximab and panitumumab have shown to be effective in metastatic CRC; however, KRAS mutation is a negative predictor of EGFR treatment response and upfront testing is recommended before starting treatment [36, 37].

As previously discussed, KRAS and BRAF mutations are mutually exclusive. Given the common signalling pathway, BRAF mutation has also been proposed to be a negative predictive marker of EGRF antibody treatment response. In the first line setting, the PRIME study evaluated the addition of panitumumab to FOLFOX. In BRAF-MT tumours, panitumumab added no benefit to survival (HR 0.9, P = 0.76) [42]. Similarly, the phase III MRC COIN trial showed no benefit in the addition of cetuximab to first-line oxaliplatin based chemotherapy, irrespective of KRAS or BRAF mutation status [43]. In the second line setting, the PICCOLO study reported no effect of panitumumab in combination with irinotecan on PFS, but a significant negative effect on OS (HR 1.84, P = 0.029). Cetuximab was also evaluated against best supportive care in the phase III CO.17 trial [44]. For BRAF MT tumours, there were no responses and no change to survival in the sample size of 13 (HR 0.84, P = 0.81).

Given the small numbers of BRAF-MT patients in these trials, there have been a number of meta-analyses evaluating the BRAF mutation as a predictive marker of EGFR therapy. Therkildsen et al. reviewed KRAS, NRAS, BRAF, PIK3CA and PTEN mutations in patients with KRAS exon 2 wild-type patients. Of the 1267 patients in 17 studies treated with either cetuximab or panitumumab in both first line and subsequent like therapies, 128 patients had BRAF V600E mutations [45]. There was a significant decrease in overall response rate (17 vs. 45%). BRAF mutation was also linked to shorter PFS (HR 2.95) and OS (HR 2.52) compared to BRAF wild-type tumours.

Pietrantonio et al. examined the impact of cetuximab and panitumumab on PFS, OS and overall response rate (ORR) [46]. This meta-analysis included 9 phase III trials and 1 phase II trial (across first-line, second-line and chemotherapy refractory settings). 463 RAS wild-type/BRAF MT CRC patients were identified. The addition of EGFR antibody therapy did not significantly improve PFS (HR 0.88, P = 0.33), OS (HR 0.91, P = 0.63) and ORR (relative risk 1.31, P = 0.25).

A further meta-analysis was published in 2015 but Rowland et al. [47]. It included 8 randomised control trials that had also been included in the analysis by Pietrantonio et al., but differed by excluding 2 trials; 1 by Tveit et al. [48] due to lack of OS and PFS data and Stintzing et al. [49] as the control arm included

bevacizumab. In addition, the statistical analysis differed as Rowland et al. compared BRAF MT patients with BRAF wild-type. 351 patients were identified with BRAF mutation, of which 330 with the V600E mutation. The HR for PFS was 0.86 for RAS wild-type/BRAF MT compared with 0.62 for RAS wild-type/BRAF wild-type tumours with a test of interaction that nears but does not reach statistical significance (P = 0.07). There was no difference for OS either, the HR for RAS wild-type/BRAF MT tumours was 0.97 compared with 0.81 for RAS wild-type/BRAF wild-type (test of interaction, P = 0.43). It concluded that there was insufficient evidence to definitively state that RAS wild-type/BRAF MT individuals derive a different treatment benefit from EGFR antibodies compared with RAS wild-type/BRAF wild-type patients.

More recently, the triplet chemotherapy regime, FOLFOXIRI, has been studied in combination with panitumumab in the VOLFI trial [50]. This was a randomised phase II trial of patients with RAS WT, unresectable metastatic CRC. 96 patients were included, of which, 16 patients with BRAF MT disease. The primary endpoint was ORR. The addition of panitumumab significantly improved ORR in the overall population (85.7 vs. 54.5%, P = 0.0013), and in the BRAF MT population, there was trend to improved ORR (71.4 vs. 22.2%, P = 0.1262).

