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***BRAF* mutations as a therapeutic target in metastatic colorectal cancer patients: a long due success**

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Colorectal cancer (CRC) continues to be a challenging cancer with an increasing incidence among young adults, which has led to changes in recommendations for screening. Consistent with this trend, CRC remains the third leading cause of cancer-related death in the USA among men and women [1]. Oncogenic driver genes among young adults appear to be different; perhaps this is associated with their distinct molecular behavior and has a resultant direct effect on the clinical course of the disease [2]. The rising incidence of young-onset advanced-stage CRC will also likely impact the molecular landscape of CRC as well as the frequency of potentially actionable molecular alterations.

Recent progress in the management of metastatic CRC patients, particularly in the field of precision medicine, will likely enhance our therapeutic options and lead to significant improvements in the outcomes of advanced-stage patients. With the use of next-generation sequencing, comprehensive molecular profiling can be conducted with relatively reasonable tumor sample sizes and within reasonable time durations (~4 weeks); this can potentially reveal actionable molecular alterations that are targetable, such as mismatch repair deficiency (MMR-D), *HER2* amplification and *BRAF* V600 mutations.

BRAF V600 mutations have long been of interest in the field of targeted therapeutics. Recently, combined approaches with EGFR blockade and *BRAF* inhibitors, with and without *MEK* inhibitors, have resulted in promising clinical outcomes. In this article, we elaborate on the clinical and molecular characteristics of *BRAF* V600 mutations in the CRC patient and discuss recent clinical data from early phase clinical trials investigating the therapeutic targeting of *BRAF* V600 mutations.

Oncogenic *BRAF* mutations at a glance

BRAF is a well-established oncogene and gain of function mutations have been identified in multiple solid tumors, including melanoma, lung and colorectal cancers [3]. *BRAF* is a member of the RAF kinase family (a serine/threonine-protein kinase family) and it has a key function in the MAP kinase signaling pathway which drives cellular growth and proliferation [4]. In the MAP kinase pathway, *BRAF* is regulated by upstream *RAS* signaling, which is, in turn, activated by receptor tyrosine kinases such as EGFR [5].

BRAF V600E mutation, which is by far the most common (80–97%) among *BRAF* mutations in CRC patients, results in substitution of glutamic acid (E) for valine (V) at position 600 in the kinase domain of the protein [3,6]. Other less common *BRAF* mutations, including V600K, V600R, K601E and G469A, have been shown to be involved in carcinogenesis in multiple solid tumors, including CRC [7,8]. However, it is important to note that their

oncogenic activity is significantly different [9] when compared with *BRAF* V600E. For example, in one preclinical study analyzing the *BRAF* mutations *in vitro*, the authors demonstrated higher oncogenic *ERK* pathway activation with *BRAF* V600E mutation as compared with *BRAF* V600K mutation and even more interestingly, decreased *BRAF* activity and *ERK* pathway activation with certain mutations such as *BRAF* D594V [9]. These data suggest that the clinical significances of *BRAF* mutations should be carefully assessed in each clinical scenario based on the specific mutation, rather than conceptually considering all *BRAF* mutations in a single, equivalent category. The mutations causing an oncogenic gain of function in the *BRAF* gene result in a constitutively-activated downstream *MEK-ERK* signaling pathway, thus inducing cell growth, proliferation and invasion [8,10].

Clinical characteristics of *BRAF* mutations in metastatic colorectal cancer patients

BRAF mutations have been identified in approximately 5–10% of CRC patients in different patient cohorts [11,12]. The frequency of *BRAF* mutation is greater in tumors with microsatellite instability (MSI) as compared with microsatellite stable (MSS) tumors, particularly in sporadic microsatellite unstable tumors that are associated with CpG island methylator phenotype [13–15]. Multiple studies have suggested worse clinical outcomes in CRC patients with a *BRAF* mutation, in particular with MSS tumors [13]. For example, in one clinical cohort study, the authors showed *BRAF* mutations may be one of the predictors of worse outcomes in early-stage CRC patients who underwent surgical resections [16]. Similarly, in metastatic disease, tumors with *BRAF* V600E mutation behave aggressively and lead to early disease progression [17,18]. Notably, *BRAF*-mutant CRC tumors with MSI appear to have better outcomes when compared with MSS patients, indicating a distinct clinical behavior, perhaps related to the immunogenic nature of tumors with MSI tumors being infiltrated by tumor-reactive lymphocytes [13,19]. Therefore, CRC patients with *BRAF* mutations should be evaluated based on their MSI status and the clinical approach to therapeutic options should be determined in a different manner as discussed below. Notably, MSI itself may result in uncommon *BRAF* mutations (unlike *BRAF* V600E driven CpG island methylator phenotype tumors) as a consequence of frameshift mutations, but their clinical significances are currently unclear.

Clinical trials investigating the efficacy of *BRAF* targeting agents in combination with EGFR blockade

BRAF mutations have been a focus for investigators as a potential target given its biological and clinical significance. Promising response rates and improvement in clinical outcomes in melanoma patients with single-agent vemurafenib, a *BRAF* inhibitor, have not been translated into clinically meaningful effects in CRC patients [20]. Unlike melanoma, CRC carries different molecular characteristics, with various alternative oncogenic pathways for activation that serve as escape mechanisms against *BRAF* inhibitors. For example, a landmark preclinical study in *BRAF*-mutant CRC showed rebound activation of EGFR signaling in the presence of *BRAF* inhibition [21]. Consistent with preclinical data, outcomes with single-agent *BRAF* inhibitors, in *BRAF*-mutant CRC, did not lead to significant clinical responses [20,22]. These findings have led to clinical trials combining *BRAF* inhibitors and EGFR blockade.

