The Role of BRAF in Metastatic Colorectal Carcinoma—Past, Present, and Future

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Abstract: With a global incidence of 1.8 million cases, colorectal cancer represents one of the most common cancers worldwide. Despite impressive improvements in treatment efficacy through cytotoxic and biological agents, the cancer-related death burden of metastatic colorectal cancer (mCRC) is still high. mCRC is not a genetically homogenous disease and various mutations influence disease development. Up to 12% of mCRC patients harbor mutations of the signal transduction molecule BRAF, the most prominent being BRAF\textsuperscript{V600E}. In mCRC, BRAF\textsuperscript{V600E} mutation is a well-known negative prognostic factor, and is associated with a dismal prognosis. The currently approved treatments for BRAF-mutated mCRC patients are of little impact, and there is no treatment option superior to others. However, the gradual molecular understanding over the last decades of the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway, resulted in the development of new therapeutic strategies targeting the involved molecules. Recently published and ongoing studies administering a combination of different inhibitors (e.g., BRAF, MEK, and EGFR) showed promising results and represent the new standard of care. In this review, we present, both, the molecular and clinical aspects of BRAF-mutated mCRC patients, and provide an update on the current and future treatment approaches that might direct the therapy of mCRC in a new era.

Keywords: colorectal cancer; multitargeted therapy; BRAF inhibitors; BRAF; MAPK

1. Introduction

Colorectal cancer (CRC) is still one of the leading cancers worldwide. With a global incidence of approximately 1.8 million cases and 700,000 cancer-related deaths per year, it is the third most prevalent form of cancer and the fourth most frequent cause of cancer-related death, only exceeded by lung, liver, and stomach cancers. By gender, CRC is the second most common cancer in women (9.2%) and the third in men (10%) [1]. Most cases of CRC are detected in Western countries (55%), but this tendency is changing due to the fast development of some countries over the past few years [2].

The lifetime risk to develop CRC is about 4% to 5% [3]. Alongside many personal traits or habits that are considered to be risk factors for developing polyps and in further sequence, CRC, the main risk factor remains age—past the fifth decade of life, the risk of developing CRC is markedly increased, while the onset of CRC below the age of fifty is rare (apart from inherited cancers) [4]. However, in recent years, the incidence in this age group increased, while there seems to be a slow decrease in the population above 50 years of age. Broader participation in screening programs is presumably the reason for these dynamics [5]. Other important risk factors are a history of inflammatory bowel
disease or the presence of a positive familial history of CRC. Increased risk due to familial history can be derived from inherited mutations or the environment [6].

Most CRC patients with metastatic disease are treated with a combination of cytotoxic and biological agents. First-line chemotherapy with palliative purposes comprises fluoropyrimidines (e.g., 5-fluorouracil (5-FU) or capecitabine) alone, or combined with leucovorin (LV), as well as other cytotoxic agents, such as oxaliplatin (5-FU/LV/oxaliplatin (FOLFOX) and capecitabine/LV/oxaliplatin (CAPOX)), or irinotecan (5-FU/LV/irinotecan (FOLFIRI: FOLIRINOX)) [7–11].

After progression, patients with a good organ function and performance status (Eastern Cooperative Oncology Group ECOG 0-1) are offered a second-line chemotherapy regime, depending on the first line. Second-line treatment for patients refractory to irinotecan consist of an oxaliplatin-containing combination, whereas patients refractory to oxaliplatin are treated with an irinotecan-containing treatment [11]. The treatment option after triplet-therapy is not clearly defined. Alternatives consists of treatment with regorafenib [12] or trifluridine/tipiracil [13].

In addition to chemotherapy, monoclonal antibodies or proteins against vascular endothelial growth factor (VEGF) [14–16] and epidermal growth receptor (EGFR) [17,18], combined with traditional chemotherapy were demonstrated to improve the outcome of mCRC.

Several drugs and combinations thereof are now available for the treatment of patients with advanced CRC, however, the optimal sequence of therapy remains to be established.

For the sake of completeness, local treatments should be mentioned as well, since they are an integral part of the multimodal concepts that could be offered to mCRC patients. Recent studies highlight their importance with either laparoscopic or open resection of liver metastasis, as well as percutaneous radiofrequency ablation, improving the survival rates in these patients. [19–22]

However, mCRC is not a genetically homogenous disease and various mutations influence disease development, treatment response, and outcome. A prominent molecular feature is the BRAF mutational status. BRAF mutations occur in 8% of all tumors, and 5–12% of the mCRC patients present with a BRAF mutation [23]. More than 90% of them harbor the BRAFV600E mutation associated with resistance to standard treatment regimens, and with a dismal prognosis [24]. In the light of the recently approved targeted therapies for the BRAFV600E-mutated mCRC, we present in this review, the molecular and clinical aspects related to this subgroup of patients.

2. BRAF—Molecular Insights and Clinical Relevance

Colon cancer development results from the sequential accumulation of genetic alterations, which drive the progression from a benign stage (adenoma) to the fully transformed phenotype [25]. These genetic alterations underlie the manifestation of the hallmarks of cancer [26], which are essential for tumor initiation and progression. Mutations in intracellular signaling pathways, which when unperturbed are required for developmental processes and proliferation, survival, and differentiation of cells during postnatal life, function as essential drivers in colon carcinogenesis. Important entities affected include Wnt, RAS-RAF, PI3K/PKB/AKT, TGF-β, p53, and DNA mismatch-repair pathways [27].

2.1. Intracellular Signaling Pathways Involved in CRC

2.1.1. RAS-RAF Pathway

BRAF is a member of the RAF kinase family, which additionally comprises ARAF and CRAF [28–30]. These serine/threonine kinases are part of an evolutionarily conserved pathway that connects the stimulation of cell surface receptors with intrinsic tyrosine kinase activity (Receptor Tyrosine Kinase, RTK, e.g., the epidermal growth factor receptor (EGFR, HER, cERBB)), with the stimulation of small G proteins of the RAS family, the activation of RAF kinases, and the downstream effectors MEK1/2 and their substrates ERK1/2. (Figure 1) Frequently, the net outcome is the transcriptional activation of genes involved in the proliferation, survival, or differentiation of cells. Signaling through this pathway plays a key role in the developmental processes but also during adult life, when components of this
cascade can be affected by mutations in human cancers usually resulting in the constitutive activation of their enzymatic activity, which relieves them from control by extrinsic factors.

**Figure 1.** Schematic illustration of the canonical Wnt/β-catenin, RAS-ERK, and PI3K/AKT/mTOR signaling pathways. In the presence of extracellular Wnt ligands, the β-Catenin degradation complex is inhibited and β-Catenin translocates to the nucleus, resulting in the activation of the target genes. Additionally, Wnt can affect the RAF-MEK-ERK signaling through the stabilization of the RAS proteins. The RAS-ERK route is stimulated through the binding of EGF to EGFR, which then allows SOS to activate RAS by exchanging GDP to GTP. GTP-bound RAS is necessary for the activation of RAF and the signal is propagated to MEK-ERK kinase, via phosphorylation. Phosphorylated ERK translocates to the nucleus and activates various transcription factors. Activated PI3K, an additional RAS target, results in the activation of PDK1 and AKT. AKT signaling, in turn activates mTOR, leading to the expression of target genes. Red asterisks indicate the gain of the function mutation, ©Silvia Eller.

Within the RAF family, BRAF is the preferred target for genetic alterations with the V600E exchange predominating. Mutation frequencies in human cancers are as high as 60% in malignant melanoma [30]. BRAFV600E or BRAFV594G exchanges that are mutually exclusive with the more frequent Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations (30–50%) are present as oncogenic drivers in 5–12% of patients with mCRC [23]. Several studies demonstrated that the BRAFV600E but no other less common BRAF mutations, are associated with a worse prognosis for these patients [31]. Stratification of CRCs based on gene expression resulted in the identification of four consensus molecular subtypes (CMS), with distinct features. CRCs carrying BRAFV600E mutations are enriched in the subgroup CMS1 associated with hypermethylation, microsatellite instability (MSI), and chromosomal instability [32].

Mutations in BRAF are most commonly associated with an increase in its kinase activity, resulting in continuous downstream signaling. Therefore, several generations of mutation-specific small molecule BRAF inhibitors were developed, initially mainly for the use in the treatment of malignant melanoma, where BRAF mutations are most common. However, the clinical response, at best, was transient but a cure was never achieved [33,34]. One major obstacle was the fast development of drug resistance through various mechanisms, which usually left the ability of the drug to inhibit BRAF kinase activity intact, but bypassed its effect on downstream signaling. Drug unresponsiveness also went along with the activation of signaling proteins outside the RAS-RAF axis [35], which is discussed here briefly. For the clinical routine, this suggests the future use of treatment regimens, which combine the
simultaneous inhibition of several signaling pathways and, in the future, also checkpoint inhibitors, as recently demonstrated for melanoma [36,37].

