Optimizing Anti-EGFR Therapy in Colorectal Cancer

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Treatment with anti-EGFR monoclonal antibodies has been successfully integrated in the continuum of care for metastatic colorectal cancer. The major challenge is the identification of patients who would benefit from treatment.

In this issue of Clinical Cancer Research, Peeters and colleagues report on the final analysis of KRAS and NRAS mutations and their impact on clinical efficacy in a randomized phase III study of FOLFIRI plus panitumumab versus FOLFIRI as second-line treatment for metastatic colorectal cancer (1). Treatment of metastatic colorectal cancer has greatly improved in the past decade with the introduction of more effective antineoplastic drugs and with the development of a therapeutic strategy that includes several lines of non-cross-resistant drugs and the wider use of potentially curative surgery for liver and, in selected cases, for lung metastases. In this context, introduction of antiangiogenic drugs, including bevacizumab, aflibercept, ramucirumab, and regorafenib, and of anti-EGFR monoclonal antibodies, such as cetuximab and panitumumab, has given a relevant contribution to improve metastatic colorectal cancer patient prognosis (2).

Given the complex molecular heterogeneity of colorectal cancer, a major challenge is to identify valuable and reliable predictive biomarkers for appropriate patient selection to optimize treatment with molecular-targeted drugs. Hypothesis-generating predictive biomarkers are best developed prospectively as companion diagnostics in the drug development process, but they can also be developed retrospectively from prospectively randomized clinical trials data (prospective-retrospective analysis), if they follow a sound methodologic path. Mutations in oncogenes and tumor-suppressor genes can carry sensitivity (positive prediction of response) or resistance (negative prediction of response) depending on the gene and on the target. A subgroup of metastatic colorectal cancers is highly dependent on EGFR signaling, and the use of EGFR inhibitors has been demonstrated effective in early lines of treatment as well as in heavily pretreated chemorefractory colorectal cancer patients. In this latter case, approximately 10% of unselected patients respond to treatment with either cetuximab or panitumumab. The identification of activating KRAS exon 2 (codons 12 and 13) gene mutations has been the first clinically relevant step to identify those patients in which the RAS pathway is constitutively active to signal cell proliferation and survival in cancer cells and, therefore, EGFR inhibition is not effective (3). These mutations account for approximately 85% to 90% of RAS mutations in colorectal cancers. Subsequently, less frequent activating mutations in KRAS exons 3 and 4 and in NRAS exons 2, 3, and 4, which are present in approximately 15% to 20% of KRAS exon 2 wild-type tumors, have been identified as other biomarkers of intrinsic cancer cell resistance to cetuximab or to panitumumab, as it was for the first time found in the randomized phase III study of FOLFOX plus panitumumab versus FOLFOX alone (4). As a result, the European Medicines Agency has restricted the use of these drugs to metastatic colorectal cancer patients with KRAS and NRAS wild-type tumors, as recommended by all major international clinical guidelines.

Peeters and colleagues were able to retrospectively analyze 85% of tumor samples for extended KRAS and NRAS mutations. These mutations were found in approximately 18% of the original KRAS exon 2 wild-type patient population in agreement with previous reports of panitumumab or cetuximab with both FOLFIRI or FOLFOX combinations in first-line trials (4, 5). The results of the present study confirm the lack of efficacy of panitumumab plus FOLFIRI in patients with any RAS mutations and clearly show that an extended molecular selection is translated in a better clinical efficacy for patients whose tumors are RAS wild type. Therefore, this is another important experimental proof that the best tool we have for selecting patients to be treated with anti-EGFR monoclonal antibodies is to exclude from treatment patients with RAS mutations.

However, not all patients with RAS wild-type tumors will respond to treatment. In fact, is conceivable that only 50% to 65% of these patients have an EGFR-dependent cancer. How to better select patients in order to identify those that most likely will benefit from anti-EGFR therapy? First, other genes that could be responsible for resistance to cetuximab or panitumumab have been identified, although it is not clear if they are negative prognostic factors rather than true predictive biomarkers. This is the case of BRAF and of PIK3CA mutations. In the present study, there is no evidence of a predictive role of BRAF mutations, whereas PIK3CA mutations were too few for any analysis. The results of a large...
retrospective European Consortium study have shown that BRAF and PIK3CA exon 20 (but not exon 9) mutations were markers of lack of efficacy of cetuximab plus irinotecan in chemorefractory colorectal cancer patients (6). Furthermore, more recently by using multiple gene assessment by next-generation sequencing (NGS), it has been shown that treatment with FOLFIRI plus cetuximab in first line was most effective in patients whose tumors were KRAS, NRAS, BRAF, and PIK3CA wild type (5).

Other relevant questions for clinical practice are the following: which is the best technique to use for evaluating the presence of these mutations? What is the clinical relevant cutoff of mutated allele(s) that could be useful to predict cancer cell resistance to treatment? Is there a difference of mutational status between primary tumor and different metastatic sites? Which tissue should we use for clinical practice? Is there a role in clinical practice for analyzing circulating free DNA from cancer cells in the plasma (liquid biopsy)? No clearly standardized procedures for KRAS/NRAS mutational testing have been established and an increasing number of quantitative and highly sensitive techniques are currently being used. However, the clinical significance of low fraction of RAS mutant alleles as resistance predictors of anti-EGFR therapy remains unclear. The authors of the CRISTAL trial used a quantitative BEAMING digital PCR (dPCR) platform and selected a 5% cutoff as an optimal predictive sensitive threshold to define KRAS and NRAS mutations (7). More recently, Laurent-Puig and colleagues have evaluated the role of minor mutant KRAS subclones in patients with metastatic colorectal cancer treated with anti-EGFR drugs using picoliter multiplex droplet dPCR. A cutoff of 1% of KRAS exon 2–mutant clones was determined as optimal to identify responding from nonresponding patients (8). In addition, a wide array of high sensitivity dPCR and NGS platforms are being developed to be able to pick up circulating tumor mutations in plasma. This approach can have two clear clinical utilities. First, it can be used to detect the emergence of RAS mutations and other molecular alterations that drive acquired resistance during anti-EGFR treatment (9, 10). Second, it is a new option for real-time diagnosis at the required time-point, for example, when lack of tumor sample availability, either primary or metastasis. All the randomized trials that have validated the predictive value of RAS mutations have been performed with available archived paraffin tumor samples, from recent or old primary tumors or metastasis individually, as there is little tumor heterogeneity when evaluating KRAS mutations in different tumor or metastases locations from the same individuals (11). More variability has been found in mutation detection from different labs in quality assessment controls (12). Therefore, expanded RAS mutation analysis for clinical practice should be performed by a certified laboratory that complies with external quality assurance programs. More studies are needed to answer remaining questions in the EGFR pathway. Further progress in the development and validation of other predictive biomarkers will undoubtedly improve the clinical efficacy of anti-EGFR treatments in metastatic colorectal cancer.

Disclosure of Potential Conflicts of Interest
R. Salazar reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Amgen, Merck Serono, and Roche. F. Ciardiello is a consultant/advisory board member for AstaZeneca, Bayer, Eli Lilly, Merck Serono, Roche, and Sanoﬁ-Aventis. No other potential conﬂicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Ciardiello
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Salazar, F. Ciardiello
Writing, review, and/or revision of the manuscript (i.e., reporting or organizing data, constructing databases): F. Ciardiello
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