Sclafani et al present data and suggest that tumor protein 53 (TP53) may be a predictive marker of cetuximab benefit in patients with rectal cancer undergoing neoadjuvant chemoradiation in this issue of the Journal (1). This article relies on an unplanned, retrospective analysis of molecular marker data on a subset of patients participating in the randomized phase II EXPERT-C clinical trial initially reported in 2012 (2). Patients with operable magnetic resonance imaging–defined high-risk rectal cancer received four cycles of capecitabine/oxaliplatin (CAPOX) followed by capcitabine chemoradiotherapy, surgery, and adjuvant CAPOX (four cycles) or the same regimen plus weekly cetuximab (CAPOX-C). The primary endpoint was complete tumor response, which was modified during conduct of the study to include only the subset of patients whose tumors were KRAS/BRAF wild type. This was quite reasonably done when the impact of KRAS status on antitumor activity of epidermal growth factor receptor (EGFR)-inhibiting monoclonal antibodies was documented (3). However, the sample size of the study was not increased to assure the intended statistical power. One hundred and fifty eligible patients were randomly assigned. Based on a subset of patients with KRAS/BRAF wild-type tumors (46 patients who received cetuximab vs 44 patients who did not receive cetuximab as a component of treatment), the authors concluded that cetuximab led to a statistically significant increase in response rate and overall survival, but the primary end point of improved complete pathologic response was not met. Further follow-up revealed that contrary to the initial report, adding cetuximab was not associated with a statistically significant improvement in progression-free survival or overall survival in either KRAS/BRAF wild-type or unselected patients (1,2). Understanding the small sample size and selection of a subset of randomized patients for analysis in the EXPERT-C trial is germane to evaluation of TP53 as a predictor of cetuximab benefit in the current study.

What is the biological rationale to suggest that TP53 might be a predictor of cetuximab effect in this setting? The loss of the protective p53 function in cells, either through mutation or posttranslational modification, leads to unchecked proliferation, growth, genomic instability and therapeutic resistance. In preclinical studies, loss of TP53 function is associated with drug and radiotherapy resistance across myriad malignancies (4). Likewise, some studies have indicated that the expression of wild-type TP53 is required for the efficacy of both radiation and chemotherapy. In a series of elegant studies, Huang and colleagues demonstrated that the response to cetuximab and radiation can be regulated through manipulation of TP53, including in parental cells or restoration of functional TP53 in resistant cells (5). The exact mechanisms for these findings remain uncertain. Intact TP53 is a known prerequisite for G0/G1-arrest and is a key component of cell cycle checkpoints through an ability to respond to several different cellular signals and induce arrest at different cell cycle points. Huether and colleagues demonstrated that cetuximab treatment up-regulates the cell cycle inhibitors p21Waf1/CIP1 and p27KIP1, which are also established transcription targets of activated TP53 (6). Thus, wild-type TP53 and cetuximab therapy might provide physiologic synergy in cell cycle arrest. Functional TP53 may further interact with cetuximab therapy through involvement in the phosphoinositide 3-kinase/AKT and mitogen-activated protein kinase pathways. Activated TP53 results in decreased AKT activity in epithelial tumors, another potential mechanism of anti-EGFR resistance (7). The authors of the current study also suggest that wild-type TP53 may promote cetuximab-induced, antibody-dependent, cell-mediated cytotoxicity and resultant cellular senescence (8). It is noteworthy that Sclafani and colleagues defined a TP53 mutation to be present in either a pre-treatment or resected specimen. With a concordance rate of only 60% between paired pre and post-treatment specimens, the impact of treatment itself on TP53 status might be relevant. Of these and other proposed mechanisms, the current study adds little to the fundamental understanding of the proposed relationship between TP53 status and cetuximab benefit. It is also important to point out that the opposite relationship between TP53 status and cetuximab outcomes has been reported. Oden–Gangloff and colleagues assessed tumor specimens from 64 patients with chemotherapy-refractory metastatic colorectal cancer who were treated with cetuximab-based chemotherapy. They found an association between TP53 mutations and improved clinical outcome, particularly in patients without KRAS mutations (9). These investigators also had a relatively small sample size and used a slightly more restrictive set of primers for TP53 limited only to exons 5–8, compared to exons 4–9 in the current study. Nearly 80% of TP53 mutations are missense mutations, unlike most other tumor suppressor genes that are typically affected by nonsense frameshift mutations (10). Given the central role that TP53 plays in assessing cellular stress and then modulating multiple responses, these missense mutations may result in oncogenic properties that extend beyond our relatively simplistic binary identification of either wild-type or mutant variants. Determining which specific
properties mutant TP53 encodes, such as the loss of activated TP53 function, the gain of new oncogenic properties, or the gain of dominant-negative properties, may hold the key to assessing this important genomic guardian as a truly useful biomarker.

Predictive markers of response in rectal cancer represent a clinical unmet need, with opportunities to consider a convergence of new technologies. Similar to breast cancer, the neoadjuvant treatment of rectal cancer represents an important opportunity to integrate clinical care with scientific discovery through the collection of serial tumor tissue biopsies and circulating tumor products. Enhanced imaging technologies including diffusion weighted MRI and PET-CT scanning hold the potential to non-invasively assess and predict the response to neoadjuvant chemoradiotherapy in patients with rectal cancer. Prospective cohort studies are needed to validate these tools and their role in personalized clinical decision making. However, molecular predictive biomarkers remain the current focal point for many reasons. These include tumor-specific markers selected by the therapeutic agents being delivered (eg, KRAS/NRAS/BRAF for anti-EGFR antibodies) as well as those more generalized to the overall treatment paradigm (eg, hypoxia-inducible factor 1α for tissue hypoxia).

It is highly unlikely that a single biomarker will serve all patients. Instead, a more comprehensive composite molecular profile will be needed. This will require that both patients and investigators participate in the prompt and standardized collection, processing and annotation of fresh blood and tissues at the beginning, during, and at the completion of therapy. Prospective studies with such critical translational processes should be prioritized and financially supported. Multiplex gene expression profiling to identify molecular predictive “signatures” of response to neoadjuvant chemoradiotherapy are currently being explored. These hold the potential to importantly assess elements of both the tumor and the microenvironment. Any proposed marker will require validation. Tumor specimens collected prospectively in randomized phase III clinical trials such as NSABP R-04 (NCT00058474), for example, may afford an opportunity to independently validate putative predictive biomarkers in rectal cancer (11).

The identification of TP53 as a potential biomarker predictive of cetuximab benefit in the neoadjuvant rectal cancer setting by Sclafani et al is an interesting preliminary observation that clearly needs confirmation. The authors are to be commended for their frank and comprehensive discussion of the limitations of their observation, and their cautious conclusion. We agree with the authors that further retrospective studies of TP53 as a predictive marker in rectal cancer should be carried out before any consideration of prospective confirmatory clinical trials.

References

Note
The authors have no conflicts of interest to disclose.

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