Editorial Commentary

The role of negative hyperselection in metastatic colorectal cancer

Jingran Ji, Marwan Fakih

Department of Medical Oncology and Therapeutics Research, City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Correspondence to: Marwan Fakih, MD. Department of Medical Oncology and Therapeutics Research, City of Hope Comprehensive Cancer Center, 1500 E Duarte Road, Duarte, CA 91010, USA. Email: mfakih@coh.org.

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Antibodies directed towards epidermal growth factor receptor (EGFR), panitumumab (Pmab) and cetuximab (Cmab), in combination with cytotoxic chemotherapy, are effective first line treatments for *RAS* wild type (WT), left-sided metastatic colorectal cancer (mCRC) (1,2). The initial trials with these agents, in addition to pooled analyses, clearly demonstrated that patients with *RAS* (predominantly *KRAS* exon 2) mutations had worse outcomes when exposed to anti-EGFR therapy compared to who with *RAS* WT disease (3). Furthermore, tumors originating on the right side of the colon up to the splenic flexure also appeared to be resistant to anti-EGFR therapy (4). The variation in clinical outcomes and response to therapy is likely due to differences in underlying disease biology, in part due to alterations in genes aside from *RAS*. Thus, how the underlying molecular profile in mCRC affects response to therapy and disease-related outcomes requires further investigation (5).

In a recent issue of *Clinical Cancer Research*, Stahler and colleagues present findings from a prespecified analysis of the multicenter, phase II, PANAMA trial evaluating the predictive and prognostic value of negative hyperselection (selecting for tumors that do not harbor gene alterations thought to confer primary resistance to anti-EGFR therapy) among patients with *RAS* WT mCRC entering the maintenance phase of treatment after having achieved a response or stable disease on induction chemotherapy plus Pmab (6). The original PANAMA trial randomized these patients to either 5-fluorouracil/leucovorin (5FU/LV) alone or 5FU/LV plus Pmab (7). Stahler *et al.* analyzed baseline tissue-based molecular testing and categorized the PANAMA trial participants into hyperselection WT and mutation (MUT) cohorts. They defined hyperselection MUT patients as anyone with disease harboring one or more of the following: pathogenic mutations in *KRAS* (those detected on NGS and not by initial study screening), *ERBB2*, *AKT1*, *ALK1*, *PIK3CA*, *PTEN*, $BRAF^{V600E}$, or HER2/neu overexpression by immunohistochemistry (IHC) score 3+ only. Those without any of these gene alterations were categorized into the hyperselection WT cohort. The primary endpoint was median progression-free survival (PFS) on maintenance treatment. Secondary endpoints included median overall survival (OS) since the start of maintenance treatment and the objective response rate (ORR) of maintenance treatment.

In the overall study population, patients in the hyperselection WT cohort had a trend towards better PFS compared to patients in the hyperselection MUT cohort, though this was not statistically significant [7.5 *vs.* 5.4 months, hazard ratio (HR) =0.75; 95% CI: 0.52–1.07, P=0.11]. There was, however, a significant difference in OS between the two groups (28.7 *vs.* 22.2 months, HR =0.53; 95% CI: 0.36–0.77, P=0.001). When broken down into treatment groups, patients with hyperselection WT tumors who received Pmab had a significantly longer PFS compared to those who received 5FU/LV alone (9.2 *vs.* 6.0 months; HR =0.66; 95% CI: 0.47–0.93, P=0.02). There was only a trend toward benefit in terms of OS (36.9 *vs.*

24.9 months; HR =0.91, 95% CI: 0.61–1.36, P=0.50) but a significantly better ORR (44.2% *vs.* 26.3%, P=0.02). For those in the hyperselection MUT group, the addition of Pmab to maintenance therapy did not confer benefit in terms of PFS, OS, or ORR.

These findings serve to reinforce the original PANAMA trial results and suggest that there may be prognostic value in the hyperselection gene alterations selected by the authors for this study. However, the predictive value of using negative hyperselection for treatment determination in the maintenance setting is not as clear. While maintenance Pmab appeared to have benefit in the hyperselection WT cohort, conclusions about anti-EGFR therapy in the hyperselection MUT cohort is limited by the small sample size. The hyperselection MUT cohort also included patients with *BRAF*^{V600E} mutant disease, which is another established mutation that confers primary resistance to anti-EGFR therapy (7-9). When these patients were separated out from the rest of the hyperselection MUT group, the remaining cohort who received Pmab showed a trend towards benefit compared to 5FU/LV alone [median PFS (mPFS) 11.6 *vs.* 5.0 months, HR =0.41; 95% CI: 0.16–1.05, P=0.07], which may suggest benefit from maintenance Pmab in non-*BRAF*^{V600E} hyperselection MUT cases.

