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# **New therapeutic opportunities based on DNA Mismatch Repair and *BRAF* Status in Metastatic Colorectal Cancer**

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*Cohen et al., DNA Mismatch Repair and BRAF Status in Metastatic Colorectal Cancer: New therapeutic opportunities?*

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**Key Words:** microsatellite instability, BRAF mutation, immune checkpoint, PD-1, PD-L1

## **Abstract**

Recently, colorectal cancer (CRC) subtyping consortium identified four consensus molecular subtypes (CMS1-4). CMS1 is enriched for deficient mismatch repair (dMMR) and *BRAF*<sup>V600E</sup> tumors. Intriguingly, this subtype has better relapse-free survival but worse overall survival after relapse compared with the other subtypes. Growing evidence is accumulating on the benefit of specific therapeutic strategies such as immune checkpoint inhibition therapy in dMMR tumors and MAPK pathway targeted therapy in tumors harboring *BRAF*<sup>V600E</sup> mutation. After reviewing dMMR prognostic value, immune checkpoints as major targets for dMMR carcinomas will be highlighted. Following, *BRAF*<sup>V600E</sup> prognostic impact will be reviewed and therapeutic strategies with the combination of cytotoxic agents and especially the combinations of BRAF and MAPK inhibitors will be discussed.

## Introduction

CRC is a biologically heterogeneous disease that arises through distinct pathways including chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP). CIMP phenotype, with some degree of overlap with CIN and MSI, represents a specific type of epigenetic instability that leads to aberrant gene silencing [1]. MSI phenotype is caused by deficient DNA mismatch repair (dMMR) function resulting from an epigenetic inactivation of *MLH1* or from a germline mutation of one of the MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) predisposing to the Lynch' syndrome (LS).

Sporadic dMMR CRC, but not LS, is frequently associated with the v-raf murine sarcoma viral oncogenes homolog B1 (*BRAF*)<sup>V600E</sup> mutation. Replacement of valine by glutamic acid at position 600 within *BRAF* gene makes mutant BRAF protein constitutively active, inducing activation of MAP kinase pathway through the phosphorylation of mitogen-activated protein kinase (MEK) downstream. Colorectal cancer subtyping consortium (CRCSC) identified four distinct consensus molecular subtypes (CMS) of CRC based on genetic and epigenetic analysis [2,3]. CMS1 includes dMMR and/or *BRAF*<sup>V600E</sup> CRC and is associated with proximal location, immune activation, older age at diagnosis and female gender. CMS2 tumors exhibit high CIN, proficient MMR (pMMR), *P53* mutation and/or WNT/MYC pathway; tumors with low CIN, *KRAS* mutation and/or phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutation fall in CMS3; CMS4 tumors are characterized by transforming growth factor (TGF)-beta and/or vascular endothelial growth factor (VEGF) pathways activation and associated with mesenchymal phenotype and younger age at diagnosis. CRC with dMMR and/or *BRAF*<sup>V600E</sup> represent subtype with a poor survival after relapse (SAR) despite a favorable disease-free survival (DFS) among the four subtypes [2]. Interestingly, dMMR CRC has been recently shown as an attractive target of immunotherapy [4].

This review is focused on dMMR and  $BRAF^{V600E}$  in metastatic CRC (mCRC). In the first part, a comprehensive overview of prognostic impact of dMMR status and of recent data of immune checkpoint modulation for dMMR mCRC is provided. In the second part, prognostic and predictive values of  $BRAF^{V600E}$  mutation are presented followed by an update of the clinical results of targeted therapeutic strategies for  $BRAF^{V600E}$  mCRC.

