INTRODUCTION

The development of drugs directed against the epidermal growth factor receptor (EGFR) in colorectal carcinoma began on the basis of findings suggesting that EGFR and its ligands are involved in the pathogenesis of human colorectal carcinoma (1). In several clinical trials, investigators have confirmed the efficacy of blocking anti-EGFR monoclonal antibodies, such as cetuximab and panitumumab, in the medical treatment of patients with metastatic colorectal carcinoma (2). Although EGFR is expressed in the majority of colon adenocarcinomas, responses to treatment with anti-EGFR monoclonal antibodies used as single agents have been observed in approximately only 10% of patients with metastatic colorectal carcinoma (2). These results suggest that mechanisms of resistance to anti-EGFR drugs are common in this disease. At the same time, these findings represent clinically relevant proof that a subgroup of colorectal carcinoma is addicted to the EGFR pathway because the blockade of this signaling pathway results in significant clinical efficacy in selected patients with metastatic colorectal carcinoma even after failure of standard cytotoxic chemotherapies.

Several different mechanisms have been hypothesized to play a role in both intrinsic (primary or \textit{de novo}) and acquired resistance to anti-EGFR drugs in metastatic colorectal carcinoma as well as in other cancer types (see Table 1 for a summary). In metastatic colorectal carcinoma, resistance to anti-EGFR therapies is likely attributable to the constitutive activation of signaling pathways acting downstream of EGFR that can result from either genetic alterations of components of intracellular signaling cascades or from the activation of receptor tyrosine kinases other than EGFR. In this regard, the authors of a number of retrospective and prospective clinical trials have demonstrated that activating mutations in codons 12 and 13 of the \textit{KRAS} gene determine intrinsic resistance to treatment with anti-EGFR monoclonal antibodies in patients with metastatic colorectal carcinoma (3). Authors have also suggested that specific activating mutations of the \textit{BRAF}, \textit{NRAS}, or \textit{PIK3CA} (located in exon 20) genes may also lead to primary resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal carcinoma (4). In addition, unfortunately, all patients with metastatic colorectal carcinoma who initially respond to anti-EGFR therapies develop resistance to these drugs. Therefore, both \textit{de novo} and acquired resistance mechanisms significantly limit the efficacy of anti-EGFR monoclonal antibodies in the medical management of patients with metastatic colorectal carcinoma.

In this scenario, novel experimental models to assess the molecular mechanisms leading to resistance to anti-EGFR agents in colorectal carcinoma and for developing novel therapeutic strategies to prevent or overcome resistance are definitely required. In this issue of \textit{Cancer Discovery}, Bertotti et al. (5) propose an innovative approach: they transplanted fragments of molecularly characterized human colorectal carcinoma samples in immune-compromised mice and expanded the cohort of mice to generate independent transplantable tumor xenografts from the same patient cancer specimen. The authors generated large xenograft cohorts from 85 patient-derived, genetically characterized metastatic colorectal carcinoma samples. These animals were defined as “xenopatients” to outline a close correlation between this experimental model and the cancer cell
characteristics in the patient. In fact, this model has the potential advantage to recapitulate the extreme molecular variability that is observed in a true clinical scenario and that cannot be represented by any human cancer cell line-derived tumor xenograft or by transgenic mice.

The authors analyzed serially transplanted tumors and found that they retained the morphologic and genomic features of their original counterparts. The response rate to the anti-EGFR antibody cetuximab in xenopatients was similar to what observed in the clinic and correlated with clinically validated predictive biomarkers. These findings suggest that the model proposed by Bertotti et al. (5) might indeed represent a novel approach to study the mechanisms of resistance to anti-EGFR therapy in metastatic colorectal carcinoma.

Furthermore, in the present study, the authors have identified HER2 gene amplification as a potential mechanism of resistance to cetuximab in metastatic colorectal carcinoma that harbor normal, wild-type KRAS/NRAS/BRAF/PIK3CA genes. In fact, HER2 gene amplification was found to occur in approximately 2% of unselected metastatic colorectal carcinoma and at a significantly greater frequency in patients with KRAS wild-type tumors that did not benefit from treatment with anti-EGFR monoclonal antibodies. Analysis of HER2-amplified tumor xenografts in “xenopatients” confirmed the role of HER2 gene amplification in cetuximab resistance and suggested that the combined inhibition of HER2 and EGFR by treatment with selective inhibitors could induce long-lasting tumor regression (5). These data suggest that activation of HER2 signaling is associated with primary resistance of metastatic colorectal carcinoma to anti-EGFR agents.

The molecular mechanisms that are involved in the primary, de novo resistance to anti-EGFR drugs are likely to play a role also in the acquired resistance. In this regard, the findings by Bertotti et al. (5) are in agreement with a recent study in which the authors demonstrated that activation of HER2 signaling mediates acquired resistance to anti-EGFR monoclonal antibodies in different human cancer cell lines, including colorectal carcinoma (6). However, two distinct mechanisms were found to be involved: HER2 gene amplification or increased secretion of the HER2 ligand heregulin. Both of these mechanisms caused acquired resistance to cetuximab treatment of human cancer cell lines by leading to persistent activation of ERK signaling (6). More importantly, HER2 gene amplification or increased heregulin secretion were found to be associated with either de novo or acquired resistance to cetuximab therapy in a retrospective analysis of chemotherapy-refractory patients with metastatic colorectal carcinoma. Expression of HER2 protein has been reported in up to 20% of metastatic colorectal carcinoma, whereas HER2 gene amplification is a rare event in this disease, occurring in only approximately 2% of the cases (6).

