The evolving landscape of BRAF inhibitors in BRAF mutant colorectal cancer and the added value of cytotoxic chemotherapy

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Comment on: Kopetz S, Guthrie KA, Morris VK, *et al.* Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406). J Clin Oncol 2021;39:285-94.

Introduction

Our understanding of the molecular profile and associated targeted therapies has revolutionised the approach to treatment of metastatic colorectal cancer (mCRC). Approximately 10% (range, 8–21%) of mCRC carry a BRAF mutation which occurs primarily (>90%) at the V600E codon and leads to overactivation of the RAS/RAF/MEK/ERK signalling [mitogen-activated protein kinases (MAPK)] pathway (1). $BRAF^{V600E}$ mutant (MT) mCRC are renowned for their poor prognosis with a median overall survival (OS) inferior ranging from 10 to 20 months with resistance to standard systemic therapy, often not even reaching second-line treatment (2,3). The exception is the subset of MSI-H/dMRR patients (up to 30% of $BRAF^{V600E}$ MT CRC) who benefit from immunotherapy with checkpoint inhibitors (CPI) following the recent Keynote-177 trial (4).

Unlike melanoma, previous trials with BRAF inhibition monotherapy (or combination with MEK inhibition) for $BRAF^{V600E}$ MT mCRC show only minimal activity. This is due to feedback upregulation of epidermal growth factor receptor (EGFR) that re-activates the oncogenic pathway bypassing BRAF. Benefit of combining BRAF and EGFR inhibitors to overcome this pharmacological escape has been seen in several trials now. The BEACON phase 3 trial (5) is the pivotal study of this approach showing that the combination of the BRAF inhibitor encorafenib with anti-EGFR treatment (cetuximab) with or without a MEK-inhibitor (binimetinib) led to significantly better OS and overall response rates (ORR) compared to irinotecan (FOLFIRI) or irinotecan with cetuximab.

One strategy to ameliorate outcomes of these patients might be to combine multiple mitogen-activated protein kinases (MAPK) targeting agents with cytotoxic agents. Encouraging preclinical data combining irinotecan with anti-BRAF molecules (6,7), led Kopetz *et al.* (8) to explore the addition of the BRAF inhibitor vemurafenib to a backbone of irinotecan and cetuximab in previously treated $BRAF^{V600E}$ MT mCRC.

Study design and summary of results

After random allocation, patients received irinotecan + cetuximab (IC), or irinotecan + cetuximab + vemurafenib (VIC). In 100 eligible patients with $BRAF^{V600E}$ MT mCRC who had one (including progression during or within 6 months after adjuvant) or two prior systemic regimens with the primary outcome being progression free survival (PFS). The primary end point PFS favoured the experimental arm (VIC) 4.2 months *vs.* the control arm (IC) 2.0 months [hazard ratio (HR) 0.50; 95% confidence interval (CI) 0.32–0.76; P=0.001]. Worth noting that patients were allowed to have had prior irinotecan, which might have contributed to the poor outcomes in the control arm. The improvement in PFS of adding vemurafenib was seen throughout all subgroups such as prior irinotecan treatment, tumour location, microsatellite status, PIK3CA mutations, and RNA profiling. Although there was a trend for improved

OS when adding vemurafenib, this was not statistically significant with OS of 9.6 months in the VIC group *vs.* 5.9 months in the IC group (HR 0.77; 95% CI: 0.50 to 1.18; P=0.23). The crossover of 21 patients (42%) from the IC to VIC group probably influenced this noting the PFS for those crossing over was 5.4 months in keeping with those treated upfront with this combination. The ORR were 17% and 4% in the VIC and IC group, whilst disease control rates were 65% and 21% respectively.