## **Mismatch repair deficiency**

### Early stage colorectal cancer

Colorectal tumors with dMMR can be detected through immunohistochemistry or polymerase chain reaction-based assay (Box 1). These tumor type is commonly associated with proximal location, high grade mucinous differentiation, and prominent lymphocyte infiltration [5]. Data show that dMMR status confers improvement DFS in patients with stage II or III CRC [6–8] and that 5-fluorouracile (5-FU) based chemotherapy is ineffective in patients exhibiting stage II dMMR tumors [6,7,9]. Other studies have shown that in patients with high-risk stage II and stage III tumors who received oxaliplatin-based adjuvant chemotherapy (*i.e.* FOLFOX), dMMR status conferred DFS benefit (André et al., in press, Journal of Clinical Oncology 2015 ) [10,11]. Interestingly, Collura et al. showed that large biallelic deletion in the T17 intron of the gene that encodes chaperone protein HSP110 sensitizes CRC to 5-FU alone or 5-FU plus oxaliplatin in the adjuvant setting and affect survival of patients with stage II-III tumors [12,13]. Given that this mutation confers an important fraction of (about 25%) of patients with stage II and III dMMR CRC these findings lead to reflection of the genetic features importance in chemosensitivity analysis of these tumors.

### Metastatic colorectal cancer

The impact of dMMR status on survival of patients with CRC has not been fully elucidated. The poor prevalence of dMMR in patients with mCRC (3%-5%) reinforces the low metastatic capacity of dMMR tumors and hampers the evaluation of dMMR status as a prognostic biomarker in mCRC. Indeed, the results from early studies of microsatellite instability and BRAF mutation on survival in mCRC remain inconclusive or inconsistent [14–17]. However, the pooled analysis of four phase III studies in first-line treatment of mCRC, the CAIRO, CAIRO2, COIN, and FOCUS studies, by Venderbosch et al. demonstrated that dMMR is associated with poorer overall survival (OS) (Table 1) [18]. Among 3063 patients with stage IV CRC, those who had dMMR tumors (5%) exhibited significantly reduced progression-free survival (PFS) and OS (HR 1.33; 95% confidence interval [CI], 1.12-1.54 and HR 1.35; 95% CI, 1.13-1.61), respectively. Although the analysis was not sufficiently powered to test the interaction between dMMR and *BRAF*<sup>V600E</sup> status, the poor prognosis of dMMR mCRC seemed to be driven by *BRAF*<sup>V600E</sup> mutation (see below) [18]. The authors also suggested that the worse prognostic value observed in *BRAF*<sup>V600E</sup> tumors may be related to the pattern of metastatic spreading. Indeed, the low frequency of liver metastasis [17] and high rate of peritoneal disease [19] have been reported in dMMR tumors.

#### Therapeutic perspectives for treatment of dMMR mCRC

The immune checkpoints therapeutic strategies, such as inhibition of anti-programmed death 1 (PD-1) receptor or its ligand (PD-L1) can be considered really breakthrough agents in the targeted treatment of dMMR mCRC. A recently published phase II trial showed that patients with dMMR CRC (*N*=11) are more responsive to anti-programmed death 1 (PD-1) antibody pembrolizumab than are pMMR CRC patients (*N*=21) [4]. The immune-related objective response rate and the 20-week immune-related PFS rate were 40% and 78% for dMMR mCRC versus 0% and 11% for pMMR mCRC, respectively. According to immune-related



response criteria, new lesions did not constitute disease progression if tumor burden, including new lesions, was stable or decreased. Hazard ratio for PFS was 0.10 ( $P < 0.001$ ) and for OS was 0.22 ( $P = 0.05$ ). Results in patients with dMMR non-CRC ( $N = 9$ ) were similar to those with dMMR tumors. Interestingly, whole-exome sequencing analysis of dMMR and pMMR tumors revealed 20-fold more somatic mutations (inducing much more tumor-specific neoantigens) in dMMR CRC as compared with pMMR CRC [4,20]. These results suggested that specific immune response elements are linked to tumor genomics that is dMMR tumors may be much more responsive to checkpoint blockage with anti-PD-1 due to their incapacity to repair DNA mismatches. Higher tumor neoantigen load was associated with tumor-infiltrating lymphocytes and improved survival outcomes [21,22].

