TITLE: Class(y) dissection of *BRAF* heterogeneity: beyond non-V600.

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Summary

Different classes of *BRAF* mutations are present in colorectal and other cancers. Non-V600 mutations are rare; however, their detection rate will increase as the use of next-generation sequencing ramps up quickly in clinical practice. Different biochemical signalling pathways are active in non-V600 *BRAF* mutant cancers and may affect treatment response.

Main Text

In this issue of *Clinical Cancer Research*, Yaeger and colleagues (1) investigated whether patients with different classes of non-V600 *BRAF* mutant colorectal cancer (CRC) might benefit from anti-epidermal growth factor receptor (EGFR) therapies. They suggested that class-2 non-V600 *BRAF* mutant tumours do not respond while class-3 non-V600 *BRAF* mutant tumours are more likely to respond to EGFR inhibition.

The importance of *BRAF* mutations is increasingly recognised in CRC: firstly, in view of the poor prognostic role in advanced disease; secondly, as negative predictive biomarker of response to anti-EGFR therapies and to single agent BRAF-inhibitors; and lastly, because of the recently established role in predicting response to chemotherapy-free combinations of cetuximab, encorafenib with or without binimetinib (1, 2). However, these findings currently apply exclusively to the most common *BRAF* mutation (V600E), highlighting that not all BRAF mutations are the same.

Non-V600 *BRAF* mutations are rare, occurring in about 2% of the metastatic CRC population (3). Comparison of their clinical and prognostic associations with those of classical V600E *BRAF* mutant tumours was previously performed in a retrospective analysis of more than 9600 patients (3). The less aggressive phenotype and significantly longer survival compared to V600E *BRAF* mutation carriers was described, justifying the potential need for different therapeutic strategies. These findings have been recently echoed by Schirippa and colleagues (4) who also described substantial differences in gene expression profiling, with non-V600 *BRAF* mutations exclusively presenting an epithelial-like profile, in contrast to V600E *BRAF* mutant tumours where the inflammatory profile is usually the most common one.

Even between non-V600 *BRAF* mutant tumours heterogeneity exists. A number of elegant pre-clinical studies have dissected biochemical properties specific to individual classes of non-V600 *BRAF* mutations (5) (**Figure 1**), however, attempts to correlate *BRAF* variants with clinical outcome have been hampered by the relative rarity of these mutations. Hence, Yaeger and colleagues established a large clinically annotated database of patients whose tumours were found to carry a non-V600 *BRAF* alteration in sequencing studies in multiple US and Japanese institutions (1).

Up to 153 patients were identified, with 5 of them carrying a non-V600 *BRAF* mutation not previously fully characterized. Therefore, for the very first time the effect of these 5 non-V600 *BRAF* mutations on downstream proteins of the phospho-ERK and phospho-MEK pathway was described using mouse embryonic fibroblasts. Four patient-derived xenograft models were also established and assessed for anti-EGFR sensitivity. In one of them, carrying a D594N *BRAF* mutation (class 3), treatment with cetuximab clearly demonstrated growth inhibition. This mutation appeared to confer a much less aggressive phenotype when compared to the other 3 models, with tumour volumes still quite small two weeks post-inoculation and slower increments in the vehicle-treated group. This is in line with the good prognostic value of this mutation previously described in CRC patients (6). Conversely, the G466V model (also class 3) demonstrated a very aggressive phenotype with high tumour volume at baseline and doubling-times of less than 10 days, which possibly led to experiment termination just before 20 days, much earlier than D594N (class 3) and K601E (class 2). The last model (T599dup, class 2) seemed also associated with more aggressive phenotype with high tumour volume in the vehicle treated group and some level of response demonstrated in the cetuximab treated group.