S1406 study also performed retrospective next-generation sequencing (NGS) and RNA sequencing on tumour tissue and prospective circulating tumour DNA (ctDNA) NGS to look for prognostic and predictive biomarkers and identify genomic mechanisms of resistance. A ctDNA analysis was possible in 69 (69%) of patients at baseline, but only in 34% at baseline and at first restaging, which stresses the challenge for collection. Eighty-seven percent of patients in the experimental arm with at minimum of two ctDNA time points demonstrated a downgrading in variant allele frequency (VAF) of BRAF[V600E] whereas no patients in the control arm did (P≤0.001), supporting increased activity of the VIC protocol. Regarding mechanisms of resistance, remarkably only one patient in the VIC arm acquired KRAS mutations (G12V and Q61L) on progression.

Concerning safety, adding vemurafenib resulted in more grade 3/4 toxicity, but notably anaemia, neutropenia and nausea or vomiting which are not specific to BRAF inhibition. This led to 22% of patients in the VIC group discontinuing treatment *vs.* 8% in the IC group. This is also in great contrast with the triplet and doublet groups of BEACON (5) where respectively 7% and 8% discontinued therapy due to adverse events and highlights the risk of combination with chemotherapy.

Discussion and commentary

It is not surprising that this study met its primary endpoint of improved PFS as the combination of BRAF and EGFR inhibition in second and further lines treatment of BRAF[V600E] mCRC has also been proven to be effective in other recent studies including the larger phase III BEACON trial (5). BEACON randomly assigned patients to receive encorafenib, binimetinib and cetuximab (triplet); encorafenib and cetuximab (doublet); or the investigators' choice of either cetuximab and irinotecan or cetuximab and FOLFIRI (folinic acid, fluorouracil, and irinotecan) (control). When we look at S1406 and BEACON, both trials showed a statistically significant benefit in median progression-free

survival (mPFS) (S1406 VIC 4.2 months *vs.* IC 2.0 months; HR 0.50 and BEACON triplet 4.3 months and doublet 4.2 months *vs.* control 1.5 months; HR 0.38 and 4.0) and ORR (S1406 17% *vs.* 4% and BEACON 26% *vs.* 20%) reflecting clear activity of BRAF inhibition together with EGFR inhibition in the later lines of treatment of BRAF[V600E] MT mCRC. When we look at older trials evaluating outcomes of BRAF MT mCRC, before the implementation of BRAF inhibitors, PFS was indeed worse. Morris *et al.* (9) retrospectively analysed 127 patients with BRAF MT mCRC who received a median of 2 lines of chemotherapy and reported a mPFS for second-line of 2.5 months (95% CI: 1.8–3.0 months), and for third-line 2.6 months (95% CI: 1.0–4.2 months).

BEACON more importantly also reported a statistically significant benefit in mOS as their primary endpoint; 9.0 months in the triplet group and 8.4 months in the doublet group *vs.* 5.4 months in the control group, both statistically significant (respectively HR 0.52; 95% CI: 0.39 to 0.70; P<0.001 and HR 0.60; 95% CI: 0.45 to 0.79; P<0.001). mOS benefit in SWOG S1406 was almost 4 months (VIC 9.6 months *vs.* IC 5.9 months; HR, 0.77, 95% CI: 0.50 to 1.18) comparable to the absolute mOS benefit in BEACON, though was not statistically significant (P=0.23) and not the primary endpoint of this phase II trial. The considerable amount (42%) of cross-over could be an explanation for this, whereas in BEACON crossover was not permitted before the cut-off date.

Notably, both S1406 as well as BEACON used a control arm (IC; FOLFIRI + cetuximab) that could be questioned given the conflicting results regarding the use of an anti-EGFR regimen in BRAF[V600E] MT mCR, especially in second or further lines (10,11). In the large FIRE-3 trial (12), a first-line study comparing FOLFIRI plus bevacizumab *vs.* FOLFIRI plus cetuximab, among BRAF[V600E] MT mCRC enrolled in this trial (N=48; 14%), cetuximab led to a higher ORR but no difference in PFS and OS between the two arms. Similarly, second-line PICCOLO trial (13), also could not demonstrate a benefit in OS when adding panitumumab to irinotecan over irinotecan alone in KRAS wild patients who had progressed on oxaliplatin, with a of 1.84 in favour of irinotecan alone in the BRAF[V600E] MT subgroup. The value of adding 5FU to this regimen is unclear, as the control arm in BEACON also included FOLFIRI + cetuximab but still reported poor PFS and OS.