These data along with the previous observation of dense immune infiltration in those tumors support the hypothesis that dMMR CRC may be an attractive target for immunotherapy [5]. Galon and colleagues first established that the immune context as defined by the type, the density, and the location of immune cells in CRCs has the prognostic value, highlighting T-helper (Th)1-related adaptive immunity (characterized by interferon- $\gamma$  production) [23–25]. This finding was confirmed by Lal et al. on an immune gene signature which delineated dMMR CRC as a specific subgroup exhibiting high expression of Th1-related immune genes and immune checkpoint-related genes [26]. Interestingly, this active immune microenvironment is counterbalanced by the overexpression of several immune checkpoint genes including PD-1, PD-L1, cytotoxic T lymphocyte antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), and indoleamine 2,3-dioxygenase (IDO) [27], making sense of immune checkpoint inhibition in dMMR CRC.

The remarkable efficacy of pembrolizumab in dMMR CRC reported in phase II study by Le et al. is an exciting discovery that opens up an entirely new field of investigations [4]. One must keep in mind that this was a very small trial where median PFS and OS for dMMR

cohorts were not reached after a median follow-up of 36 weeks, thus larger study will be needed to confirm the findings and clarify specific issues such as effect on OS and PFS in dMMR cohort. Of note, PD-L1 expression within tumor or microenvironment was not significantly associated with either better PFS or OS. Further studies are needed to explore the PD-1 and PD-L1 expression and tumor-infiltrating immune cells as potential biomarkers of efficacy in dMMR mCRC. A phase II clinical trial of nivolumab (another anti-DP-1 antibody) and nivolumab plus ipilimumab in patients with dMMR mCRC is ongoing (ClinicalTrials.gov Identifier: NCT02060188). Moreover, the relation between  $BRAF^{V600E}$  status and efficacy of immune checkpoints inhibition in sporadic cancers with dMMR also warrants further exploration. Combining immunotherapy with targeted therapy in  $BRAF^{V600}$  dMMR mCRC may be an interesting approach which should be investigated. Finally, immune checkpoint inhibitors should be evaluated for efficacy in patients with constitutional MMR deficiency syndrome (CMMRD). CMMRD, variant of LS, is a rare condition that results from bi-allelic germline mutations of the MMR genes and is associated with a broad spectrum of childhood cancers, including CRC, hematologic malignancies, and brain tumors [28].

### ***BRAF*-mutated colorectal cancer**

The serine/threonine-protein kinase BRAF is a downstream signaling protein in the EGFR-mediated MAPK pathway that exerts its oncogenic effect through the induced phosphorylation and activation of MEK. As with *RAS* mutations, mutation of codon 600 within the activation segment of the kinase domain of BRAF causes constitutive activation of the MAPK pathway. Approximately 10% of CRC are *BRAF*-mutated, with V600E mutation in 87%-93% of cases. The V600E *BRAF*-mutated CRC are located predominantly in the right side of the colon, in older women, and typically arise from serrated adenomas and exhibit

high grade of differentiation.  $BRAF^{V600E}$  mutation is strongly associated with dMMR (20%-70% of dMMR tumors versus 5-10% of pMMR tumors) [18,29] and indicates a sporadic origin [30]. The gold standard for diagnostic analysis of a  $BRAF^{V600E}$  mutation is currently direct sequencing. However, the clinical impact of the very rare non- $BRAF^{V600E}$  mutations, such as  $BRAF$  codons 594 and 596 mutations, has been infrequently evaluated. The analysis of ten patients with mCRC harboring  $BRAF$  codons 594 or 596 mutated tumors suggested different pathological characteristics and clinical outcomes from these of  $BRAF^{V600E}$  tumors [31]. Longer OS was reported in patients with  $BRAF$  codons 594 or 596 mutated tumors when compared with  $BRAF^{V600E}$  mutated CRCs [median OS: 62.0 versus 12.6 months; hazard ratio: 0.36 (95% confidence interval 0.20-0.64),  $P = 0.002$ ].