2.1.2. PI3K-PKB Pathway

Apart from the RTK-RAS-RAF-MEK-ERK pathway, multiple other intracellular signaling cascades can become drivers for tumor development. In the context of CRC, these include PI3K [38] and Wnt [39,40] signaling. The lipid kinase phosphatidylinositol 3-kinase (PI3K), binds the small RAS G proteins through the same effector domain as the RAF kinases, and thus, RTK signaling might result in the concomitant activation of the RAF and PI3K. Membrane-derived lipids generated by PI3K are essential for the activation of a family of kinases called AKT1-3 or PKBα,β,γ, which fulfill important functions in proliferation survival and differentiation [41] (Figure 1). PI3K/PKB/AKT contribute to oncogenic signaling, following the loss or inactivation of the phosphatase PTEN, which normally would terminate PI3K signaling (tumor suppressor), the mutational activation or amplification of PI3K, or mutation of AKT/PKB [38,42].

2.1.3. Wnt Pathway

The Wingless-related integration site (Wnt) pathway is another evolutionarily conserved signaling cascade frequently implicated in oncogenesis. Wnt is a family of lipoglycoprotein ligands, which bind to the frizzled (FZD) family receptors. One main downstream effect of receptor activation is the stabilization of the cytosolic protein β-catenin, which regulates the expression of many cancer-relevant proteins (Figure 1). The adenomatous polyposis coli (APC) protein is part of the destruction complex, which is required to maintain low β-catenin levels in the absence of Wnt signaling. APC is mutated in 90% of all CRC patients, and frequently cooperates with mutations in KRAS and BRAF. Although deregulation of Wnt signaling is associated with the subgroup CMS2 of CRCs [32], several findings support the potential of simultaneously targeting both pathways in CRC. Stimulation by Wnt was shown to activate signaling through RAF-MEK-ERK and to assist in the stabilization of RAS proteins, thereby enhancing downstream signaling [43]. Furthermore, WNT5A promotor methylation and BRAFV600E mutation are associated in CRC patients [44].

2.2. Targeted Therapies for CRC

The molecular understanding of the underlying genetic landscape of CRC provided the rationale basis for novel therapeutic approaches. This, in particular, includes the clinical use of small molecule inhibitors of mutant BRAF (e.g., encorafenib [45]) and of MEK1,2 (e.g., binimetinib [46]), combined with the inhibition of PI3K [47], or the epidermal growth factor receptor (e.g., cetuximab, [48]). EGFRs (HERs/cERBBs) are prototypic RTK receptors, upstream of RAF and PI3K signaling. Overexpression and mutation contribute to tumor progression. Evidence of cERBB2/HER2 amplification and mutation in CRC suggests it to be a potential therapeutic target [49].

2.3. Clinical Relevance of Molecular Testing in CRC

Since many genetic subtypes of CRC are associated with specifically targeted treatment options, molecular testing has become clinical routine (Figure 2). Reports of the predictive value of various mutational status highlighted the clinical relevance of molecular testing in CRC patients, in the last decade. Half of the patients with advanced CRC harbor a KRAS or a neuroblastoma N-Ras (NRAS) tumor gene mutation. These mutations are negative predictive biomarkers with regards to the treatment response to the anti-EGFR monoclonal antibodies cetuximab or panitumumab [17,18,50,51]. Since RAS proteins belong to the main effectors of EGFR signaling, the presence of mutationally-activated RAS might bypass the effect of inhibiting EGFR signaling. Therefore, only patients with RAS wild-type mCRC should receive a therapy that includes anti-EGFR treatment.
For localized, non-metastatic CRC, there are currently no data supporting the analyses of other disease markers than the microsatellite instability/DNA mismatch repair (MSI/MMR) status. While MSI/MMR status determination is important to rule out hereditary non-polyposis CRC (HNPCC, Lynch syndrome) and to identify patients with a low risk of recurrence, B-RAF, and KRAS analysis seems to not add further information in the treatment decision-making process [53].

In contrast, for mCRC, the standard panel of molecular markers comprises the MSI/MMR status, RAS, HER-2, and BRAF [9,54]. Due to the high immunogenicity shown in MSI tumors, MSI/MMR status determination is important to identify patients who will benefit from immune checkpoint inhibitors [52]. As already mentioned, RAS mutations identify patients resistant to anti-EGFR therapies. Furthermore, recent data identified HER-2 amplification as a possible treatment target in mCRC patients, not responding to standard chemotherapy lines [55]. Last but not the least, BRAF mutations are the focus of recent and current clinical trials, where specific targeted approaches are tested in mutated mCRC.

More than 90% of mutations in BRAF-mutated cancers occur in codon 600 (V600E mutation). The so-called non-V600E-BRAF mutations in codon 594 and 596, account for less than 5% [56]. The reported incidence of BRAFV600E mutation varies between 5% and 12% [57–62], even though recent registry data report 21% of mCRC patients harboring BRAF mutations [63]. The differences arise from differences in the tumor stages included in the reporting papers, with a stronger decline in the advanced tumor stages, due to their worse prognosis. Interestingly, RAS and BRAF mutations are mutually exclusive, and are reported to occur together in only 0.001% of patients [64].

Non-V600E-BRAF mutations define a clinically distinct subtype of CRC. These mutations occur more frequently in the left-sided colon and rectum, are associated less with peritoneal metastases, and were shown to be associated with a microsatellite stable (MSS) status. Even though they result generally in a significantly better overall survival (OS) (median 62.0 vs. 12.6 months; HR 0.36, p = 0.002) [65,66], non-V600E-BRAF-mutated CRC can be subdivided in two classes, with respect to their anti-EGFR treatment response, the RAS-independent activating (class 2) and the RAS-dependent activating non-V600E-BRAF mutation (class 3) [67].

In contrast, the BRAFV600E mutation in colon cancer occurs more frequently in women and elderly patients, in proximal tumor locations, and in tumors arising from serrated adenomas and
with mucinous differentiation. It is also associated with a higher rate of lymph node metastases and peritoneal dissemination [60,68–70]. From a molecular point of view, in up to 50%, it is associated with high microsatellite instability MSI-H [71]. Patients with BRAF\textsuperscript{V600E} mutation survive, on average, less than half as long as patients with BRAF wild-type mCRC. [59,60,72]

2.3.1. Prognostic Value of BRAF\textsuperscript{V600E}

BRAF\textsuperscript{V600E} mutation is known as a negative prognostic marker.

Regarding non-metastatic CRC, the evaluation of more than 1300 specimens in the PETACC-3 trial, revealed BRAF\textsuperscript{V600E} mutation as marker for significantly worse OS (HR 1.78, 95% CI 1.15–2.76); however, it did not influence recurrence-free survival (RFS) (HR 1.30; 95% CI 0.87–1.95) [73]. Domingo et al. observed a shorter relapse-free survival for BRAF\textsuperscript{V600E} mutated patients (HR 2.21, 95% CI 1.47–3.29) [74], in a population combining the QUASAR 2 trial and an Australian community-based series. More recent data from the PETACC-8 and N0147 trials confirmed the negative prognostic value for both, time to recurrence (TTR) (HR 1.27, 95% CI 1.04–1.56) and OS (HR 1.49, 95% CI 1.20–1.86) [53].

The frequent occurrence of MSI in BRAF\textsuperscript{V600E} mutation, poses the question of whether the MSI status could act as a possible opposite prognostic factor in the BRAF\textsuperscript{V600E}-mutated patients. Indeed, despite the small number of events, PETACC-3 trial data suggest that MSI-H status overrules the prognostic value of the BRAF\textsuperscript{V600E} mutation status (RFS: HR 1.26, 95% CI 0.59–2.70; OS: HR 1.53, 95% CI 0.63–3.70) [73,75]. The analysis of 1913 stage II specimens of the QUASAR trial showed that the BRAF\textsuperscript{V600E} mutation status did not influence the better RFS in the MSI/MMR tumors (HR 0.48, 95% CI 0.27–0.85) [76]. Similarly, recent data including PETACC-8 and the N0147 trial with 4411 patients confirmed BRAF\textsuperscript{V600E} mutation as a negative prognostic marker in stage III MMS patients (TTR: HR 1.54, 95% CI 1.23–1.92; OS: HR 2.01, 95% CI 1.56–2.57); however, with no prognostic influence on MSI patients (TTR: HR 0.94, 95% CI 0.58–1.51; OS: HR 1.26, 95% CI 0.78–2.04) [53]. Results from the intergroup trial CALGB 89803, reflect the difficult task of interpreting these data. Categorization according to BRAF, as well as MSI status, suggested opposing prognostic effects of BRAF\textsuperscript{V600E} mutation and MSI-H, however, no difference reached statistical significance [77]. In contrast, the analysis of stage III colon cancer patients of the N0147 trial did not support these findings [78].

The negative impact of BRAF\textsuperscript{V600E} mutation was also reported for patients with advanced CRC. A pooled analysis including more than 3000 patients of the CAIRO, CAIRO 2, COIN, and FOCUS trial, showed in patients with BRAF\textsuperscript{V600E} mutation, both worse progression-free survival (PFS) (HR 1.34, 95% CI 1.17–1.54) and OS (HR 1.91, 95% CI 1.66–2.19) [79]. Data from the AIO 0207 trial showed that the BRAF\textsuperscript{V600E} mutation remains a negative prognostic marker, with a significantly worse OS in right and left-sided colon cancer [80]. Similarly, data from the FIRE-3 study and the MRC FOCUS trial confirm a worse prognosis for PFS and OS in this patient group [59,81].