Prompted by the pre-clinical observation that different non-V600 *BRAF* mutations may confer variable degrees of sensitivity to anti-EGFR treatments, the authors tested the predictive/prognostic value of class 2 and class 3 *BRAF* mutations in CRC patients treated with EGFR inhibitors. The results suggested that class 2 mutation carriers have limited or no benefit while patients whose tumour harbour a class 3 mutation show 50% response rate with some durable responses when treated with anti-EGFR monoclonal antibodies (1). These observations are certainly intriguing and hypothesis-generating but do not completely align with currently available literature in the field. Jonhson et al. evaluated the association between atypical *BRAF* mutations and response to EGFR inhibitors and found no complete or partial

responses in any of the eleven non-V600 *BRAF* mutant patients in their cohort; among the six patients with stable disease, four were class 3, one was class 2 and one unknown suggesting a very limited benefit for EGFR inhibitors in non-V600 *BRAF* mutant CRC patients in general (7). In a different report, Wang et al. observed a partial response to single agent panitumumab in a *KRAS* $^{\text{wild-type}}$ *BRAF* $^{\text{G469V}}$ (class 2) mutant CRC patient (8).

Several confounding factors may account for the heterogeneous pattern of response to EGFR inhibitors observed across the different studies (1,7-8). Indeed, the small number of patients, the frequent use of EGFR monoclonal antibodies in combination with chemotherapy, the presence of different chemotherapy backbones, the unbalanced number of previous therapies (with different expected response rates according to line of treatment) and the absence of a control group may well justify discordant findings in these reports.

The use of next-generation platforms based on extended targeted panels or whole exome sequencing is rapidly growing in routine clinical practice thus we should expect an increase in the detection of relatively rare non-V600 *BRAF* mutations in CRC patients. While the prospective collection of well-annotated datasets may, in future, help to clarify the predictive value of these classes of mutations, several questions relative to the clinical value of non-V600 *BRAF* mutants in current clinical practice remain. Should clinicians take into account the occurrence of non-V600 *BRAF* mutation in their decisional algorithm? Do the data from Yaeger and colleagues provide a framework to design future clinical trials in this setting?

In our opinion, current evidence is not robust enough to justify the use of class 2 *BRAF* mutations as negative biomarkers for the selection of CRC patients' candidate for EGFR monoclonal antibodies. Class 2 mutations represent a small subgroup within the already rare population of non-V600 *BRAF* CRC. Due to conflicting data on their predictive value, and lack of definitive evidence confirming that the addition of EGFR monoclonal antibodies has a detrimental effect in these patients, omitting anti-EGFR therapies cannot be recommended. As suggested by the authors (1), future trials should tackle the biology underpinning class 2 *BRAF* mutations and test type II RAF inhibitors or the combination of cetuximab and MEK inhibitors in order to improve outcome in this subset of patients.

Class 3 mutations co-occur with *RAS* mutations in more than 30% of cases and, in this scenario, the presence or absence of a *RAS* mutation would dictate the clinician decision to add anti-EGFR agents. In those patients with *RAS* wild-type and class 3 *BRAF* mutations that rely on receptor tyrosine kinase activation, anti-EGFR monoclonal antibodies can be considered. In this context, sequencing or multi-omics analyses may identify actionable drivers that could be targeted using EGFR, MET or MEK inhibitors alone or in combination and future trials should move in this direction.

In summary, even though the retrospective nature of the study and some caveats in the design prevent from drawing firm conclusions, the study reported by Yaeger and colleagues highlights the paramount importance of reverse translation studies matching clinical and pre-clinical data to identify molecular vulnerabilities and inform the design of future trials opening the way to diversified therapeutic approaches in class 2 and class 3 non-V600 *BRAF* mutant patients.