Thus, while there exists a significant body of evidence that suggests minimal clinical benefit of EGFR antibody treatment in BRAF MT metastatic CRC, it is not definitive and therefore remains an option for therapy in discussion with the patient. This primarily relates to anti-EGFR as the sole biological agent however anti-EGFR therapy may have a definite role when combined with additional biological agents such as BRAF inhibitors as discussed below.

6.3 BRAF inhibition in mCRC

BRAF represents a therapeutic target in cancer as, unlike KRAS, it is a relatively unidirectional MEK-ERK effector. Inhibition of BRAF with vemurafenib (PLX4032) has been demonstrated to significantly benefit patients with unresectable or metastatic BRAF V600E MT melanoma, improving progression free survival and OS, with a response rate of 48% [51]. In sharp contrast, BRAF inhibition in mCRC is disappointing. An expansion phase II study examined vemurafenib in patients with BRAF MT mCRC who have had at least one line of prior therapy [52]. Of the 21 patients treated, 1 patient had a partial response and 7 other patients had stable disease by RESIST criteria. The median PFS was only 2.1 months and ORR of 5%. Although there were signs of efficacy, the authors concluded that single-agent vemurafenib did not show any meaningful clinical activity in patients with BRAF V600E MT mCRC.

These results were similar to a histology-independent phase II "basket" trial of vemurafenib. 122 patients with BRAF V600 MT malignancies were enrolled into 7 prespecified cohorts, including 37 with mCRC [53]. Vemurafenib, as a single agent, was given to 10 patients with mCRC. Response was poor, with 50% having stable disease and the rest progressing on therapy. The remaining 27 patients with mCRC received combination of vemurafenib and cetuximab, and the results will be discussed later in the chapter.

There are several mechanisms of resistance identified that reduce the efficacy of BRAF inhibition in mCRC. For example Prahallad et al. identified that BRAF inhibition with vemurafenib in mCRC cells causes a rapid activation of EGFR through an ERK-dependent negative feedback loop [54]. Unlike in melanoma, CRC cell lines express high levels of activated EGFR. Blockade of EGFR with either EGFR monoclonal antibodies or small-molecule kinase inhibitors (gefitinib and erlotinib) was showed to work synergistically with BRAF inhibition.

More recently, it has been showed that BRAF inhibition can also lead to up regulation of other receptor tyrosine kinases including human epidermal growth factor receptor (HER) 2 and HER3 [55].

Activation of the phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway has also been implicated in BRAF inhibition resistance [56]. PI3K signalling is activated by direct mutational activation or amplification of PIK3CA and AKT1 or loss of PTEN [57]. Approximately 40% of CRC have been shown to have alterations in 1 of 8 PI3K pathway genes, which are almost always mutually exclusive to each other [58]. Genotyping of BRAF MT CRC has showed concomitant PI3KCA and PTEN mutations [59].

The Wnt/β-catenin pathway is also involved in cell proliferation, differentiation and survival and interacts with the RAS-RAF-MEK-ERK pathway at multiple points. It has been identified as an important step in tumourigenesis and alterations in the Wnt pathway have been identified more frequently in BRAF V600E MT CRC patient samples, potentially representing an alternative pathway of tumour development when BRAF is inhibited [60].

Based on these findings, BRAF inhibition has been combined with a number of different agents in order to attempt to overcome resistance and improve response.

6.4 BRAF and EGFR inhibition

In the fore-mentioned phase II "basket" trial, the effect of vemurafenib and cetuximab evaluated in 27 patients with BRAF V600 MT mCRC. The result was marginally improved compared to single-agent treatment. One patient had a partial response (4% ORR) and 69% had stable disease. Median PFS was 3.7 months and median OS was 7.1 months. A pilot trial with combination panitumumab and vemurafenib included 15 patients with BRAF V600E mCRC who had received at least 1 prior line of therapy [61]. 2 patients had confirmed partial response and 6 patients had stable disease, including 2 patients with stability lasting over 6 months. The treatment was well tolerated with fatigue and rash being the most frequently observed adverse events.