In contrast to stage II and stage III cancer, a recent pooled analysis including more than 3000 patients, suggests that the MMR status does not influence the prognostic value of the BRAF\textsuperscript{V600E} mutation in advanced CRC (advCRC) [79].

The prognostic value of the BRAF\textsuperscript{V600E} mutation is also reflected in the outcome of resectable colorectal liver metastases. A recent multicenter analysis reports a 93.9% recurrence rate, over a median follow-up period of almost 50 months, with an estimated 5-year OS rate of 18.2% [82]. Still, the observed long-term survivors highlight the necessity of a more granular stratification aimed at identifying patients suitable for specific local treatments. These stratifications should be based on the clinical markers [83], as well as on additional molecular-marker-like alterations in the SMAD family, as proposed by Lang et al. in their extended clinical score [84].

2.3.2. Predictive Value of BRAF\textsuperscript{V600E}

While KRAS mutation status is now widely accepted as a predictive marker for resistance towards anti-EGFR treatment [50], the predictive role of BRAF\textsuperscript{V600E} mutation towards chemoresistance is still
under debate. Of note, the low prevalence of this mutation makes it difficult to establish it as a predictive marker.

Already earlier trials like the MRC FOCUS trial reported that the BRAF
\textsuperscript{V600E} mutant tumors had a worse prognosis but no predictive value for PFS or OS, neither in the irinotecan/FU group nor in the oxaliplatin/FU group ($p = 0.16$ and $p = 0.30$), highlighting, however, that these results should not preclude those patients from intensified treatments [59]. Current guidelines of intensified chemotherapy regimens for patients bearing BRAF\textsuperscript{V600E} mutation, rely on the results of the phase III TRIBE study. In that study, the treatment with LV/5-FU/ oxaliplatin/ irinotecan (FOLFOXIRI) plus bevacizumab showed a significantly better OS and PFS, compared to FOLFIRI plus bevacizumab in the intention to treat (ITT) population, and a relevant clinical, despite not statistically significant, advantage in median OS (19.4 vs 10.7 months; HR 0.54, 95\% CI 0.24–1.20). Again, the mutation had no predictive value (HR 1.89, 95\% CI 0.38–8.78) [85].

The impact of anti-VEGF treatment in this subset of patients is not yet clear. Results from the phase III AGITG MAX trial showed that the BRAF\textsuperscript{V600E} mutation did not predict the effectiveness of Bevacizumab, if added to capecitabine (OS: $p = 0.32$ PFS: $p = 0.46$, for the interaction of BRAF status and the assigned treatment status) [86]. In the same line, the phase III study RAISE, failed to show any statistically significant predictive value of the BRAF mutation status, however, the OS and PFS doubled in patients treated with the VEGF receptor 2 antibody ramucirumab [87]. Similarly, the VELOUR trial showed a trend towards a significant increase of OS in the BRAF\textsuperscript{V600E}-mutated patients treated with afiblercept. Of note, this difference was even more pronounced than in the RAS mutant and RAS wild-type subgroups, suggesting that BRAF\textsuperscript{V600E}-mutated patients benefit from afiblercept [15].

Since BRAF is a downstream signaling protein of the EGFR-mediated mitogen-activated protein kinases (MAPK) pathway, the efficacy of anti-EFGR treatments was also challenged for the BRAF\textsuperscript{V600E} mutations. In contrast to the KRAS mutation status, the results were not that conclusive. The PRIME study showed that the addition of panitumumab to FOLFOX4 did not result in better PFS and OS for BRAF-mutated patients (HR 0.58, 95\% CI 0.29–1.15; HR 0.90, 95\% CI 0.46–1.76, respectively). Another important finding was, that the negative predictive value for PFS and OS observed in the patient group with either RAS or BRAF mutations was driven by the RAS-mutated patients [88,89]. The different way of interpreting the data was well reflected in two recent metanlyses. Despite both studies stating that BRAF\textsuperscript{V600E} mutation had no predictive value on median PFS or median OS, one study suggests mandatory BRAF mutation assessment before initiating anti-EGFR treatment [64], whereas the second study concludes that there is not enough evidence to support mandatory assessment [90]. The addition of panitumumab to irinotecan in a 2nd line treatment (PICCOLO trial) was suggested to even have a detrimental effect (PFS: HR 1.40, 95\% CI 0.82–2.39; OS: HR 1.84, 95\% CI 1.10–3.08). Due to the low case number, the authors define their results as exploratory [91]. In contrast, the VOLF trial report encouraging data, however, there were only 16 BRAF\textsuperscript{V600E}-mutated patients included. The addition of panitumumab to modified FOLFOXIRI (mFOLFOXIRI), resulted in significantly higher overall response rate (ORR) as compared to mFOLFOXIRI alone (OR 21, 95\% CI 1.5–293.2) [92]. Recent findings suggest that further stratifications by other predictive factors like tumor sidedness, might reveal patient subsets where the BRAF mutation status is predictive for treatment response to anti-EGFR treatment [93].

According to the current ESMO guidelines, the preferred choice for 1st line treatment in fit, BRAF\textsuperscript{V600E} mutant patients, is the triplet chemotherapy FOLFIRI plus bevacizumab [9]. The German S3-guidelines also suggest an aggressive triplet treatment; however, they also point at the discordant results regarding the targeted anti-VEGF therapies and argue based on a recent subgroup analysis of the FIRE-3 trial that these patients might not benefit from either anti-EGFR- or anti-VEGF-based strategies [94]. The guidelines are now also challenged by the recently published results of the TRIBE2 trial. In this trial, the mentioned triplet cytotoxic regimen plus bevacizumab, did not show any significant benefit in the BRAF-mutated patients, as compared to the cytotoxic doublets in combination with bevacizumab [95].
With respect to the targeted treatments, both the German and the NCCN guidelines include the recent developments in the BRAF-targeted therapies [54,96].

3. Targeting BRAF in the mCRC Treatment

After the failure of 1st line of treatment, unfortunately, the subsequent lines only have a minimal effect on tumor development. Most of the time, patients experience rapid progressive disease (PD). There were many attempts to overcome further tumor progression, highlighting the unmet medical need for this group of patients [97,98].

Recently, BRAF inhibitors such as vemurafenib, dabrafenib, and encorafenib, revolutionized the treatment of BRAF$^{V600E}$ metastatic melanoma, initially in monotherapy or, currently, in combination with other drugs. Ongoing studies are also striving to reproduce these results in patients with mCRC.

3.1. Monotherapy–The Broken Promise

Unlike other tumors with BRAF$^{V600E}$ mutations, like melanoma [99–101], non-small-cell lung cancer [102] and papillary thyroid cancer [103], BRAF inhibition in BRAF$^{V600E}$ mutant mCRC showed only marginal clinical activity in the early treatment course.

Kopetz et al. led one of the first trials with a BRAF inhibitor, in previously-treated BRAF-mutated mCRC, using the recommended phase II dose of vemurafenib for melanoma (960 mg b.i.d.) in an expansion cohort [45]. A total of 21 mCRC patients with confirmed BRAF$^{V600E}$ mutations CRC were included. A confirmed partial response (PR) lasting 21 weeks and seven cases of stable disease lasting at least 8 weeks were reported. Median PFS was 2.1 months (range, 0.4–11.6 months), with two patients being progression-free for more than 6 months. Median OS was 7.7 months (range, 1.4–13.1 months).

In a phase I basket trial of dabrafenib, 11 mCRC patients were included, however, only 9 had a BRAF-mutant evaluable disease. Of these, PR was observed in only 1 patient, while in 7 patients, there was a stable disease [104].

Similarly, treatment with encorafenib, which had a more prolonged pharmacodynamic activity than the other approved BRAF inhibitors, did not show encouraging results. In a phase I escalation study, none of the included 18 patients achieved PR or a complete response (CR). After a median treatment duration of 11 weeks, 14 patients had to discontinue the treatment, most of them due to PD. Median PFS was 4.0 months [105].

The lack of clinical effectiveness of BRAF inhibitor monotherapy is currently explained by two observations. In vitro studies suggest that BRAF inhibition causes a rapid feedback activation of EGFR because of the missing negative feedback mechanism driven by ERK1/2 activation, and, in contrast to melanomas, CRC express higher EGFR levels [106,107]. As a consequence, EGFR activates MEK1/2 through several escape mechanisms, e.g., bypassing BRAF via other RAF family members or via activation of the PI3K/AKT pathway, finally resulting in missing the tumor response [108] (Figure 3).
3.2. Multitarget Approaches to Overcome Resistance in BRAF Mutated mCRC

To overcome the limited activity in the BRAFV600E-mutated mCRC, different approaches were tested in several studies that simultaneously target various signaling entities, combining BRAF inhibitors, e.g., with anti-EGFR monoclonal antibodies and MEK inhibitors (Figure 4).

3.2.1. Targeting BRAF and MEK

As it is well known that a combination of BRAF and MEK inhibition proved to be more effective in melanoma than only BRAF inhibition, the same approach was evaluated in the BRAF-mutated mCRC.
Corcoran et al. analyzed the combination of the selective BRAF inhibitor *dabrafenib* with *trametinib*, a selective MEK inhibitor, in patients with histologically confirmed BRAF<sup>V600E</sup> or BRAF<sup>V600K</sup>-mutant mCRC, in a phase I/II trial [109]. A total of 43 patients were treated with dabrafenib (150 mg twice daily) plus trametinib (2 mg daily), 17 of whom were enrolled onto a pharmacodynamic cohort, undergoing mandatory biopsies, before and during treatment.

