Biomarkers and efficacy: are we nearly there yet?

At the moment, several studies provide strong evidence that the genotyping of tumors is mandatory in patients with metastatic colorectal cancer (CRC) before treatment with anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies.

In this issue of *Annals of Oncology*, the update of the Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer (OPUS) phase II study assesses the efficacy and safety of cetuximab combined with first-line oxaliplatin-based chemotherapy for metastatic colorectal cancer patients [1]. The influence of KRAS and BRAF mutation status on primary and secondary end points of the study was evaluated.

The preliminary data of the study have already been published and confirm that KRAS mutation status is a powerful predictive factor in relation to the efficacy of cetuximab treatment [2].

Along with efficacy data in terms of response rate, overall survival (OS) and progression-free survival (PFS) according to KRAS and BRAF mutation status, the present analysis also reports the incidence of toxic effects in the intention to treat (ITT) and biomarker populations.

It should be remarked that the potential bias initially associated with the retrospective evaluation of the mutational status of KRAS in the OPUS study seems irrelevant today, since the difference between the ITT population and the population assessable for KRAS, that are made up of 337 versus 315 (93%) patients respectively, is negligible.

Although all patients with a KRAS mutation are presently excluded from treatment with anti-EGFR antibodies, the quantitative PCR-based assays used in the OPUS study could identify only 7 of 12 known KRAS gene mutations in exon 2 (codons 12 and 13) and none in exon 3 (codons 59, 61 and 63).

Moreover, some reports suggest that specific KRAS mutations have a prognostic role but not even a predictive role with regard to anti-EGFR therapies. KRAS mutations at codon 13 are associated with a worse prognosis in sporadic CRC, as reported for the first time in a prospective study carried out by our group [3] and recently confirmed in a Japanese series [4]. Furthermore, an individual patient data pooled analysis indicated that some KRAS codon 13-mutated patients may be sensitive to cetuximab treatment when compared with patients with other KRAS-mutated tumors, and that all codon 13 responders carry the p.G13D mutation [5].

The choice for the most appropriate method in KRAS mutation analysis remains a complex challenge, as it is still not known what level of test sensitivity is required in order to provide useful and predictive information in clinical practice.

Direct dideoxy DNA sequencing and the PCR-based assays would seem to be the most reliable techniques, but the detection limit of these two methods is ~20% and 10% mutant alleles, respectively [6]. Some researchers attempted to find more sensitive methods, such as pyrosequencing, so as to obtain the same results with fewer cells from the specimens. In a brief report by Santini et al. [7], 3 of 29 KRAS wild-type (by real-time PCR) patients who received an anti-EGFR therapy proved to be mutant by pyrosequencing. All of these mutated patients showed partial response according to RECIST criteria as best response during cetuximab-based chemotherapy, thus suggesting that a small percentage of KRAS-mutated tumor cells does not impair cetuximab activity.

It has been hypothesized that there is a possible switch of a CRC from wild type to mutant KRAS form with the result that the mutation status of the primary tumor might not be adequate to predict the response of metastases to anti-EGFR treatment. In fact, several reports have now demonstrated a high level of concordance between KRAS mutation in the primary tumor and liver metastasis in CRC so that both primary tumors and liver metastases can be used for KRAS mutation analysis. More specifically, our group, in a series of 99 patients found a high concordance between primary and related metastases, mostly involving the liver, in terms of KRAS mutational status [8]. This report has recently been confirmed in a large and homogenous Dutch study, which found a discordance between primary tumors and corresponding liver metastases in only 11 of 305 cases (3.6%) [9].

Overall, KRAS mutation seems to be responsible in 35%–45% cases for resistance to anti-EGFR antibodies. Another group of patients, representing 5%–10% of the total number of CRC patients, carry the BRAF mutation, which is mutually exclusive with KRAS mutations. Although is difficult to reach a definite conclusion concerning the possible predictive or prognostic utility of BRAF, because of the limited sample size, it nevertheless seems that BRAF mutations may play a strong negative prognostic role and only a slight role in predicting resistance to anti-EGFR antibodies.

BRAF mutational status has been evaluated in patients from the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer and OPUS studies and no significant efficacy difference between the treatment arms (chemotherapy with and without Cetuximab) in the wild-type KRAS/mutated BRAF group has been found [10]. Nevertheless, these patients seem to benefit from the addition of Cetuximab, with an increase of OS and a doubling of PFS rates. Furthermore, there is clearly a worse outcome in mutated BRAF in comparison with wild-type BRAF patients independently of treatment with Cetuximab, which supports the hypothesis of
a possible negative prognostic role of BRAF mutations. In the CAIRO-2 study, a similar pattern was observed in a large series of metastatic colorectal cancer (mCRC) patients treated with chemotherapy and Bevacizumab. It was seen that the BRAF mutation was associated with a worse outcome, both in terms of PFS and of OS, independently of the addition of Cetuximab to the treatment [11]. To date, therefore, the negative predictive value of mutations of BRAF is suggested only by single-arm retrospective analyses on tissue samples from patients treated with anti-EGFR antibodies [12–14], while the significant negative prognostic value seems now to have been established [4, 15].

The absence, however, of KRAS/BRAF mutations does not guarantee an increased likelihood of response to these drugs, since wild-type KRAS/BRAF status is not sufficient to confer sensitivity to anti-EGFR monoclonal antibodies. As a result, the investigation of other biomarkers such as EGFR copy number and messenger RNA expression levels of EGFR ligands epiregulin and amphiregulin, phosphatase and tensin homolog loss or mutations in NRAS and exon 20 PIK3CA may be useful to further refine the responder population. However, the data regarding their validity as predictive factors of responsiveness are less consistent and the prospective studies necessary to validate additional tests in selecting patients for anti-EGFR monoclonal antibodies can take several years because of the slow recruitment of patients harboring a rare mutation.

Up till now, clinical evidence all points towards the identification of the KRAS mutation as the only evaluated and reproducible predictive factor of resistance to anti-EGFR antibodies. Indeed, future research will test clinical efficacy of combined therapies simultaneously targeting the EGFR and the RAS/RAF/MAPK signaling pathways for mCRC patients in the context of mutational networks affecting the EGFR pathway.

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disclosure

The authors declare no conflict of interest.

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