#### Prognostic impact of $BRAF^{V600E}$ mutation in early CRC

The  $BRAF^{V600E}$  mutation is present in 6%-15% [8,32–36]. Analysis of the QUASAR study, Intergroup 0-135, and MOSAIC trials data did not show any significant prognostic impact of  $BRAF$  status on DFS (André et al., in press, Journal of Clinical Oncology 2015) [8,36]. In a pooled analysis of the NSABP C-07 and C-08 trials,  $BRAF^{V600E}$  mutation was associated with poor OS (HR 1.46,  $P < 0.0002$ ) and poor SAR (HR 2.31  $P < 0.0001$ ) and not DFS (HR, 1.02,  $P = 0.86$ ) [37]. The translational study on the PETACC-3, EORTC 40993, and SAKK 60-00 trial showed that  $BRAF$  tumor V600E mutation significantly decrease OS in patients with pMMR tumors (HR 2.2,  $P = 0.0003$ ) [32]. Furthermore, a pooled analysis of a large cohort of 3934 patients with pMMR stage III CRC from the PETACC-8 and N0147 trials showed that  $BRAF^{V600E}$  was an independent predictor of shorter time to recurrence, SAR, and OS [38]. These results strongly suggest that  $BRAF^{V600E}$  mutation is associated with poor prognosis in patients with pMMR tumors. However, the positive prognostic impact of dMMR status in stage II and III CRCs seems not significantly related to the presence of  $BRAF^{V600E}$  mutation.

The strong inter-relation between  $BRAF^{V600E}$  mutation and dMMR status is particularly interesting given that these two biomarkers exhibit opposite prognostic effects in mCRC. Thus dMMR status may reduce the risk of recurrence induced by  $BRAF^{V600E}$  CRCs, but its positive prognostic value may be eclipsed by the impact of  $BRAF^{V600E}$  after relapse, explaining the association of  $BRAF^{V600E}$  mutation with SAR and OS but not with DFS.

#### Prognostic impact of $BRAF^{V600E}$ mutation in mCRC

$BRAF^{V600E}$  is associated with a higher frequency of peritoneal and distant lymph node metastases and a decreased lower rate of lung metastases compared to  $BRAF$  wild-type tumors ( $BRAF^{WT}$ ) [17,19]. This distinct pattern of metastatic spread may be a possible explanation for the poor prognostic impact of  $BRAF^{V600E}$  in mCRC. Indeed, the analysis of the NSABP C-07 and C-08 trials reported by Gavin et al. clearly showed that  $BRAF^{V600E}$  was associated with poor prognosis after relapse in stage II and III CRC [37]. In a pooled analysis of the CAIRO, CAIRO-2, COIN, and FOCUS trials, prevalence of  $BRAF^{V600E}$  was 8.2% in mCRC [18]. Median PFS and OS were significantly reduced for patients with  $BRAF^{V600E}$  compared with  $BRAF^{WT}$  tumors (HR 1.34,  $P<0.001$  versus HR 1.91,  $P<0.001$ ). In pMMR mCRC stratified by  $BRAF^{V600E}$  status, the median OS was significantly decreased in patients with  $BRAF^{V600E}$  compared to those with  $BRAF^{WT}$  tumors (11.3 months versus 17.3 months; HR 1.94,  $P<0.001$ ). In dMMR mCRC, no statistically significant difference was observed for OS between two groups of patients (11.7 months in  $BRAF^{V600E}$  versus 15 months in  $BRAF^{WT}$  tumors; HR 1.51, 95% CI 0.93-2.46). But in patients with  $BRAF^{V600E}$  mCRC OS was poor, regardless of their dMMR status (HR 1.05, 95% CI 0.68-1.63). In  $BRAF^{WT}$  mCRC, the median OS was no significantly different between patients with dMMR and pMMR tumors (HR 1.22; 95% CI 0.91-1.65). Thus, pejorative prognostic impact of dMMR status in mCRC seems to be driven by the  $BRAF^{V600E}$  status.