The improved outcomes observed among patients with these non-*RAS* or *BRAF* gene alterations are not surprising as absolute resistance to anti-EGFR therapy has not been documented in the setting of select molecular findings, such as PIK3 pathway alterations. For example, de Roock *et al.* had previously reported potential benefit from anti-EGFR therapy in patients with *PIK3CA* exon 9 alterations but not in those with exon 20 mutations (10). Furthermore, HER-2 overexpression is not an absolute biomarker of resistance to anti-EGFR therapy, although higher HER-2 copy numbers may infer relative resistance to anit-EGFR therapy and improved sensitivity to HER-2 targeting (11-13). To that end, preliminary published results from SWOG S1613 have shown similar response rates and PFS when comparing Cmab plus irinotecan to trastuzumab plus pertuzumab regimens in HER-2 amplified mCRC in the second- and third-line settings (14).

Stahler's study was also not powered to account for the impact of sidedness on the hyperselection WT and hyperselection MUT populations, a variable that has been well-validated as a prognostic and predictive biomarker when it comes to anti-EGFR therapy (3,4,15,16). Only 9% of the hyperselection MUT cohort were right sided, limiting the ability to determine the impact of sidedness in this population. While 40% of the hyperselection MT cohort were right-sided, the small sample size of this population still does not allow a powered assessment of the impact of the site of disease. Notably, there were a few other imbalances between the two cohorts in addition to tumor sidedness, including gender (30% female in hyperselection WT cohort *vs.* 52% female in MUT cohort) and site of metastatic disease (47% liver-limited disease in hyperselection WT cohort *vs.* 28% in MUT cohort). These differences in the two cohorts further limit how much weight can be placed on the impact of negative hyperselection in relation to the reported outcomes. Finally, the small sample also limited evaluation of other rarer gene alterations as possible resistance mechanisms such as *ALK*, *NTRK*, *ROS1*, *MET* amplification, and *RET* rearrangements which have been included in prior studies evaluating negative hyperselection (17-20).

Despite these limitations, Stahler and colleagues are the first to report on the potential predictive value of hyperselection status on the efficacy of continuing anti-EGFR therapy in the maintenance setting. Prior to this, there was only one other study looking at the predictive value of negative hyperselection at the time of induction (17). Shitara *et al.* reported a secondary analysis of the PARADIGM Trial which randomized patients with *RAS* WT mCRC to first-line 5FU/LV/oxaliplatin (FOLFOX) and Pmab versus FOLFOX + bevacizumab. Baseline circulating tumor DNA (ctDNA) was used instead of primary tissue sequencing. Gene alterations used to define hyperselection status included *KRAS*, *NRAS*, *PTEN*, and extracellular domain *EGFR* mutations, *HER2* and *MET* amplifications, and *ALK*/*RET*/*NTRK1* fusions. The study found that patients without the hyperselection gene alterations treated with Pmab had longer median OS (mOS) (41.4 *vs.* 34.4 months, HR =0.75; 95% CI: 0.62–0.92) compared to those who were treated with bevacizumab, whereas those with one or more hyperselection gene alterations had a trend toward shorter mOS (18.7 *vs.* 22.2 months, HR =1.14, 95% CI: 0.84–1.54). The study also supported the prognostic value of negative hyperselection, showing that patients without any of the hyperselection gene alterations had better PFS and OS than those who harbored one or more alterations. Yet, like Stahler *et al.*, this analysis was not well powered to answer the question of benefit or lack of in the various negative hyperselection gene alteration cohorts, therefore preventing definitive conclusions regarding the exclusion of such patients from anti-EGFR therapy.

Previously, others have reported on the prognostic value

of various gene alteration panels for negative hyperselection, but none had cohorts randomized to anti-EGFR therapy versus no therapy. Cremolini *et al.* first tested the "primary resistance in RAS and BRAF wild-type metastatic colorectal cancer patients treated with anti-EGFR monoclonal antibodies" (PRESSING) panel (which included *HER2/MET* amplifications, *ALK/ROS1/NTRK1–3/RET* fusions, *PTEN* inactivating mutations and *PIK3CA* mutations) in a case-control study comparing patients with mCRC who were sensitive versus resistant to anti-EGFR therapy (18). They found that those with anti-EGFR therapy resistant tumors were more likely to have PRESSING panel alterations (51.1% *vs.* 2.1%, P<0.001).