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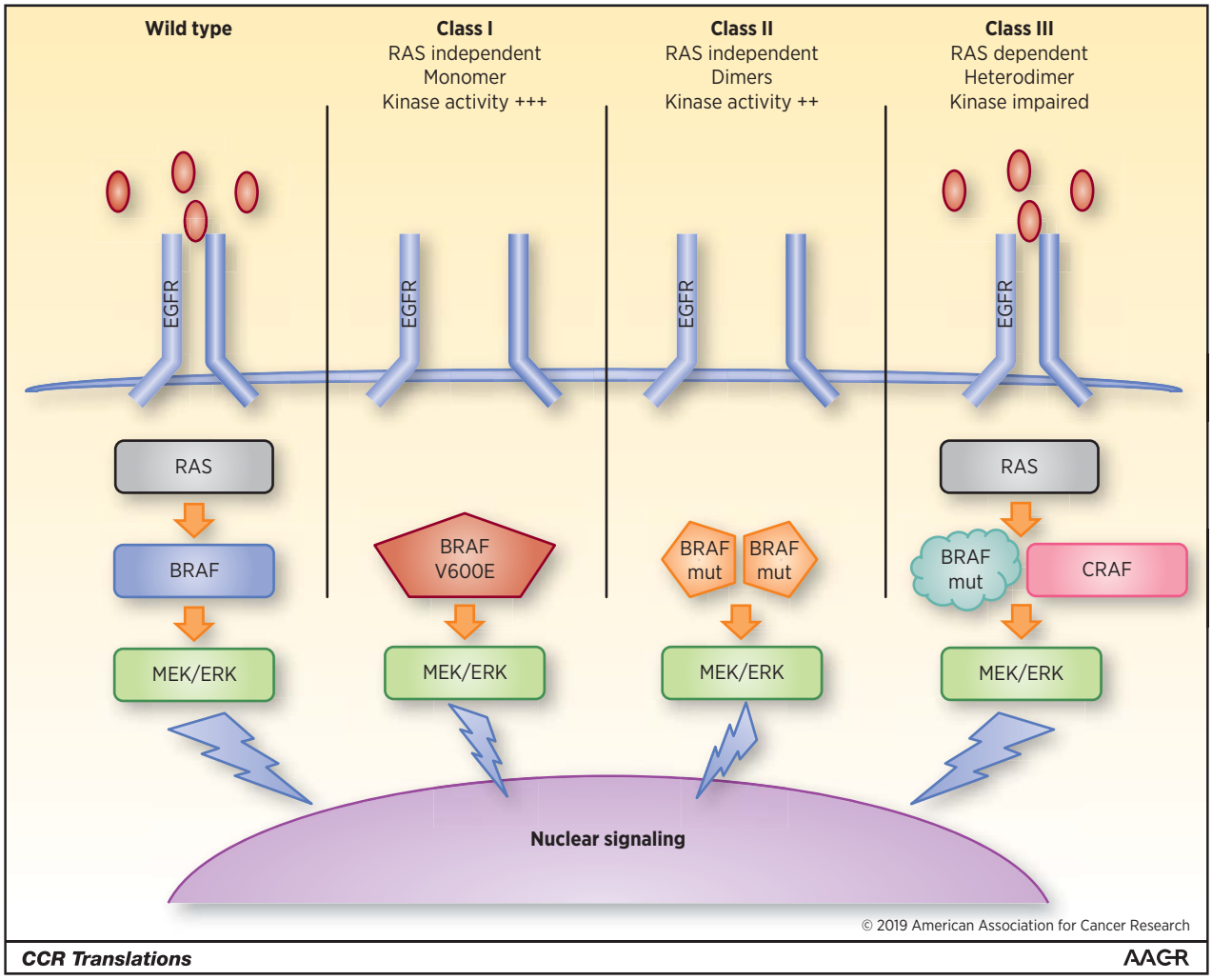
Figure 1. Signalling pathways in different classes of *BRAF* mutations. V600 *BRAF* mutations (Class 1) are independent of RAS signalling and work as monomers. Non-V600 Class 2 *BRAF* mutants are also independent of RAS but signal as constitutive dimers. Non-V600 Class 3 *BRAF* mutations have low or no kinase activity and depend on RAS activation acting as amplifiers of the RAS signalling pathway.



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