The prevalence of  $BRAF^{V600E}$  in dMMR mCRC observed by Venderbosch (a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies) [18] was higher than that reported in early-stage dMMR CRC (34.6% versus 24% in the PETACC-3 trial, 30% in the QUASAR trial) [8,32]. The observed increase of prevalence between localized and advanced dMMR CRC reinforces  $BRAF^{V600E}$  mutation as a significant poor prognostic factor in dMMR tumors.

#### Predictive impact of $BRAF^{V600E}$ status in mCRC

Constitutively active  $BRAF^{V600E}$  mutations are almost always mutually exclusive of  $KRAS$  mutations. The activating mutations in  $KRAS$  induce constitutive Ras/MAPK signaling, which cannot be suppressed by epidermal growth factor receptor (EGFR) inhibition. It has been suggested that  $BRAF^{V600E}$  may be predictive of resistance to anti-EGFR monoclonal antibody therapies, although this association remains controversial, but its negative predictive prognostic value has been established. The analysis of patients with  $BRAF^{V600E}$  mCRC of the CRYSTAL phase III trial showed no significant difference, but a trends of better PFS or OS between patients treated with FOLFIRI alone and FOLRIRI plus cetuximab in first-line setting (HR 0.93, 95% CI 0.42-2.06 and HR 0.91, 95% CI 0.51-1.62) [39]. Similar results were observed in the PRIME and FIRE-3 phase III trials [40–42]. Anti-EGFR monoclonal antibody survival benefit was observed in patients with  $BRAF^{V600E}$  tumors (Table 2). In pre-treated  $BRAF^{V600E}$  mCRC,  $BRAF^{V600E}$  status did not discriminate between responders and non-responders to anti-EGFR therapy [43–47]. In addition, there is insufficient evidence to conclude that patients mCRC do not benefit from anti-EGFR monoclonal antibody in the presence of  $BRAF^{V600E}$  as shown in the meta-analysis of randomized controlled trials of CRC patients reported by Rowland and colleagues [48]. Based on these findings, there is yet no sufficient data that would justify the exclusion of anti-EGFR monoclonal antibody therapy for patients with  $RAS^{WT}/BRAF^{V600E}$  mCRC.

### Targeting $BRAF^{V600E}$ mCRC

Considering that patients with  $BRAF^{V600E}$  mCRC have a lower probability of receiving further lines of chemotherapy because of their poor prognosis [49], further intensification of standard therapy may be a pragmatic and an efficient therapeutic approach to overcome this issue. The intensification of the treatment with the triplet regimen FOLFOXIRI (5-FU, leucovorin (LV), oxaliplatin, and irinotecan) in combination with bevacizumab has been evaluated for chemotherapy-naïve CRC patients in phase II and III studies with encouraging results [50-54]. In the phase II study by Masi et al. median PFS was 12.8 months and OS was 23.8 months for FOLFOXIRI/bevacizumab in  $BRAF^{V600E}$  mutated patients ( $n=10$ ) [50]. The addition of bevacizumab to FOLFOXIRI regimen increased treatment efficacy in the a pooled analysis of 25  $BRAF^{V600E}$  patients reported by Salvatore and colleagues [51]. At a median follow-up of 34.1 months, the pooled set of patients showed a median PFS of 11.8 months and a median OS of 23.8 months. In the phase III TRIBE trial patients were randomized either to FOLFOXIRI/bevacizumab or FOLFIRI/bevacizumab. In this study, patients with  $BRAF^{V600E}$  mCRC showed a non-significant increase of OS when treated with FOLFOXIRI/bevacizumab (19 months versus 10.7 months; HR 0.54, 95% CI 0.24-1.20). FOLFOXIRI/bevacizumab treatment was tolerable with 8.8% febrile neutropenia and 18.8% grade 3-4 diarrhea [52-54]. These data suggest that FOLFOXIRI plus bevacizumab is a serious and valid option for the first-line treatment of chemotherapy-naïve fit patients with  $BRAF^{V600E}$  mCRC.