Five patients (12%) achieved a PR or better, including one (2%) CR, with a duration of response >36 months; 24 patients (56%) achieved stable disease as the best confirmed response. With a median PFS of 3.5 months, the efficacy was greater than the median PFS of 2.5 months, observed with standard chemotherapy [110].

### 3.2.2. Targeting BRAF and EGFR

In a recent open-label phase one study, 20 BRAF<sup>V600E</sup> mutant patients were treated with the BRAF inhibitor *dabrafenib* and the anti-EGFR monoclonal antibody *panitumumab*. Two patients achieved a CR or PR, while 16 patients had stable disease, resulting in a tumor control of 90%. Again, the median PFS was 3.5 months (95% CI, 2.8–5.8) [111].

A similar response rate (RR) (13%) and similar PFS 3.2 months (95% CI, 1.6–5.3) could be achieved with the combination of the BRAF inhibitor *vemurafenib* with *panitumumab*, in a pilot study involving 15 patients; despite being well-tolerated, clinical activity of this treatment was modest [48].

These results suggest that EGFR-independent mechanisms might lead to MAPK reactivation in BRAF and EGFR-targeted treatment strategies.

The SWOG 1406 study analyzed the addition of *vemurafenib* to the anti-EGFR (*cetuximab*)/chemotherapy (irinotecan) combination. The ORR improved dramatically in the triple-therapy, compared to the dual-therapy (16% vs. 4%), however, even though PFS was significantly longer (4.4 months (95% CI: 3.6–5.7) vs. 2.0 months (95% CI: 1.8–2.1); p < 0.001), this combination showed only moderate clinical effectiveness [112].

### 3.2.3. Targeting BRAF and EGFR and PI3K

Interesting results were observed in a dose-escalation trial, where BRAF-mutated mCRC patients were administered the BRAF inhibitor *encorafenib* and the anti-EGFR monoclonal antibody *cetuximab*, with (28 patients) or without the PI3Kα inhibitor *alpelisib* (26 patients) [113]. Both treatment regimens resulted in a similar clinical efficacy. In the dual-combination and in the triple-combination, the ORR was 19% (one CR, four PR), and 18% (5 PR), respectively. However, the median PFS was similar to the before-mentioned treatments, with 3.7 months (95% CI, 2.8–12) for the dual- and 4.2 months (95% CI, 4.1–5.4) for the triple-combination.

### 3.2.4. Targeting BRAF and MEK and EGFR

Up to now, two published triple-combinations inhibited BRAF, MEK, and EGFR.

The first trial, an open-label phase I study, analyzed the efficacy of BRAF and EGFR inhibition with *dabrafenib* and *panitumumab* combined with the MEK inhibitor *trametinib*, in 91 BRAF<sup>V600E</sup> mutant mCRC patients. Of note, 23 patients did not have any prior line of therapy. This treatment strategy resulted in 19 patients experiencing CR or PR, and 59 patients having a stable disease. The median PFS was 4.2 months (95% CI, 4.0–5.6) and the OS was 9.1 months (95% CI, 7.6–20.0 months, estimable but not mature). The triple-combination was characterized by a 70% grade 3/4 adverse events [111].

In October 2019, the group of Tabernero published the results of the BEACON trial, the largest clinical study in this patient population. The trial met all its endpoints and is now included in NCCN (National Comprehensive Cancer Network) guidelines, as a recommended treatment after failure of one or two prior lines [114].

In this open-label phase 3 trial, they enrolled 665 patients with BRAF<sup>V600E</sup>-mutated mCRC, who showed disease progression after one or two previous regimens. Patients were randomly assigned in a 1:1:1 ratio. The triplet-therapy group received the BRAF inhibitor *encorafenib*, the MEK inhibitor *binimetinib*, and *cetuximab*. The doublet-therapy group was treated with *encorafenib* and *cetuximab*,...
and the third group received either cetuximab and irinotecan, or cetuximab and FOLFIRI, according to the investigators’ choice (the control group).

The median OS was 9.0 months in the triplet-therapy group and 5.4 months in the control group (hazard ratio [HR] for death, 0.52; 95% confidence interval [CI], 0.39 to 0.70; \( p < 0.001 \)). The confirmed RR was 26% (95% CI, 18–35) in the triplet-therapy group and 2% (95% CI, 0–7) in the control group (\( p < 0.001 \)). Of note, the RR in patients with only one prior line of therapy was 34% (95% CI, 23–47). The median PFS was 4.3 months (95% CI, 4.1–5.2). The dual-combination achieved similar results, with a median OS of 8.4 months (HR for death vs. control, 0.60; 95% CI, 0.45–0.79; \( p < 0.001 \)). Adverse events of grade 3 or higher occurred in 58% of patients in the triplet-therapy group, in 50% in the doublet-therapy group, and in 61% in the control group.

An updated analysis of the study confirmed the clinical efficacy of these treatment regimens. Doublet and triplet-therapy achieved a median OS of 9.3 months (95% CI, 8.0–11.3) and 9.3 months (95% CI, 8.2–10.8), respectively, compared to 5.9 months (95% CI, 5.1–7.1) in the control group, showing for the first time a significant survival benefit of the targeted therapies in the BRAF\textsuperscript{V600E} mutant mCRC patients, as compared to the standard chemotherapy options.

However, there are also some criticisms about the design of the trial. A portion of patients in the control arm might have never received an oxaliplatin-containing regimen (e.g., FOLFOX), but they merely received two irinotecan-containing regimens, consecutively. Another criticism is the missing report about the exact treatment regimens in the control arm, and about previously administered and following treatment lines. Additionally, the non-provided information about the time lag between the diagnosis of the metastatic disease and study enrollment might have affected the study results.

Finally, there is the crucial question of whether triplet was better than doublet. Even though the trial was not powered to compare the 2 regimens, there was only 0.6 months longer median OS in the triplet therapy group. However, due to the higher incidence of grade 3/4 toxicity, the European Medicines Association so far only approved the cetuximab/encorafenib combination.

**4. Future Perspectives**

The encouraging results of the BEACON trial highlight the importance of multitargeted approaches in this specific patient population, also demonstrating, however, that there are other escape mechanisms of the tumor, leading to a still poor prognosis in BRAF\textsuperscript{V600E} mutant mCRC patients. The observation of a better RR in patients with only one prior line, raises the question of the timing. In this regard, this chemotherapy-free combination is being explored frontline in the ANCHOR clinical trial (NCT03693170).

Intervening in the Wnt/\( \beta \)-catenin signaling, represents another potential future treatment option. Wnt was shown to activate signaling through RAF-MEK-ERK targeting [43], e.g., the S100 calcium-binding protein A4 (S100A4) [115]. S100A4 is associated with metastasis formation and reduced OS in CRC [116]. The phase II NIKOLO trial (NCT02519582) will test the efficacy of the antihelminthic drug niclosamide in controlling the progression of mCRC, via reduced expression of S100A4 [117].

Another important aspect of the BRAF\textsuperscript{V600E} mutant mCRC patients is the already mentioned co-occurrence of deficient-MMR, in up to 50%. Considering the encouraging results with the immune checkpoint inhibitors involving programmed cell death-1 (PD-1) protein-like pembrolizumab and nivolumab in MSI CRC patients [118], immunotherapy is to be considered in the BRAF\textsuperscript{V600E} mutant mCRC patients as well. In this regard, a positive correlation between the BRAF\textsuperscript{V600E} mutation and programmed death ligand-1 (PD-L1) was also recently described [119]. In the recently published KEYNOTE-146 (pembrolizumab) open-label phase II study, 14 out of 124 MSI-H/dMMR, included CRC patients that harbored a BRAF mutation. In 6 of these patients, an overall response could be observed [120]. Similarly, in the CheckMate 142 trial, the use of nivolumab, with or without the anti-CTLA4-antibody ipilimumab, resulted in an ORR of up to 55% [121,122]. Table 1 summarizes current trials addressing multitargeted approaches in BRAF mutated advCRC and mCRC.
**Table 1.** Summary of current ongoing trials including patients with BRAF mutation.