Other combinations of BRAF and EGFR inhibitors have also been investigated including vemurafenib plus erlotinib [62], encorafenib (LGX818, a highly selective ATP-competitive small molecule RAF kinase inhibitor) plus cetuximab [63] and dabrafenib (a small molecule kinase BRAF inhibitor) plus panitumumab [64]. Response rates in these trials range from 4 to 23%. To improve this outcome, the combination has been combined with chemotherapy in the randomised phase 2 SWOG 1406 study [65]. Interim results of this trial were presented in 2017. The combination of irinotecan and cetuximab with or without vemurafenib was examined in 106 patients. Median PFS was significantly improved with the addition of vemurafenib (4.4 vs. 2.0 months, P < 0.001). Response rate increased from 4 to 16% (P = 0.09). However, there was an increase in grade 3 and 4 adverse events including neutropenia, anaemia and nausea. It was noted that no new safety signals. The data on median OS was immature. Based on these findings, this treatment regime has been included in treatment guidelines [36].

6.5 BRAF and MEK inhibition

BRAF and MEK inhibition has been combined in melanoma with greater efficacy and so has been evaluated in the BRAF MT mCRC population. 43 patients were treated with dabrafenib and trametinib [66]. 1 patient achieved a complete response and 4 patients had a partial response (ORR 12%). 24 patients achieved stable

disease (56%). During-treatment biopsies in 9 patients showed reduced levels of ERK compared with pre-treatment biopsies. It is suggested that combination BRAF and MEK inhibition could be a potential therapeutic backbone for the addition of other agents including EGFR inhibitors.

6.6 BRAF, MEK and EGFR inhibition

Given the role of MEK and ERK in EGFR activation leading to BRAF inhibitor resistance, the triplet combination of BRAF, MEK and EGFR inhibitors have been investigated. Corcoran et al. reported on a trial involving 3 cohorts, dabrafenib and panitumumab (n = 20), dabrafenib, trametinib and panitumumab (n = 91), and trametinib and panitumumab (n = 31) [67].

The ORR for triplet therapy was 21%, compared with 0% with trametinib and panitumumab and 10% with dabrafenib and panitumumab. With the increase in response rate, there was also a corresponding increase in adverse events. 70% of patients on triplet therapy had a grade 3 or 4 adverse event. 18% of patients had an adverse event resulting in study discontinuation, 54% had an adverse event that resulted in dose reduction, and 71% of patients had an adverse event that led to dose interruption or delay. Skin toxicity including rash and dermatitis acneiform occurred in 90% of patients, with 21% having grade 3 or 4 adverse events. Paired pre-treatment and on-treatment biopsies demonstrated that triplet combination produced greater inhibition of ERK than the dabrafenib and panitumumab doublet or the dabrafenib and panitumumab doublet.

It has been suggested that BRAF inhibitors may offset the dermatologic toxicity resulting from MEK or EGFR inhibitors. Mondaca et al. reported on a case of BRAF V600E MT metastatic CRC on clinical trial with dabrafenib, trametinib and panitumumab [68]. Dabrafenib dose reductions for neutropenia were associated with increased skin toxicity, which subsequently improved with increasing the dose. This case highlights the importance of dose intensity of BRAF inhibitors with used in combination regimens.

6.7 Other therapeutic strategies and current trials

Current therapeutic investigations in the BRAF MT mCRC field involve multiple targeted therapies aimed at overcoming acquired resistance to MAPK pathway inhibition.

One such combination is encorafenib, cetuximab and alpelisib. Alpelisib (BYL719) specifically inhibits the alpha subunit of PI3K. A phase 1b dose escalation study included 2 arms, encorafenib plus cetuximab vs. triplet therapy with encorafenib, cetuximab and alpelisib [69]. Triplet therapy was showed to be active with an ORR of 18% and impressively a disease control rate of 92.8%. This combination has been investigated further in a phase 2 trial [63]. 102 patients with refractory BRAF MT CRC were randomised to doublet or triplet therapy. Progression free survival was the primary endpoint. Interim data following 73 events were released and showed no statistical difference between doublet and triplet therapy with HR 0.69 (P = 0.064) and median PFS of 4.2 vs. 5.4 months. Grade 3 and 4 adverse events were higher in the triplet arm, including anaemia and hyperglycaemia. Further investigations with other PI3K inhibitors are currently underway; however, the efficacy of PI3K inhibition remains unclear (NCT01337765, NCT01363232).