There remain a number of questions as to optimal therapy for BRAF[V600E] MT mCRC, such as the most favourable BRAF targeting agent. Preclinical models have

shown that encorafenib has greater potency compared with other BRAF inhibitors such as vemurafenib an dabrafenib (14). Unfortunately, as yet there are no clinical trials with head-to-head comparison in the management of BRAF^{V600E} MT mCRC

Another important question is the added value of chemotherapy in these regimens. The rationale for adding irinotecan in S1406 came from preclinical and clinical data using patient-derived xenografts to illustrate the potential of irinotecan to add to the activity of BRAF and EGFR inhibition. In a phase IB study from Hong *et al*. (7), the combination of vemurafenib with cetuximab and irinotecan demonstrated a 35% response rate and promising PFS for setting up this phase II trial. This theory is further reinforced by older data, including the BOND (15) study, that confirmed the synergistic effect of combining irinotecan and cetuximab in patients with irinotecan-refractory mCRC (16). They reported a significantly higher ORR in the cetuximab-irinotecan group compared with the cetuximab group (22.9% *vs.* 10.8%; P=0.007), as well as a longer median time to progression (TTP) (4.1 *vs.* 1.5 months, respectively). This demonstrates the augmentation irinotecan offers to anti EGFR blockade in a BRAF unselected population, which when considering the synergism anti EGFR has shown to BRAF blockade, strengthens the argument for combining targeting agents with this chemotherapy backbone. However, in the case of S1406 the addition of irinotecan seems to lead to similar outcomes to those reported for the non-chemotherapy experimental groups in BEACON (5). Furthermore, there is increased toxicity in this trial with the addition of irinotecan.

Next steps for BRAF MT mCRC

Although recent approval of the combination of cetuximab and encorafenib in second or further-lines has improved outcomes of BRAF^{V600E} MT mCRC, still only 60% of these patients make it to second-line treatment due to the combative nature of this disease (3). Currently, there is ongoing debate regarding first-line treatment recommendations for BRAF^{V600E} MT mCRC. FOLFOXIRI plus bevacizumab has been considered more beneficial than doublet regimens plus bevacizumab, as shown in the subgroup analysis of phase III TRIBE study (17). However, this advantage was not confirmed in the subsequent TRIBE 2 study (18) and also the meta-analysis (19) from the same group did not report a benefit of triplet over doublet for patients with BRAF^{V600E} mCRC.

It is therefore crucial to optimise first-line options by investigating the role of BRAF inhibitors, including enrolment in clinical trials. Founded on the results from BEACON, the ongoing (though not recruiting) phase II ANCHOR-CRC trial (NCT03693170) is evaluating the role of BRAF inhibition in first-line setting. Their preliminary results have already shown a favourable overall response rate (ORR) of 47.8% in the single-arm group receiving encorafenib, binimetinib plus cetuximab in first-line setting for BRAF^{V600E} MT mCRC. However, the mPFS of only 4.9 months (95% CI: 4.4–8.1 months) seems to be quite poor compared to other first-line trials. For example, mPFS of the BRAF subgroup in TRIBE (17) was 5.5 months (95% CI: 1.6–11.2 months) in the FOLFIRI + bevacizumab arm and 7.5 months (95% CI: 5.1–15.0 months; HR 0.57) in the FOLFOXIRI + bevacizumab arm. To improve outcomes in first-line setting, cytotoxic chemotherapy could be associated to these regimens. The ongoing three-arm phase III BREAKWATER trial (NCT04607421) will hopefully answer the questions regarding the value of adding chemotherapy in the upfront setting. This trial is comparing first-line encorafenib + cetuximab ± chemotherapy (FOLFOX or FOLFIRI) *vs.* investigator's choice of standard chemotherapy. The strengths of this trial are that it will also compare irinotecan *vs.* oxaliplatin in the experimental arm and that there is a head-to-head comparison with current standard of care chemotherapy + bevacizumab. We may expect to see a better safety profile in the experimental groups with chemotherapy compared to S1406, as patients in first-line are often in a better general condition and less prone to accumulated haematological toxicity.