| Targets | Compounds | Study Design | Phase | Inclusion Criteria | Participants | Primary Endpoints | Registration Number |
|---------|-----------|--------------|-------|--------------------|--------------|-------------------|---------------------|
| BRAF + EGFR | vemurafenib + cetuximab + FOLFIRI | Open-label, single-arm | II | advCRC or recCRC | 30 | ORR | NCT03727763 |
| BRAF + EGFR + MEK | encorafenib + cetuximab + binimetinib | Open-label, single-arm | II | first-line treatment in mCRC | 95 | ORR | NCT03693170 |
| BRAF + MEK + PD-1 | encorafenib + binimetinib + nivolumab | Open-label, single-arm | I/II | MSS mCRC, ≥1 treatment lines | 38 | ORR (a) radiographic response (b) best investigator-assessed response (c) treatment-related grade ≥ 3 AEs | NCT04044430 |
| BRAF + MEK + PD-1 | dabrafenib + trametinib + PDR001 | Open-label, single-arm | II | mCRC, ≥0 treatment lines | 25 | ORR (b) treatment-related grade ≥ 3 AEs | NCT03668431 |
| BRAF | oral LGX818 | Open-label, single-arm | I | mCRC/mMelanoma | 107 | treatment-related grade ≥ 3 AEs | NCT01436656 |
| BRAF + EGFR + PI3K | (a) LGX818 + cetuximab (b) LGX818 + BYL719 + cetuximab | Open-label, parallel assignment | Ib/II | mCRC, ≥1 treatment lines | 156 | (a) dose-limiting toxicities (b) PFS | NCT01719380 |
| BRAF | oral ABM-1310 | Open-label, sequential assignment | I | adv or met solid tumors including mCRC | 27 | (a) Maximum Tolerated Dose (b) Recommended Phase 2 Dose | NCT04190628 |
| BRAF + EGFR + PD1 | encorafenib + cetuximab + nivolumab | Open-label, single-arm | I/II | MSS mCRC, ≥1 ≤2 treatment lines | 38 | ORR (b) treatment-related grade ≥ 3 AEs | NCT04017650 |
| BRAF + MEK | LGX818 + MEK162 | Multicenter, open-label | Ib/II | adv or met melanoma, mCRC, ≥1 treatment lines | 127 | (a) dose-limiting toxicities (b) ORR | EudraCT Number: 2011-005875-17 |
| EGFR or VEGF | (a) cetuximab + FOLFOXIRI (b) bevacizumab + FOLFOXIRI | Randomized | II | 1st line mCRC | 108 | ORR | EudraCT Number: 2015-004849-11 |
5. Conclusions

Patients with mCRC harboring BRAF^{V600E} mutations are still burdened with a dismal prognosis compared to patients without this mutation. Current treatment options in these patients have insufficient clinical efficacy. The advent of treatment regimens addressing molecular targets in the signaling pathway is going to improve upfront treatment options, if not replace those based on cytotoxic agents.

The emergence of resistant subclones or escape mechanisms harboring MAPK-activating alterations might be a major driver for treatment failure and clearly shows that there is still a long way to go. Future strategies aimed at sustaining clinical benefit by suppressing these resistance mechanisms should include a deeper understanding of the molecular pathways, as well as combined approaches, not only addressing the targets of various intracellular signaling pathways, but also other currently available molecular characteristics like MMR.

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Abbreviations

- 5-FU 5-fluorouracil
- advCRC advanced CRC
- CI confidence interval
- CR complete response
- CRC colorectal cancer
- ECOG Eastern Cooperative Oncology Group
- EGFR epidermal growth receptor
- FOLFIRI LV/5-FU/irinotecan
- FOLFIRINOX LV/5-FU/irinotecan/oxaliplatin
- FOLFOX LV/5-FU/oxaliplatin
- FOLFOXIRI LV/5-FU/oxaliplatin/irinotecan
- HR hazard ratio
- ITT intention to treat
- KRAS Kirsten rat sarcoma
- LV leucovorin
- MAPK mitogen-activated protein kinases
- mCRC metastatic colorectal cancer
- mFOLFOXIRI modified FOLFOXIRI
- MMR DNA mismatch repair
- MSI microsatellite instable
- MSI-H microsatellite instable-high
- MSS microsatellite stable
- NRAS neuroblastoma N-Ras
- ORR overall response rate
- OS overall survival
- PD progressive disease
- PD-1 programmed cell death-1
- PD-L1 programmed death ligand-1
- PR partial response
- PKB protein kinase B
- PFS progression-free survival
References

1. Colorectal Cancer Statistics. World Cancer Research Fund. 2018. Available online: https://www.wcrf.org/dietandcancer/cancer-trends/colorectal-cancer-statistics (accessed on 21 October 2020).
2. Brody, H. Colorectal cancer. *Nature* 2015, 521, S1. [CrossRef] [PubMed]
3. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2016. *CA Cancer J. Clin.* 2016, 66, 7–30. [CrossRef] [PubMed]
4. Levin, B.; Lieberman, D.A.; McFarland, B.; Smith, R.A.; Brooks, D.; Andrews, K.S.; Dash, C.; Giardiello, F.M.; Glick, S.; Levin, T.R.; et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J. Clin.* 2008, 58, 130–160. [CrossRef] [PubMed]
5. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin.* 2019, 69, 7–34. [CrossRef]
6. Johns, L.E.; Houlston, R.S. A systematic review and meta-analysis of familial colorectal cancer risk. *Am. J. Gastroenterol.* 2001, 96, 2992–3003. [CrossRef]
7. Van Cutsem, E.; Nordlinger, B.; Cervantes, A.; ESMO Guidelines Working Group. Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. *Ann. Oncol.* 2010, 21 (Suppl. 5), v93–v97. [CrossRef]
8. Van Cutsem, E.; Cervantes, A.; Nordlinger, B.; Arnold, D.; ESMO Guidelines Working Group. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 2014, 25 (Suppl. 3), iii1–iii9. [CrossRef] [PubMed]
9. Van Cutsem, E.; Cervantes, A.; Adam, R.; Sobrero, A.; Van Krieken, J.H.; Aderka, D.; Aguilar, E.A.; Bardelli, A.; Benson, A.; Bodoky, G.; et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann. Oncol.* 2016, 27, 1386–1422. [CrossRef]
10. Argilié, G.; Tabernero, J.; Labianca, R.; Hochhauser, D.; Salazar, R.; Iveson, T.; Laurent-Puig, P.; Quirke, P.; Yoshino, T.; Taieb, J.; et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 2020, 31, 1291–1305. [CrossRef]
11. Venook, A. Critical evaluation of current treatments in metastatic colorectal cancer. *Oncologist* 2005, 10, 250–261. [CrossRef]
12. Grothey, A.; Van Cutsem, E.; Sobrero, A.; Siena, S.; Falcone, A.; Ychou, M.; Humblet, Y.; Bouché, O.; Mineur, L.; Barone, C.; et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): An international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013, 381, 303–312. [CrossRef]
13. Mayer, R.J.; Van Cutsem, E.; Falcone, A.; Yoshino, T.; Garcia-Carbonero, R.; Mizunuma, N.; Yamazaki, K.; Shimada, Y.; Tabernero, J.; Komatsu, Y.; et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N. Engl. J. Med.* 2015, 372, 1909–1919. [CrossRef] [PubMed]
14. Tabernero, J.; Yoshino, T.; Cohn, A.L.; Obermannova, R.; Bodoky, G.; Garcia-Carbonero, R.; Ciuleanu, T.E.; Portnoy, D.C.; Van Cutsem, E.; Grothey, A.; et al. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): A randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol.* 2015, 16, 499–508. [CrossRef]
15. Loupakis, F.; Cremolini, C.; Masi, G.; Lonardi, S.; Zagonel, V.; Salvatore, L.; Cortesi, E.; Tomasello, G.; Ronzoni, M.; Spadi, R.; et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N. Engl. J. Med.* 2014, 371, 1609–1618. [CrossRef] [PubMed]

16. Pratyaksha, W.; Valentina, P.; Ben, V.; Peter, K.; Evaristo, M.; Mark, J.G.; Razvan-Ovidiu, D.C.; Meinolf, K.; John, A.B.; Anca, C.M.; et al. Velour trial biomarkers update: Impact of RAS, BRAF, and sidedness on aflibercept activity. *J. Clin. Oncol.* 2017, 35, 3538.

17. Karapetis, C.S.; Khambata-Ford, S.; Jonker, D.J.; O’Callaghan, C.J.; Tu, D.; Tebbutt, N.C.; Simes, R.J.; Chalchal, H.; Shapiro, J.D.; Robitaille, S.; et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N. Engl. J. Med.* 2005, 359, 1757–1765. [CrossRef]

18. Van Cutsem, E.; Peeters, M.; Siena, S.; Humblet, Y.; Hendlisz, A.; Neyns, B.; Canon, J.L.; Van Laethem, J.L.; Maurel, J.; Richardson, G.; et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J. Clin. Oncol.* 2007, 25, 1658–1664. [CrossRef] [PubMed]

19. Nordlinger, B.; Sorbye, H.; Glimelius, B.; Poston, G.J.; Schlag, P.M.; Rougier, P.; Bechstein, W.O.; Primrose, J.N.; Walpole, E.T.; Finch-Jones, M.; et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): Long-term results of a randomised, controlled phase 3 trial. *Lancet Oncol.* 2013, 14, 1208–1215. [CrossRef]

20. Primavesi, F.; Stättner, S.; Jäger, T.; Göbel, G.; Presl, J.; Tomanová, K.; Buchner, S.; Maglione, M.; Resch, T.; Hutter, J.; et al. Progressive Oncological Surgery Is Associated with Increased Curative Resection Rates and Improved Survival in Metastatic Colorectal Cancer. *Cancers* 2019, 11, 218. [CrossRef] [PubMed]

21. Braunwarth, E.; Perathoner, A.; Stättner, S.; Maglione, M. Laparoscopic liver surgery for colorectal liver metastases—A narrative review of the recent literature. *Laparosc Surg.* 2020, in press. [CrossRef]