However, it is possible that HER2 signaling might be involved in the acquired resistance to anti-EGFR monoclonal antibodies in a greater fraction of patients. In fact, subclones of tumor cells carrying HER2 gene amplification might not be detectable at diagnosis. However, these cells can be selected by treatment with anti-EGFR drugs, and, therefore, may be responsible of the recurrence of the disease. In addition, it cannot be excluded that HER2 gene amplification might occur in tumor cells under the selective pressure of therapy. Finally, the finding that increased heregulin secretion can lead to HER2-mediated resistance suggests that HER2 gene amplification is not essential and that increased HER2 signaling can induce resistance to anti-EGFR drugs in absence of HER2 gene amplification. The aforementioned summarized findings also indicate that blockade of HER2 might prevent or revert resistance to anti-EGFR monoclonal antibodies in selected patients. Indeed, combinations of different agents that selectively target EGFR (cetuximab or gefitinib) and HER2 (lapatinib or pertuzumab) were able to significantly inhibit the growth of cetuximab-resistant colorectal carcinoma cells in experimental models (5, 6). Although these data are exciting and open new possibilities for the treatment of patients with resistance to anti-EGFR drugs, prospective clinical trials are required to assess whether this finding can be translated in effective anticancer treatments in patients with metastatic colorectal carcinoma.

Previous findings demonstrated that treatment of human breast cancer cells that co-express EGFR and HER2 with the EGFR tyrosine kinase inhibitor gefitinib and the anti-HER2 monoclonal antibody trastuzumab resulted in a synergistic

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anti-tumor effect (7, 8). Following these findings, a phase I/II clinical trial of trastuzumab plus gefitinib in human breast cancer patients with HER-2–expressing tumors was completed (9). However, the few responses observed were in previously untreated patients (2/28). These results led the investigators to conclude that further use of combinations of trastuzumab plus EGFR tyrosine kinase inhibitors in patients with breast cancer would not be justified.

The results of this study could have been flawed because of several factors. The dose of gefitinib used, 250 mg/day, was largely insufficient to block the activation of wild-type EGFR. However, this dose was chosen because of the unexpected toxicity observed at the 500-mg level (grade 3 diarrhea) (9). More importantly, the patients were not selected on the basis of the level of EGFR expression, and only 6 of the 28 patients enrolled were found to express EGFR. Therefore, these negative findings do not preclude the clinical evaluation of combinations of anti-EGFR and anti-HER2 drugs in patients with metastatic colorectal carcinoma. However, the toxicity of such combinations needs to be further explored in phase I clinical trials to confirm the feasibility of this approach in patients with metastatic colorectal carcinoma.

Another therapeutic strategy that could be used to treat colorectal carcinoma resistant to anti-EGFR agents might be represented by combinations of anti-EGFR drugs with inhibitors of intracellular signaling pathways. In this regard, the study by Yonesaka et al. (6) indicated that ERK signaling is a main pathway involved in HER2-induced acquired resistance to cetuximab. Interestingly, involvement of ERK signaling in the acquired resistance to gefitinib of breast cancer cells has been previously demonstrated (10). In this respect, a potentially effective combination could be the use of anti-EGFR drugs and MEK inhibitors. However, MEK inhibitors as single agents are unlikely to exhibit a significant antitumor effect mostly because of the activation of compensatory signaling loops between the MEK/ERK and PI3K/AKT pathways. A more effective therapeutic strategy could be the combined blockade of both the MEK/ERK and the PI3K/AKT pathways. Significant steps toward personalized treatment of patients with metastatic colorectal carcinoma have been taken in the past few years. The demonstration that patients who carry mutations of the KRAS gene do not benefit from treatment with anti-EGFR agents represented an important innovation for medical oncology (2, 3). However, an increasing number of molecular alterations have been more recently hypothesized to be involved in resistance to anti-EGFR drugs in colorectal carcinoma: mutations in BRAF, NRAS, and PIK3CA; loss of expression of PTEN; and now, activation of HER2 signaling through HER2 gene amplification and/or increased heregulin stimulation. These findings suggest that the resistance to anti-EGFR agents involves a complex network of molecular alterations that seem to be in most of the cases mutually exclusive, although it cannot be excluded that subclones of tumor cells with different molecular features might coexist in the same tumor, as recently hypothesized for non–small cell lung cancer and represent the “seed” for the development of anti-EGFR treatment resistance.

The complexity and the heterogeneity of the molecular alterations that are being identified as resistance mechanisms to anti-EGFR therapies suggest that a comprehensive molecular characterization of metastatic colorectal carcinoma will likely be necessary in the near future to choose the most appropriate therapy for each patient. This approach has become feasible with the development of new technologies to study molecular alterations in cancer, which might reveal a complete molecular portrait of the tumor. The findings of innovative and elegant preclinical studies such as the report from Bertotti et al. (5) in this issue of Cancer Discovery represent a further significant step to personalized medicine for patients with metastatic colorectal carcinoma.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Grant Support
The research programs in the authors’ laboratories are supported by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC).

Received October 6, 2011; accepted October 6, 2011; published online November 17, 2011.

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HER2 Signaling and Resistance to the Anti-EGFR Monoclonal Antibody Cetuximab: A Further Step toward Personalized Medicine for Patients with Colorectal Cancer

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