In an early-phase clinical trial, vemurafenib was combined with irinotecan (180 mg/m²) and an EGFR monoclonal antibody, cetuximab (500 mg/m²) known as VIP regimen. This treatment plan resulted in an objective response rate (ORR) and median progression free survival of 35% and 7.7 months, respectively, in metastatic *BRAF* V600E mutant CRC patients who were previously treated with cytotoxic agents [23]. The most common adverse events noted with this triplet therapy were – expectedly – fatigue (89%), diarrhea (84%), nausea (79%) and rash (74%). In another Phase I trial, dabrafenib (a *BRAF* inhibitor) and trametinib (an *MEK* inhibitor) with panitumumab (an EGFR monoclonal antibody) were investigated in previously-treated metastatic CRC patients with a *BRAF* mutation, for both safety and early clinical efficacy analysis [24]. The triple combination of dabrafenib, trametinib and panitumumab led to improved clinical responses with an ORR of 21% as compared with the combination of dabrafenib and panitumumab (an ORR of 10%) or trametinib and panitumumab (no objective responses).

The safety lead-in of a Phase III trial (the BEACON trial) investigated the combination of encorafenib (a *BRAF* inhibitor) and binimetinib (a *MEK* inhibitor) with cetuximab in previously treated metastatic *BRAF*-mutant CRC patients [25]. In this study, patients received triplet therapy as a combination of encorafenib (300 mg daily), binimetinib (45 mg twice daily) and cetuximab at 400 mg/m² (first dose) followed by 250 mg/m² per week as a 28-day cycle regimen. The authors reported very promising ORR (41%) although only a limited number of patients were evaluated at the time of the report (a total of 29 patients). The authors also reported that an additional nine

patients had durable disease control, further indicating promising therapeutic efficacy. Based on these findings, the US FDA granted breakthrough therapy designation to this triplet therapy [26]. In this study, the toxicities related to this triplet therapy were manageable and the most common adverse events were fatigue (14%), anemia (10%), increased CPK (10%) and AST (10%). Most recently, the authors reported the final results of the safety lead-in part of this trial and identified a progression free survival and overall survival of 8.0 and 15.3 months, respectively [27]. At this time, National Comprehensive Cancer Network guidelines have been updated for these different approaches targeting *BRAF* mutations in metastatic CRC patients [26].

It is important to note that the therapeutic approach for *BRAF*-mutant CRC with MSI-High (MSI-H) disease should be different. *BRAF*-mutant CRC patients with MMR-D/MSI-H disease should be considered for immune checkpoint inhibitor-based therapy, which includes nivolumab, nivolumab with ipilimumab or pembrolizumab, based on the recent evidence with promising clinical responses [28–30]. Therefore, the MMR-D/MSI-H should be evaluated in all *BRAF*-mutant CRC patients, if they have not already been assessed at the time of initial diagnosis.

Conclusion & future perspective

With the recent progress in targeted cancer therapeutics, the combination approaches targeting *BRAF* mutations and associated feedback loops have yielded significant clinical responses; in fact, one of these combinations (encorafenib, binimetinib and cetuximab) has been granted breakthrough therapy designation, based on the promising results in early reports of the BEACON trial [27,31]. The other aforementioned combinations have also been implemented in National Comprehensive Cancer Network guidelines and can be considered in *BRAF*-mutant CRC patients. Perhaps in the near future, all the aforementioned combined therapeutic options targeting *BRAF* mutations in CRC patients will likely be approved by the FDA. However, it is important to note that, the studies discussed above have evaluated these patients in chemotherapy-refractory settings, and therefore these agents should be used as a subsequent line of therapy based on the current evidence. In the first-line setting, FOLFOXIRI in combination with bevacizumab should be considered in patients with good performance status [32]. Notably, upfront classification of *BRAF*-mutant CRC patients based on their MMR-D/MSI-H status is required, as patients with MMR-D/MSI-H disease in the setting of a *BRAF* mutation may benefit from immune checkpoint inhibitors (pembrolizumab, nivolumab or nivolumab with ipilimumab) and achieve durable clinical responses [28,29]. It is also important to note that these agents have already been approved by the FDA for this subset of CRC patients.

Even though these promising results will likely improve the outcomes in this aggressive subset of CRC patients, there are resistance mechanisms that develop progressively. In a translational study, resistance mechanisms were investigated in patients with *BRAF*-mutant CRC treated with *BRAF*/MEK inhibitors and multiple aberrations were identified that reconstituted MAP kinase signaling, including *BRAF* and *KRAS* amplification and a *MEK1* mutation [33]. These data suggest that additional blockade(s) downstream of the MAP kinase pathway could potentially further enhance the efficacy of *BRAF*-targeting agents and may prevent development of resistance that can be attributed to MAP kinase signaling. Perhaps performing molecular profiling of circulating tumor DNA upon progression may advance our understanding related to mechanisms of acquired resistance. Moreover, molecular profiling may potentially uncover other actionable genes that can be targeted in a clinical trial setting. As discussed above, the significance of other uncommon *BRAF* mutations (besides *BRAF* V600E and *BRAF* V600K) in CRC patients is unknown and may or may not be related to the driver gene. Interestingly, a recent study reported the benefit of the use of EGFR-directed therapies without *BRAF* inhibitors in CRC patients with *BRAF* non-V600 mutations [34]. This recent finding suggests that *BRAF* mutations should not be lumped into a single category and each case should be evaluated individually based on the class of *BRAF* mutation [9].

Detection of other clinically relevant genomic alterations in CRC patients may also provide important data regarding other driver genes, e.g., *KRAS*, *PIK3CA* and *HER2*. Therefore, we propose that all *BRAF*-mutant metastatic CRC patients should undergo next-generation based molecular profiling for better characterization of the molecular signature of the tumor and to best determine the therapeutic actions.

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