Morano *et al.* then used the PRESSING panel in a secondary analysis of the Valentino trial to study the prognostic value of negative hyperselection (19). The patients in this study were *RAS/BRAF* WT and had been randomized to Pmab alone or Pmab plus 5FU/LV after induction FOLFOX plus PMAB. They found that patients with PRESSING-positive tumors had worse response rates (59.2% *vs.* 75.3%, P=0.03), PFS (7.7 *vs.* 12.1 months, P<0.001), and OS (2-year rate: 48.1% *vs.* 68.1%, P=0.021) compared to those with PRESSING-negative tumors. More recently, Pietrantonio *et al.* also conducted a similar analysis on the PANDA trial which randomized elderly patients to FOLFOX plus PMAB or 5FU/LV plus PMAB induction (21). They also reported that patients with PRESSING positive tumors had worse outcomes compared to those with PRESSING negative tumors.

The same group then created a PRESSING2 panel that added even rarer genomic alterations to the original PRESSING panel including *ERBB3*, *NF1*, *MAP2K*, *AKT2* mutations, *PTEN/NF1* loss, *ERBB3*, *FGFR2*, *IGF1R*, *KRAS*, *ARAF*, and *AKT1–2* amplification, and *EGFR* rearrangements. Randon *et al.* studied the prognostic value of negative hyperselection using the PRESSING2 panel and showed superior outcomes among those treated with anti-EGFR therapy who were negative for PRESSING and PRESSING2 mutations compared to those who were only negative for PRESSING mutations (mOS of 49.9 *vs.* 22.6 months; HR =2.98; 95% CI: 1.49–5.96) (20). This "ultraselected" group of patients clearly had exceptional outcomes, though no conclusions can be made about the impact of anti-EGFR therapy in this cohort.

The clinical utility of negative hyperselection still requires further prospective investigation. It is fair to state, based on prospective clinical trials, that $BRAF^{V600E}$ mutations should be excluded from anti-EGFR therapy, unless coupled with a BRAF inhibitor (8,9). Hence, the big question today is: which other alterations or characteristics should exclude the use of anti-EGFR therapy in the first line or subsequent lines of therapy? Despite the PARADIGM data, sidedness is still a valid biomarker for the integration of anti-EGFR therapy in the first-line treatment of RAS/BRAF-WT patients. This is now validated through numerous randomized phase 3 trials, even if retrospectively (3,4,15,16). The analysis of negative hyperselection in the PARADIGM trial based on ctDNA was exploratory in nature and was not included as a primary or secondary endpoint on the original trial. Additional research should be performed in patients with right-sided disease to better define if there are particular molecular signatures that can predict benefit from anti-EGFR therapy. Such studies are better conducted in 3rd line settings after irinotecan, oxaliplatin, and fluoropyrimidine failure as a clinical benefit in such settings will be more conclusive.

Among *RAS* and *BRAF* WT disease, irrespective of sidedness, relative resistance has also been suggested for HER-2 overexpression, *PIK3CA/AKT/MAP2K/PTEN* mutations, microsatellite instability high (MSI-H) status, mucinous carcinomatosis, *GNAS* alterations, and many others (10,13,22-24), However, there continues to be a lack of level one evidence that any of these alterations impairs benefit from anti-EGFR therapy. Answering such questions may require the pooling of data from several prospective clinical trials and conducting sophisticated multivariate analyses as prospectively powered randomized studies limited to these alterations will be impossible. Meanwhile, a reasonable approach would be to limit the use of anti-EGFR therapy to 3rd line treatment settings among those with *RAS/BRAF* WT disease that have one or more of these "negative hyperselection" gene alterations.

In summary, the analysis presented by Stahler *et al.* adds to the growing body of evidence that negative hyperselection for gene alterations outside of *RAS* is prognostic and may even be predictive of better outcomes from anti-EGFR therapy. We commend the authors for carrying out this detailed analysis in a unique patient population receiving maintenance therapy and exploring the role of using negative hyperselection to guide the continuation of anti-EGFR therapy for patients with *RAS* WT mCRC in the maintenance setting.

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Footnote

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