Other potential targets include BRAF and AKT inhibition [70], BRAF, EGFR and HER2 inhibition [55], ERK inhibition alone or in combination with BRAF inhibition [71] and Wnt/β-catenin pathway inhibition (NCT02278133) **Table 2**.

ClinicalTrials. gov number	Therapeutic strategy	Agents investigated	Study design	Status
NCT02928224	BRAF + EGFR + MEK inhibition	Encorafenib + cetuximab ± binimetinib	Phase 3, randomised, open label	Recruiting
NCT02906059	Chemotherapy + selective Wee 1 inhibitor	Irinotecan + AZD1775	Phase 1b	Recruiting
NCT01351103	PORCN inhibitor + immunotherapy	LGK974 ± PDR001	Phase 1	Recruiting
NCT01640405	Antiangiogenesis agent + cytotoxic chemotherapy	FOLFOX + bevacizumab vs. FOLFOXIRI + bevacizumab	Phase 3 open label	Active, not recruiting
NCT01750918	BRAF + EGFR + MEK inhibition	Dabrafenib + panitumumab vs. dabrafenib + trametinib + panitumumab Dabrafenib + panitumumab vs. dabrafenib + trametinib + panitumumab vs. 5-fluorouracil-based chemotherapy + monoclonal antibody Trametinib + panitumumab	4 part phase 1/2, open label	Active, not recruiting
NCT01719380	BRAF + EGFR + PI3K inhibition	Encorafenib + cetuximab + alpelisib	Phase 1b/2, open label, dose escalation	Active, not recruiting

Updated 7th November 2018.

Table 2.
Ongoing studies investigating different treatment strategies for BRAF MT mCRC.

Immunotherapy also plays a role in the management of metastatic CRC [36]. Pembrolizumab and nivolumab are immune check point inhibitors against programmed death 1 (PD-1) that have demonstrated significant activity against MSI-high mCRC [72, 73]. Given the strong association between MSI-high and BRAF MT CRC, this represents a possible therapeutic option. The initial trial of pembrolizumab in MSI-high CRC did not include BRAF MT cases; however, a case report does suggest activity in the MSI-high BRAF MT population [74].

Nivolumab and combination nivolumab with ipilimumab (cytotoxic T-lymphocyte associated protein 4 inhibitor) in MSI-high/dMMR CRC was examined in the phase 2 CheckMate 142 study [73, 75]. 12 of the 74 patients receiving nivolumab harboured a BRAF mutation. An objective response was seen in 3 patients (25%) and 9 patients achieved disease control for greater than 12 weeks. The ORR for combination immunotherapy was greater at 55% in patients with MSI-high BRAF MT CRC, and disease control rate of 79%. Safety data was not reported by mutation status, however, appeared manageable, with 32% experiencing a grade 3 or 4 adverse event, most commonly raised AST. Discontinuation due to a treatment related adverse event was 13%.

7. Conclusion

BRAF V600E mutations are present in 7–10% of CRC. It represents a population with poor prognosis and a particular clinical phenotype, being more prevalent in

women, older than 70 years of age, associated with poorly differentiated histology and right-sided tumours. Chemotherapy with the addition of anti-angiogenesis agent remains the current standard of care in the first line metastatic setting. More aggressive, triplet chemotherapy (FOLFOXIRI) may be appropriate in the selected patient. BRAF inhibition has been extensively investigated for second line therapy and beyond and when in combination with EGFR, MEK and PI3K inhibitors have increased response rates, however, PFS and OS remains poor. Ongoing research remains important to improve outcomes in BRAF MT CRC.

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