Vemurafenib, an oral inhibitor of the mutant BRAF kinase achieved minimal clinical activity in  $BRAF^{V600E}$  advanced melanoma patients, showing a significant benefit in response rates, PFS, and OS [55,56]. However, vemurafenib alone achieved minimal clinical activity in  $BRAF^{V600E}$  mCRC patients [57]. Prahallad and colleagues demonstrated that  $BRAF^{V600E}$  inhibition causes a rapid feedback activation of EGFR in CRC cell lines [58]. Based on this

observation, the combination of vemurafenib with an anti-EGFR therapy or a MEK inhibitor may be more effective way to overpass BRAF inhibitor primary resistance in  $BRAF^{V600E}$  mCRC in the clinical setting. Two recent studies showed that the combination of a BRAF inhibitor dabrafenib and a MEK inhibitor trametinib significantly improved OS in patients with  $BRAF^{V600E}$  metastatic melanoma. The combination significantly improved OS compared with single-agent inhibition [59,60]. Moreover, dual BRAF-MEK inhibition by dabrafenib and trametinib was shown to reduce the single-agent cutaneous toxicities probably due to their opposing effects on cellular functions and signaling. In cells expressing  $BRAF^{WT}$  such as epidermal keratinocytes, BRAF inhibitors binding to one member of RAF homo/heterodimers inhibit one promoter, but paradoxically transactivate the drug-free protomer, which results in increased downstream the RAF-MEK-ERK signaling, while the combination of EGFR and MEK inhibitors effectively block the MAPK signaling pathway [61,62]. Several drug combinations are currently tested in  $BRAF^{V600E}$  mCRC (Table 3). Combined inhibition with dabrafenib and trametinib showed limited activity in  $BRAF^{V600E}$  stage IV mCRC with a response rate of 12%, including one prolonged complete response (> 22 months) in phase I/II trial [63]. The further logical next step for treatment optimization was the “horizontal” and “vertical” inhibition of MAPK pathway with the triplet therapy including dabrafenib, trametinib, and panitumumab [64]. Atreya et al. conducted phase I/II trial of this combination in BRAF mutated mCRC. Overall, 11%, 36%, and 54% of patients treated with the combination of panitumumab and dabrafenib, the triple regimen, and the combination of trametinib plus panitumumab, respectively, required dose reductions or interruptions. The greatest proportion of serious dermatologic toxicities with the combination of panitumumab and trametinib without the BRAF inhibitor were reported, thus that addition of dabrafenib to the doublet lessened skin-related toxicity. Overall, 26% of confirmed response rates with one confirmed complete response were observed [64]. The triplet therapy therefore appears to be

more active in  $BRAF^{V600E}$  mCRC than the double combination of BRAF and MEK inhibitors, but is associated with significant skin toxicities. The combination of panitumumab with vemurafenib for  $BRAF^{V600E}$  mCRC has shown interesting hints of enhanced clinical activity in a pilot trial by Yaeger et al. [65]. Two of 12 evaluable patients (13%) had confirmed long-lasting partial responses. The combination was well tolerated with less cutaneous toxicity than expected with either agent. The activity of the triplet combination of vemurafenib with cetuximab and irinotecan for the treatment of 19 BRAF-mutated mCRC was evaluated in a phase Ib trial [66]. The majority of patients (74%) had received prior irinotecan and nearly half had prior exposure to cetuximab. Confirmed response rate was 53% and median PFS was 7.7 months. These results form the basis for the ongoing phase 2 Intergroup Study S1402 trial of irinotecan and cetuximab with or without vemurafenib in cetuximab-naïve  $BRAF^{V600E}$  patients (ClinicalTrials.gov Identifier: NCT02164916). MO29112 is an ongoing phase II trial evaluating a biomarker-driven maintenance strategy for FOLFOX plus bevacizumab first-line therapy in patients with  $BRAF^{V600E}$  mCRC (ClinicalTrials.gov Identifier: NCT02291289). Patients with  $BRAF^{V600E}$  mCRC without progressive disease after induction with the FOLFOX plus bevacizumab combination are randomized to receive either 5-FU/LV with cetuximab and vemurafenib or fluoropyrimidine (5-FU/LV or capecitabine) plus bevacizumab. Patients without targetable tumor biomarker will receive fluoropyrimidine and bevacizumab with or without MPDL3280A (anti-PD-L1 antibody).