22. Schullian, P.; Johnston, E.W.; Putzer, D.; Laimer, G.; Waroschitz, G.; Braunwarth, E.; Amann, A.; Maglione, M.; Bale, R. Stereotactic radiofrequency ablation (SRFA) for recurrent colorectal liver metastases after hepatic resection. *Eur. J. Surg. Oncol.* 2020, in press. [CrossRef]

23. Holderfield, M.; Deuker, M.M.; McCormick, F.; McMahon, M. Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. *Nat. Rev. Cancer* 2014, 14, 455–467. [CrossRef] [PubMed]

24. Ardekani, G.S.; Jafarnejad, S.M.; Tan, L.; Saeedi, A.; Li, G. The prognostic value of BRAF mutation in colorectal cancer and melanoma: A systematic review and meta-analysis. *PLoS ONE* 2012, 7, e47054. [CrossRef]

25. Kinzler, K.W.; Vogelstein, B. Lessons from hereditary colorectal cancer. *Cell* 1996, 87, 159–170. [CrossRef]

26. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The Next Generation. *Cell* 2011, 144, 646–674. [CrossRef] [PubMed]

27. The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012, 487, 330–337. [CrossRef]

28. Zebisch, A.; Czernilofsky, A.P.; Keri, G.; Smigelskaite, J.; Sill, H.; Troppmair, J. Signaling through RAS-RAF-MEK-ERK: From basics to bedside. *Curr. Med. Chem.* 2012, 19, 646–674. [CrossRef]

29. Zebisch, A.; Troppmair, J. Back to the roots: The remarkable RAF oncogene story. *Eur. J. Surg. Oncol.* 2019, 45, 130–1330. [CrossRef]

30. Wellbrock, C.; Karasarides, M.; Marais, R. The RAF proteins take centre stage. *Nat. Rev. Mol. Cell Biol.* 2012, 13, 875–885. [CrossRef]

31. Morris, V.K.; Bekaii-Saab, T. Improvements in Clinical Outcomes for BRAF^{V600E} Mutant Metastatic Colorectal Cancer. *Clin. Cancer Res.* 2014, 20, 4435–4441. [CrossRef]

32. Guinney, J.; Dienstmann, R.; Wang, X.; de Reynies, A.; Schlicker, A.; Soneson, C.; Marisa, L.; Roepman, P.; Nymundanda, G.; Angelino, P.; et al. The consensus molecular subtypes of colorectal cancer. *Nat. Med.* 2015, 21, 1350–1356. [CrossRef] [PubMed]

33. Schadendorf, D.; van Akkooi, A.C.J.; Berking, C.; Griewank, K.G.; Gutzmer, R.; Hauschild, A.; Stang, A.; Roesch, A.; Ugurel, S. Melanoma. *Lancet* 2018, 392, 971–984. [CrossRef]

34. Czamecka, A.M.; Bartnik, E.; Fiedorowicz, M.; Rutkowski, P. Targeted Therapy in Melanoma and Mechanisms of Resistance. *Int. J. Mol. Sci.* 2020, 21, 4576. [CrossRef] [PubMed]

35. Wong, D.J.; Ribas, A. Targeted Therapy for Melanoma. In *Cancer Treatment and Research Book Series*; Kaufman, H.L., Mehnert, J.M., Eds.; Springer: Cham, Switzerland, 2016; Volume 167, pp. 251–262. [CrossRef]
36. Gutzmer, R.; Stroyakovskiy, D.; Gogas, H.; Robert, C.; Lewis, K.; Protsenko, S.; Pereira, R.P.; Eigentler, T.; Ruthkowsk, P.; Demidov, L.; et al. Atezolizumab, vemurafenib, and cobimetinib as first-line treatment for unresectable advanced BRAFV600 mutation-positive melanoma (IMspire150): Primary analysis of the randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2020, 395, 1835–1844. [CrossRef]

37. Shin, M.H.; Kim, J.; Lim, S.A.; Lee, K.M. Current Insights into Combination Therapies with MAPK Inhibitors and Immune Checkpoint Blockade. *Int. J. Mol. Sci.* 2020, 21, 2531. [CrossRef] [PubMed]

38. Yang, J.; Nie, J.; Ma, X.; Wei, Y.; Peng, Y.; Wei, X. Targeting PI3K in cancer: Mechanisms and advances in clinical trials. *Mol. Cancer* 2019, 18, 26. [CrossRef]

39. Polakis, P. The many ways of Wnt in cancer. *Curr. Opin. Genet. Dev.* 2007, 17, 45–51. [CrossRef]

40. Zhan, T.; Rindtorff, N.; Boutros, M. Wnt signaling in cancer. *Onco Targets Ther.* 2017, 36, 1461–1473. [CrossRef]

41. Hemmings, B.A.; Restuccia, D.F. PI3K-PKB/Akt pathway. *Cold Spring Harb. Perspect. Biol.* 2012, 4, a011189. [CrossRef]

42. Zhang, J.; Roberts, T.M.; Shivdasani, R.A. Targeting PI3K signaling as a therapeutic approach for colorectal cancer. *Gastroenterology* 2011, 141, 50–61. [CrossRef]

43. Jeong, W.J.; Ro, E.J.; Choi, K.Y. Interaction between Wnt and PI3K/AKT signaling pathway in colorectal cancer. *Expert Opin. Drug Discov.* 2020, 15, 745–754. [CrossRef] [PubMed]

44. Rawson, J.B.; Mrkonjic, M.; Daftary, D.; Dicks, E.; Buchanan, D.D.; Parfrey, P.S.; Young, J.P.; Pollett, A.; Green, R.C.; et al. Promoter methylation of Wnt5a is associated with microsatellite instability and BRAF V600E mutation in two large populations of colorectal cancer patients. *Br. J. Cancer* 2011, 104, 1906–1912. [CrossRef] [PubMed]

45. Kopetz, S.; Desai, J.; Chan, E.; Hecht, J.R.; O’Dwyer, P.; Maru, D.; Morris, V.; Janku, F.; Dasari, A.; Chung, W.; et al. Phase II Pilot Study of Vemurafenib in Patients With Metastatic BRAF-Mutated Colorectal Cancer. *J. Clin. Oncol.* 2015, 33, 4032–4038. [CrossRef]

46. Tran, B.; Cohen, M.S. The discovery and development of binimetinib for the treatment of melanoma. *Expert Opin. Drug Discov.* 2020, 15, 745–754. [CrossRef] [PubMed]

47. Aasen, S.N.; Parajuli, H.; Hoang, T.; Feng, Z.; Stokke, K.; Wang, J.; Roy, K.; Bjerkvig, R.; Knappskog, S.; Thorsen, F. E. Wnt signaling in cancer. *Curr. Opin. Genet. Dev.* 2017, 40, 3992–3995. [CrossRef] [PubMed]

48. Daniel, S.; et al. Targeting HER2 in colorectal cancer: The landscape of amplification and short variant mutations in ERBB2 and ERBB3. *Cancer* 2018, 124, 1358–1373. [CrossRef] [PubMed]

49. Ross, J.S.; Fakhri, M.; Ali, S.M.; Elvin, J.A.; Schrock, A.B.; Suh, J.; Vergilio, J.A.; Ramkissoon, S.; Severson, E.; Daniel, S.; et al. Targeting HER2 in colorectal cancer: The landscape of amplification and short variant mutations in ERBB2 and ERBB3. *Cancer* 2018, 124, 1358–1373. [CrossRef] [PubMed]

50. Li,” 2020. 

51. Ameador, R.G.; Wolf, M.; Peeters, M.; Van Cutsem, E.; Siena, S.; Freeman, D.J.; Juan, T.; Sikorski, R.; Suggs, S.; Radinsky, R.; et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 2008, 26, 1626–1634. [CrossRef]

52. Dienstmann, R.; Salazar, R.; Tabernero, J. Molecular Subtypes and the Evolution of Treatment Decisions in Metastatic Colorectal Cancer. *Annu. Rev. Clin. Oncol.* 2018, 38, 231–238. [CrossRef]

53. Taieb, J.; Le Malicot, K.; Shi, Q.; Penault-Llorca, F.; Bouché, O.; Tabernero, J.; Mini, E.; Goldberg, R.M.; Folprecht, G.; Van Laethem, J.L.; et al. Prognostic Value of BRAF and KRAS Mutations in MSI and MSS Stage III Colon Cancer. *J. Natl. Cancer Inst.* 2017, 109. [CrossRef] [PubMed]

54. S3-Leitlinie Kolorektales Karzinom. Langversion 2.1.—Januar 2019. Available online: https://www.awmf.org/uploads/tx_szleitlinien/021-007OL_S3_Kolorektales-Karzinom-KRK_2019-01.pdf (accessed on 20 October 2020).