Role of prognostic and predictive biomarkers and possible mechanisms of resistance or the molecular complexity of BRAF^{V600E} CRC

ctDNA via liquid biopsy in CRC, as in many other malignancies, can aid in early diagnosis, detection of minimal residual disease and monitoring treatment response, assess different types of molecular alterations responsible for tumour transformation and heterogeneity that give rise to resistance and assist the decision of the most suitable therapies (20). As it was shown in the phase 1B study from the same group, in S1406, serial ctDNA BRAF^{V600E} testing appeared to be a sensitive indicator of treatment response with 87% of the experimental arm demonstrating a downgrade in VAF of BRAF^{V600E} while no patients in the control arm did (P≤0.001). However, it should be noted

that in S1406, correlative studies were underpowered due to limited patients providing ctDNA. Remarkably, ctDNA response rate was very high, 87% compared to only 17% radiologically. This also correlated with Hong's findings that decrease in ctDNA predicts radiological response (7). Furthermore, Corcoran *et al*. (21) demonstrated in their phase I study investigating the combination of BRAF and EGFR inhibition with dabrafenib + panitumumab ± MEK inhibition with trametinib in BRAF^{V600E} MT mCRC, that the percentage of ctDNA dropping seemed to correlate with response.

The molecular complexity of BRAF^{V600E} MT CRC and the dynamic changes under biological pressure of targeted agents could also be captured by serial ctDNA testing and may reveal mechanisms of resistance via alternations of the MAPK signalling pathway. In the trial by Corcoran *et al*. (21), almost half of patients (48%) developed RAS mutations in ctDNA at the time of progression, and this might even have been an underestimation. Moreover, multiple subclonal RAS mutations were revealed in many (33%) of these patients at progression, emphasizing the ability of tumour heterogeneity in the context of acquired resistance to therapy. Also, Kopetz and colleagues suggested that many BRAF^{V600E} MT CRCs may already possess tumour subclones with 1 or more RAS mutations prior to therapy, with the potential for rapid development of heterogeneous resistant subclones under treatment pressure. Yet, in S1406, only 1/50 patients on the experimental arm showed acquired KRAS mutations (G12V and Q61L) on progression. The authors assume that the mechanism of resistance induced by a combination of cytotoxic chemotherapy with targeted therapy may vary from regimens consisting of only targeted therapy. The currently ongoing first-line BREAKWATER trial will further explore this hypothesis by investigating the correlation between ctDNA genetic alterations and clinical outcome.

Conclusion and future strategies

The S1406 trial confirms the previously proven benefit of BRAF inhibitors in combination with anti-EGFR treatment in second- (and third-line) treatment of BRAF^{V600E} MT CRC, more specific in combination with chemotherapy. The added value of chemotherapy remains unclear and may result in increased toxicity given more than double of patients discontinued the experimental group in S1406 compared to triplet and doublet group in BEACON (5). However, we should take caution when comparing these two

trials directly. Ultimately, further clinical trials are required to answer this question.

Despite there being unanswered questions in second- and third-line therapy, many patients with BRAF^{V600E} MT mCRC still do not reach second-line. Hence, the key question is whether to move these regimens to first-line treatment to improve prognosis. The ongoing BREAKWATER trial (NCT04607421) will provide further exploration of this hypothesis and will also assess the added value of chemotherapy in first-line, including a comparison of irinotecan *vs*. oxaliplatin based chemotherapy in the initial safety run in phase.

As ctDNA BRAF^{V600E} MT evolution appears to be predictive of treatment response and given the tumour heterogeneity and emergence of MAPK pathway alterations known to drive resistance in BRAF^{V600E} MT mCRC during treatment, the inclusion of ctDNA analysis in future treatment trials and ideally normal practice will assist management decisions for this complex group of patients.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Digestive Medicine Research*. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dmr.amegroups.com/article/view/10.21037/dmr-21-99/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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