Considering that poor prognosis conferred by  $BRAF^{V600E}$ , clinical trial enrollment should be systematically considered and planned so that patients with tumors harboring this mutation have the opportunity to receive these innovative-targeted therapies.

## Conclusion



Given the high frequency of CRC worldwide, dMMR and  $BRAF^{V600E}$  CRC still constitutes meaningful group of patients (9 to 11% of patients with mCRC). New therapeutic strategies are urgently needed for those patients. dMMR and  $BRAF^{V600E}$  are closely interlinked, with specific prognostic and predictive values for both of them. Considering the metastatic setting, new horizons have been opened in the field of dMMR mCRC through immune checkpoint inhibitors, which may be probably the next revolution for patients with dMMR carcinomas. Further studies will have to confirm these attractive results and investigate biological mechanisms underlying sensitivity of dMMR carcinomas to immunotherapy. Dramatic prognosis conferred by  $BRAF^{V600E}$  mutation stresses the urge for new therapeutic strategies. Targeting  $BRAF^{V600E}$  appears challenging in the context of mCRC and combinations of BRAF inhibitors with other MAPK inhibitors and cytotoxic agents have to be evaluated. For these reasons, patients with dMMR or  $BRAF^{V600E}$  mCRC have to be systematically identified because of the potential innovative therapeutic opportunities offered in the ongoing clinical trials.

## **Boxes**

Box 1: How to determine dMMR status?

DNA mismatch repair (MMR) machinery consists of a complex of proteins MutL homolog 1 (MLH1), MutS homolog 2/ (MSH2/), and postmeiotic segregation increased 2 (PMS2), which form heterodimers MutL $\alpha$  (MLH1-PMS2) and MutS $\alpha$  (MSH2-MSH6). Mutations of the MMR genes result in loss of expression of the corresponding protein and may be detected by immunohistochemistry (IHC). Considering the predominance of *MLH1* and *MSH2* gene mutations in Lynch syndrome and the epigenetic inactivation of *MLH1* in sporadic dMMR colorectal cancers (CRC), IHC with anti-MLH1 and anti-MSH2 antibodies can be used to detect MMR-deficient (dMMR) tumors [67]. MLH1 and MSH2-negative tumors are systematically associated with loss of MLH1/PMS2 and MSH2/MSH6 function (MSH6 unstable in the absence of MSH2), respectively [68]. Isolated absence of MSH6 or PMS2 protein do not always produce loss of their corresponding partner, however these are rare cases. A potential drawback to IHC is that it may fail to detect dMMR cases with missense mutations, which not always correlate with loss of protein expression. Nevertheless, MMR IHC analysis is as effective for detecting dMMR CRC as microsatellite genotyping [69].