55. Tosi, F.; Sartore-Bianchi, A.; Lonardi, S.; Amatu, A.; Leone, F.; Ghezzi, S.; Martino, C.; Bencardino, K.; Bonazzina, E.; Bergamo, F.; et al. Long-term Clinical Outcome of Trastuzumab and Lapatinib for HER2-positive Metastatic Colorectal Cancer. *Clin. Colorectal Cancer* 2020, 19, 256–262.e2. [CrossRef] [PubMed]

56. Cantwell-Dorris, E.R.; O’Leary, J.J.; Sheils, O.M. BRAFV600E: Implications for carcinogenesis and molecular therapy. *Mol. Cancer Ther.* 2011, 10, 385–394. [CrossRef] [PubMed]
57. Maughan, T.S.; Adams, R.A.; Smith, C.G.; Meade, A.M.; Seymour, M.T.; Wilson, R.H.; Idziaszczyk, S.; Harris, R.; Fisher, D.; Kenny, S.L.; et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: Results of the randomised phase 3 MRC COIN trial. *Lancet* 2011, 377, 2103–2114. [CrossRef]

58. Souglakos, J.; Philips, J.; Wang, R.; Marwab, S.; Silver, M.; Tzard, M.; Silver, J.; Ogino, S.; Hooshmand, S.; Kwak, E.; et al. Prognostic and predictive value of common mutations for response to treatment and survival in patients with metastatic colorectal cancer. *Br. J. Cancer* 2009, 101, 465–472. [CrossRef] [PubMed]

59. Richman, S.D.; Seymour, M.T.; Chambers, P.; Elliott, F.; Daly, C.L.; Meade, A.M.; Taylor, G.; Barrett, J.H.; Quirke, P. 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, 5931–5937. [CrossRef] [PubMed]

60. Tran, B.; Kopetz, S.; Tie, J.; Gibbs, P.; Jiang, Z.Q.; Lieu, C.H.; Agarwal, A.; Maru, D.M.; Sieber, O.; Desai, J. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer* 2011, 117, 4623–4632. [CrossRef]

61. Yokota, T.; Ura, T.; Shibata, N.; Takahari, D.; Shitara, K.; Nomura, M.; Kondo, C.; Mizota, A.; Utsunomiya, S.; Muro, K.; et al. BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. *Br. J. Cancer* 2011, 104, 856–862. [CrossRef]

62. Tie, J.; Gibbs, P.; Lipton, L.; Christie, M.; Jorissen, R.N.; Burgess, A.W.; Croxford, M.; Jones, I.; Langland, R.; Kosmider, S.; et al. Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAFV600E mutation. *Int. J. Cancer* 2011, 128, 2075–2084. [CrossRef]

63. Sorbye, H.; Dragomir, A.; Sundström, M.; Pfeiffer, P.; Thunberg, U.; Bergfors, M.; Aasebo, K.; Eide, G.E.; Ponten, F.; Qvortrup, C.; et al. High BRAF Mutation Frequency and Marked Survival Differences in Subgroups According to KRAS/BRaf Mutation Status and Tumor Tissue Availability in a Prospective Population-Based Metastatic Colorectal Cancer Cohort. *PLoS ONE* 2015, 10, e0131046. [CrossRef]

64. Pietrantonio, F.; Petrelli, F.; Coinu, A.; Di Bartolomeo, M.; Bortolomeo, K.; Baggio, C.; Cabiddu, M.; Iacovelli, R.; Bossi, L.; Donati, V.; et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: A meta-analysis. *Eur. J. Cancer* 2015, 51, 587–594. [CrossRef] [PubMed]

65. Jones, J.C.; Renfro, L.A.; Al-Shamsi, H.O.; Schrock, A.B.; Rankin, A.; Zhang, B.Y.; Kasi, P.M.; Voss, J.S.; Leal, A.D.; Sun, J.; et al. Non-V600BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *J. Clin. Oncol.* 2017, 35, 2624–2630. [CrossRef] [PubMed]

66. Cremolini, C.; Di Bartolomeo, M.; Amatu, A.; Antoniotti, C.; Moretto, R.; Berenato, R.; Perrone, F.; Tamborini, E.; Aprile, G.; Lonardi, S.; et al. BRAF codons 594 and 596 mutations identify a new molecular subtype of metastatic colorectal cancer at favorable prognosis. *Ann. Oncol.* 2015, 26, 2092–2097. [CrossRef]

67. Yaeger, R.; Otte, P.; Mertens, A.; van de Vijver, M.; van't Veer, L.; van der Graaf, Y.; Yu, C.; Cozzio, A.; Mikkelsen, T.; et al. Response to Anti-EGFR Therapy in Patients with BRAF non-V600-Mutant Metastatic Colorectal Cancer. *Clin. Cancer Res.* 2019, 25, 7089–7097. [CrossRef] [PubMed]

68. Wang, J.; Shen, J.; Huang, C.; Cao, M.; Shen, L. Clinicopathological Significance of BRAFV600E mutation in colorectal cancer: An updated meta-analysis. *J. Cancer* 2019, 10, 2323–2341. [CrossRef]

69. Samowitz, W.S.; Albertsen, H.; Sweeney, C.; Herrick, J.; Caan, B.J.; Anderson, K.E.; Wolff, R.K.; Slattery, M.L. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. *J. Natl. Cancer Inst.* 2006, 98, 1731–1738. [CrossRef]

70. Day, F.; Muranyi, A.; Singh, S.; Shanmugam, K.; Williams, D.; Byrne, D.; Pham, K.; Palmieri, M.; Tie, J.; Grogan, T.; et al. A mutant BRAF V600E-specific immunohistochemical assay: Correlation with molecular mutation status and clinical outcome in colorectal cancer. *Target. Oncol.* 2015, 10, 99–109. [CrossRef]

71. Samowitz, W.S.; Sweeney, C.; Herrick, J.; Albertsen, H.; Levin, T.R.; Murtaugh, M.A.; Wolff, R.K.; Slattery, M.L. Poor survival associated with the BRAFV600E mutation in microsatellite-stable colon cancers. *Cancer Res.* 2005, 65, 6063–6069. [CrossRef]

72. Van Cutsem, E.; Köhne, C.H.; Láng, I.; Folprecht, G.; Nowacki, M.P.; Cascinu, S.; Schepotin, I.; Maurel, J.; Cunningham, D.; Tejpar, S.; 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, 2011–2019. [CrossRef]
73. Roth, A.D.; Tejpar, S.; Delorenzi, M.; Yan, P.; Fiocca, R.; Klingbiel, D.; Dietrich, D.; Biesmans, B.; Bodoky, G.; Barone, C.; 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. [CrossRef]

74. Domingo, E.; Camps, C.; Kaisaki, P.J.; Parsons, M.J.; Mouradov, D.; Pentony, M.M.; Makino, S.; Palmieri, M.; Ward, R.L.; Hawkins, N.J.; et al. Mutation burden and other molecular markers of prognosis in colorectal cancer treated with curative intent: Results from the QUASAR 2 clinical trial and an Australian community-based series. Lancet Gastroenterol. Hepatol. 2018, 3, 635–643. [CrossRef]

75. Klingbiel, D.; Saridaki, Z.; Roth, A.D.; Bosman, F.T.; Delorenzi, M.; Tejpar, S. Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: Results of the PETACC-3 trial. Ann. Oncol. 2015, 26, 126–132. [CrossRef] [PubMed]

76. Hutchins, G.; Southward, K.; Handley, K.; Magill, L.; Beaumont, C.; Stahlschmidt, J.; Richman, S.; Chambers, P.; Seymour, M.; Kerr, D.; et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J. Clin. Oncol. 2011, 29, 1261–1270. [CrossRef] [PubMed]

77. Ogino, S.; Shima, K.; Meyerhardt, J.A.; McLeod, N.J.; Ng, K.; Hollis, D.; Saltz, L.B.; Mayer, R.J.; Schaefer, P.; Whittom, R.; et al. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: Results from intergroup trial CALGB 89803. Clin. Cancer Res. 2012, 18, 890–900. [CrossRef] [PubMed]

78. Sinicrope, F.A.; Mahoney, M.R.; Smyrk, T.C.; Thibodeau, S.N.; Warren, R.S.; Bertagnolli, M.M.; Nelson, G.D.; Goldberg, R.M.; Sargent, D.J.; Alberts, S.R. 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. [CrossRef]

79. Venderbosch, S.; Nagtegaal, I.D.; Maughan, T.S.; Smith, C.G.; Cheadle, J.P.; Fisher, D.; Kaplan, R.; Quirke, P.; Seymour, M.T.; Richman, S.D.; 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. [CrossRef]

80. Noepel-Duennebacke, S.; Arnold, D.; Hertel, J.; Tannapfel, A.; Hinke, A.; Hegewisch-Becker, S.; Reinacher-Schick, A. Impact of the Localization of the Primary Tumor and RAS/BRAF Mutational Status on Maintenance Strategies After First-line Oxaliplatin, Fluoropyrimidine, and Bevacizumab in Metastatic Colorectal Cancer: Results From the AIO 0207 Trial. Clin. Colorectal Cancer 2018, 17, e733–e739. [CrossRef]

81. Neumann, J.; Heinemann, V.; Engel, J.; Kirchner, T.; Stintzing, S. The prognostic impact of CDX2 correlates with the underlying mismatch repair status and BRAF mutational status but not with distant metastasis in colorectal cancer. Virchows Arch. 2018, 473, 199–207. [CrossRef]

82. Kobayashi, S.; Takahashi, S.; Takahashi, N.; Masuishi, T.; Soji, H.; Shinozaki, E.; Yamaguchi, T.; Kojima, M.; Gotohda, N.; Nomura, S.; et al. Survival Outcomes of Resected BRAF V600E Mutant Colorectal Liver Metastases: A Multicenter Retrospective Cohort Study in Japan. Ann. Surg. Oncol. 2020, 27, 3307–3315. [CrossRef]

83. Loupakis, F.; Intini, R.; Cremolini, C.; Orlandi, A.; Sartore-Bianchi, A.; Pietrantonio, F.; Pella, N.; Spallanzani, A.; Dell’Aquila, E.; Scartozzi, M.; et al. A validated prognostic classifier for V600E BRAF-mutated metastatic colorectal cancer: The ‘BRAF BeCool’ study. Eur. J. Cancer 2019, 118, 121–130. [CrossRef]

84. Lang, H.; Baumgart, J.; Heinrich, S.; Tripke, V.; Passalaqua, M.; Maderer, A.; Galle, P.R.; Roth, W.; Kloth, M.; Moehler, M. Extended Molecular Profiling Improves Stratification and Prediction of Survival After Resection of Colorectal Liver Metastases. Ann. Surg. Oncol. 2019, 27, 799–805. [CrossRef] [PubMed]

85. Cremolini, C.; Loupakis, F.; Antoniotti, C.; Lupi, C.; Sensi, E.; Lonardi, S.; Mezi, S.; Tomasetto, G.; Ronzoni, M.; Zaniboni, A.; et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: Updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. 2015, 16, 1306–1315. [CrossRef]
86. Price, T.J.; Hardingham, J.E.; Lee, C.K.; Weickhardt, A.; Townsend, A.R.; Wrin, J.W.; Chua, A.; Shivasami, A.; Cummins, M.M.; Murone, C.; 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. [CrossRef] [PubMed]

87. Yoshino, T.; Portnow, D.C.; Obermannová, R.; Bodoky, G.; Prausová, J.; Garcia-Carbonero, R.; Ciuleanu, T.; Garcia-Alfonso, P.; Cohn, A.L.; Van Cutsem, E.; et al. Biomarker analysis beyond angiogenesis: RAS/RAF mutation status, tumour sidedness, and second-line ramucirumab efficacy in patients with metastatic colorectal carcinoma from RAISE—A global phase III study. *Ann. Oncol.* 2019, 30, 124–131. [CrossRef] [PubMed]

88. Douillard, J.Y.; Oliner, K.S.; Siena, S.; Tabernerio, J.; Burkes, R.; Barugel, M.; Humblet, Y.; Bodoky, G.; Cunningham, D.; Jassem, J.; et al. Panitumumab—FOLFOX4 treatment and Ras mutations in colorectal cancer. *N. Engl. J. Med.* 2013, 369, 1023–1034. [CrossRef] [PubMed]

89. Ciardiello, F.; Normanno, N.; Martinelli, E.; Troiani, T.; Piscionti, S.; Cardone, C.; Nappi, A.; Bordonaro, A.R.; Racchiglio, M.; Lambiase, M.; et al. Cetuximab continuation after first progression in metastatic colorectal cancer (CAPRI-GOIM): A randomized phase II trial of FOLFOX plus cetuximab versus FOLFOX. *Ann. Oncol.* 2016, 27, 1055–1061. [CrossRef]

90. Rowland, A.; Dias, M.M.; Wiese, M.D.; Kichenadasse, G.; McKinnon, R.A.; Karapetis, C.S.; Sorich, M.J. 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, 112, 1888–1894. [CrossRef]

91. Seymour, M.T.; Brown, S.R.; Middleton, G.; Maughan, T.; Richman, S.; Gwyther, S.; Lowe, C.; Seligmann, J.F.; Wadsley, J.; Maisey, N.; 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.* 2013, 14, 749–759. [CrossRef]

92. Geissler, M.; Klingler, T.; Knothrenschil, J.R.; Tannapfel, A.; Guegler, J.; Siefert, L.; Kanzler, S.; Held, S.; Heinemann, V.; Reinhofer-Schick, A.; et al. 1st-line mFOLFOXIRI + Panitumumab vs. FOLFOXIRI treatment of Ras wt mCRC: A randomized phase II VOLF1 trial of the AIO (KRK-0109). *Ann. Oncol.* 2018, 29 (Suppl 8), viii150–viii204. [CrossRef]

93. Roberto, M.; Marchetti, P.; Arrivi, G.; Di Pietro, F.R.; Casciniu, S.; Gelsomino, F.; Caputo, F.; Cerma, K.; Ghidini, M.; Ratti, M.; et al. The treatment paradigm of right-sided metastatic colon cancer: Harboring BRAF mutation makes the difference. *Int. J. Colorectal Dis.* 2020, 35, 1513–1527. [CrossRef]

94. Stintzing, S.; Miller-Phillips, L.; modest, D.P.; von Weikersthal, L.F.; Decker, T.; Kiani, A.; Vehling-Kaiser, U.; Al-Batran, S.E.; Heintges, T.; Kahl, C.; 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, 79, 50–60. [CrossRef] [PubMed]

95. Cremolini, C.; Antoniotti, C.; Rossini, D.; Lonardi, S.; Loupakis, F.; Pietrantonio, F.; Bordonaro, R.; Latiano, T.P.; Tamburini, E.; Santini, D.; et al. Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFOXIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): A multicentre, open-label, phase 3, randomised, controlled trial. *Lancet Oncol.* 2020, 21, 497–507. [CrossRef] [PubMed]

96. National Comprehensive Cancer Network®, NCCN Clinical Practice guidelines in Oncology (NCCN Guidelines®): Colon Cancer: Version 4.2020. Available online: https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf (accessed on 21 October 2020).

97. Loupakis, F.; Cremolini, C.; Salvatore, L.; Masi, G.; Sensi, E.; Schirripa, M.; Michelucci, A.; Pfanner, E.; Brunetti, I.; Lupi, C.; et al. FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer. *Eur. J. Cancer* 2014, 50, 57–63. [CrossRef] [PubMed]

98. Seligmann, J.F.; Fisher, D.; Smith, C.G.; Richman, S.D.; Elliott, F.; Brown, S.; Adams, R.; Maughan, T.; Quirke, P.; Cheadle, J.; 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. [CrossRef] [PubMed]

99. Dummer, R.; Ascierto, P.A.; Gogas, H.J.; Arance, A.; Mandala, M.; Liszkay, G.; Garbe, C.; Schadendorf, D.; Krajsova, I.; Gutzmer, R.; et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): A multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* 2018, 19, 603–615. [CrossRef]
115. Dahlmann, M.; Okhrimenko, A.; Marcinkowski, P.; Osterland, M.; Herrmann, P.; Smith, J.; Heizmann, C.W.; Schlag, P.M.; Stein, U. RAGE mediates S100A4-induced cell motility via MAPK/ERK and hypoxia signaling and is a prognostic biomarker for human colorectal cancer metastasis. *Oncotarget* **2014**, *5*, 3220–3233. [CrossRef] [PubMed]

116. Boye, K.; Nesland, J.M.; Sandstad, B.; Mælandsmo, G.M.; Flatmark, K. Nuclear S100A4 is a novel prognostic marker in colorectal cancer. *Eur. J. Cancer* **2010**, *46*, 2919–2925. [CrossRef] [PubMed]

117. Burock, S.; Daum, S.; Keilholz, U.; Neumann, K.; Walther, W.; Stein, U. Phase II trial to investigate the safety and efficacy of orally applied niclosamide in patients with metachronous or synchronous metastases of a colorectal cancer progressing after therapy: The NIKOLO trial. *BMC Cancer* **2018**, *18*, 297. [CrossRef] [PubMed]

118. Ganesh, K.; Stadler, Z.K.; Cercek, A.; Mendelsohn, R.B.; Shia, J.; Segal, N.H.; Diaz, L.A., Jr. Immunotherapy in colorectal cancer: Rationale, challenges and potential. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 361–375. [CrossRef]

119. Rosenbaum, M.W.; Bledsoe, J.R.; Morales-Oyarvide, V.; Huynh, T.G.; Mino-Kenudson, M. PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumor-infiltrating lymphocytes. *Mod. Pathol.* **2016**, *29*, 1104–1112. [CrossRef] [PubMed]

120. Le, D.T.; Kim, T.W.; Van Cutsem, E.; Geva, R.; Jäger, D.; Hara, H.; Burge, M.; O’Neil, B.; Kavan, P.; Yoshino, T.; et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability—High/Mismatch Repair—Deficient Metastatic Colorectal Cancer: KEYNOTE-164. *J. Clin. Oncol.* **2020**, *38*, 11–19. [CrossRef]

121. Overman, M.J.; McDermott, R.; Leach, J.L.; Lonardi, S.; Lenz, H.J.; Morse, M.A.; Desai, J.; Hill, A.; Axelson, M.; Moss, R.A.; et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): An open-label, multicentre, phase 2 study. *Lancet Oncol.* **2017**, *18*, 1182–1191. [CrossRef]

122. Overman, M.J.; Lonardi, S.; Wong, K.Y.M.; Lenz, H.J.; Gelsomino, F.; Aglietta, M.; Morse, M.A.; Van Cutsem, E.; McDermott, R.; Hill, A.; et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair—Deficient/Microsatellite Instability—High Metastatic Colorectal Cancer. *J. Clin. Oncol.* **2018**, *36*, 773–779. [CrossRef]

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