MMR deficiency results in microsatellite instability (MSI). Microsatellites are highly repetitive DNA sequences of one to six nucleotides distributed throughout the genome, which are frequently copied incorrectly. The MMR system is responsible for their detection and correction. Genotyping microsatellites through polymerase chain reaction (PCR)-based assay is the standard method to detect MSI. First guidelines on MSI analysis recommended a reference panel (Bethesda panel) of five microsatellites comprising two mononucleotide repeats (BAT-26 and BAT-25), and three dinucleotide repeats (D2S123, D5S346, and D17S250) [70]. This method is comparing the differences in allelic sizes obtained from tumor

and normal DNA; MSI being defined by instability for at least two of the five microsatellites. However, the Bethesda panel has several limitations, mainly due to the difficulty to interpret PCR amplification of dinucleotide markers. Therefore, the revised Bethesda guidelines recommend the use of mononucleotide repeats instead of dinucleotide repeats [71]. An alternative pentaplex-PCR assay comprising five quasi-monomorphic mononucleotide repeats (BAT-26, BAT-25, NR-21, NR-24, and NR-27) has been developed; MSI status being defined by the instability of three microsatellites or more. This optimized pentaplex PCR assay (Pentaplex Promega©) is at least as sensitive and specific for detection of MSI status as the Bethesda panel and obviate the need for normal matching DNA for comparison [67].

**Tables**

**Table 1** Prognostic value of deficient mismatch repair in metastatic colorectal cancer

Author	No. of patients (dMMR/pMMR)	OS (months)		P-value
		dMMR	pMMR	
Brueckl et al. [14]	43 (7/36)	33	19	0.021
Des Guetz et al. [15]	40 (9/31)	16	22.5	0.16
Koopman et al. [16]	515 (18/497)	10.2	17.9	0.41
Tran et al. [17]	350 (40/310)	11.1	22.1	0.001
Venderbosch et al. [18]	3063 (153/2910)	13.6	16.8	0.001

**Table 2** *BRAF*<sup>V600E</sup> mutation predictive value for anti-EGFR monoclonal antibody therapy

Trial	No. of patients	PFS		OS	
		Median (months)	Hazard ratio [95% CI]; <i>P</i> -value	Median (months)	Hazard ratio [95% CI]; <i>P</i> -value
<b>CRYSTAL</b> [39]	59				
FOLFIRI	33	5.6		10.3	
FOLFIRI + cetuximab	26	8.0	0.93 [0.42-2.06]; 0.87	14.1	0.91 [0.51-1.62]; 0.74
<b>PRIME</b> [40]	53				
FOLFOX	29	5.4		9.2	
FOLFOX + panitumumab	24	6.1	0.58 [0.29-1.15]; 0.12	10.5	0.90 [0.46-1.76]; 0.76
<b>FIRE-3</b> [42]	48				
FOLFIRI + bevacizumab	-	6.0		13.7	
FOLFIRI + cetuximab	-	4.9	0.87 [0.49-1.57]; 0.65	12.3	0.87 [0.47-1.61]; 0.65

**Table 3** Targeted therapies in clinical development for *BRAF*<sup>V600E</sup> metastatic colorectal cancer

<b>Treatment</b>	<b>Clinicaltrial.gov Identifier</b>	<b>Phase</b>	<b>No. of patients</b>	<b>OR (%)</b>	<b>SD (%)</b>	<b>PFS (months)</b>
Vemurafenib [57]	NCT00405587	I	19	5	21	3.7
Vemurafenib [72]	NCT01524978	II	10	0	-	-
Vemurafenib + panitumumab [65]	NCT01791309	I/II	15	13	53	3.2
Vemurafenib + cetuximab [72]	NCT01524978	II	11	-	36	-
Vemurafenib + cetuximab + CPT11 [66]	NCT01787500	Ib	19	35	-	7.7
Dabrafenib + trametinib [63]	NCT01750918	I/II	43	12	51	3.5
Dabrafenib + panitumumab [64]	NCT01750918	I/II	20	10	80	3.4
Dabrafenib + trametinib + panitumumab [64]	NCT01750918	I/II	35	26	60	4.1
Encorafenib [73]	NCT01436656	I	18	0	67	4
Encorafenib + cetuximab [74]	NCT01719380	I	26	23	-	3.7
Encorafenib + cetuximab + BYL719 [74]	NCT01719380	I	28	32	-	4.3

OR: objective response; SD : stable disease; PFS: progression-free survival

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••• Of outstanding importance

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