EDITORIAL

What Should We Do Better? Lessons from Negative Results of a Biomarker Validation Study

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Over the past decade, the growing knowledge of colorectal cancer (CRC) molecular characteristics and complex genomic makeup has prompted a widespread effort to identify predictive biomarkers for available therapeutic agents while supporting the rationale for the development of new targeted therapies and treatment combinations. Pharmacogenomics represents an irreplaceable tool to tailor patient treatment based on germline and somatic genetic variations that can predict drug response and risk of toxicities. Several pharmacogenomic biomarkers, including single nucleotide polymorphisms (SNPs) in genes involved in key oncogenic mechanisms, drug metabolism, and tumor-microenvironment interaction, have shown promising results in different settings so far; nevertheless, their clinical validation is often lacking.

To date, a limited number of practice-changing predictive biomarkers have been integrated in the therapeutic decision making for CRC, including RAS mutational status in patients who are candidates for anti-EGFR monoclonal antibodies (1), and more recently the use of microsatellite instability (MSI) or mismatch repair deficiency (MMR-d) to select patients for immune checkpoint inhibitors in the metastatic setting (2,3). Several pharmacogenomic biomarkers, including single nucleotide polymorphisms (SNPs) in genes involved in key oncogenic mechanisms, drug metabolism, and tumor-microenvironment interaction, have shown promising results in different settings so far; nevertheless, their clinical validation is often lacking.

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Oxaliplatin is a core component of the chemotherapy backbone for CRC treatment, with proven activity both in the adjuvant (in combination with a fluoropyrimidine) and in the metastatic setting (combined with other agents and targeted drugs). Our group has been the first to study the impact of germline polymorphisms in the DNA repair pathway highlighting the association between XPD SNPs and ERCC1 levels and oxaliplatin activity in CRC and other tumor types (5–7). Despite these encouraging preliminary data, ERCC1 gene expression recently failed to be confirmed in a dedicated prospective trial, the phase II MAVERICC study (8), where ERCC1 levels in mCRC patients treated with mFOLFOX6-bevacizumab did not predict resistance to oxaliplatin nor were they associated with a differential outcome from FOLFIRI-bevacizumab compared to mFOLFOX6-bevacizumab.

Several other candidate gene polymorphisms have been proposed as predictors for clinical outcome in CRC patients treated with platinum-based regimens (9,10). In this issue of JNCI, Gray and colleagues report the results of a validation analysis of pattern recognition receptor polymorphisms as predictors of oxaliplatin benefit in CRC (11). The authors aimed to verify previous evidence on the predictive value of common loss of function (LOF) polymorphisms in FPR1 (rs867228), TLR3 (rs3775291), and TLR4 (rs4986790/rs4986791) in patients treated with anthracycline- and oxaliplatin-based chemotherapy (12–14). These genes encode receptors mediating the innate immune system recognition of endogenous ligands presented by dying cells (15,16), which appears to be especially relevant in the context of oxaliplatin-induced cell death (14,17). Two large, well-defined, prospectively treated cohorts from the SCOT (n = 2929) (18) and COIN/COIN-B trials (n = 1948) (19), encompassing both early-stage and metastatic disease, were included in this retrospective biomarker analysis. The authors found no evidence of an association between any SNP and disease-free survival in the SCOT cohort, or with overall survival in either cohort, irrespective of the genetic model imposed. Prespecified subgroup analyses stratified by TLR3 and TLR4 functional status revealed no evidence of an association between FPR1 status and clinical outcomes, while an additional analysis according to radiological response to oxaliplatin-based chemotherapy revealed no difference in the proportions of functional and LOF alleles between responders and nonresponders for either FPR1 rs867228.
(P = .90, two-sided, χ² test), TLR3 rs3775291 (P = .68, two-sided, χ² test), or TLR4 rs4986790 (P = .64, two-sided Fisher exact test). The authors conclude that they found no evidence that constitutional LOF SNPs in FPR1, TLR3, or TLR4 are associated with differential benefit from oxaliplatin, suggesting that these SNPs are unlikely to be clinically useful biomarkers.

The negative results of this study raise, once again, the issue of the level of evidence of positive genotyping results obtained in small dataset and retrospective series of patients. The authors, in fact, argue that the discordance between their results and those from previous studies may be explained by the increased risk of false-positive associations in smaller cohorts, and in the case of FPR1, an apparent misclassification of the functional and LOF alleles in previous analyses (11). However, they recognize that a limitation of their work is the lack of testing for an association between the selected SNPs and outcome in specific molecular subgroups, particularly those with enhanced immunogenicity such as MMR-d tumors. Growing evidence supports the influence of tumor molecular subtypes (consensus molecular subtypes (CMS)) and tumor characteristics such as MSI status on chemosensitivity. Indeed, retrospective data from several studies have consistently reported a lack of efficacy from 5-fluorouracil treatment in MSI-high stage II patients (20), while the benefit from oxaliplatin-based adjuvant chemotherapy appears to be independent from MSI status (21–23). In the metastatic setting, on the other hand, recent data suggest a greater activity of irinotecan-based chemotherapy compared to oxaliplatin-based chemotherapy in MSI-high tumors (24). Retrospective analyses have suggested a lack of benefit from oxaliplatin for the mesenchymal-like CMS4 tumor phenotype (25), which has been recently supported by preclinical studies exploring models of CMS in large panels of CRC cell lines, primary cultures, and patient-derived xenografts (26,27). Furthermore, gender-specific genomic profiling has been shown to predict outcome in mCRC patients treated with 5-fluorouracil and oxaliplatin (28). Therefore, the predictive value of genetic polymorphisms should be tested in clinically relevant subgroups to avoid missing possibly meaningful results in molecularly defined patient populations. In this era of precision medicine, in fact, optimizing therapeutics for specific subsets of patients based on patient and tumor characteristics is crucial to improve outcomes and minimize unrequired toxicities.

Conflicting results from different studies and lack of prospective validation warrant caution in the generalization of positive preliminary SNPs data, as Gray and colleagues’ study exemplifies (11). Nevertheless, the constant effort in dissecting the pharmacogenomic basis of drug activity and efficacy is paramount to improve patient selection and drive personalized treatment strategies and novel drug development. Moving from a single-gene approach to molecular signatures comprising multiple genetic and/or genomic and transcriptional data across multiple relevant pathways may better mirror the complexity of the tumor-host interaction, exploiting novel high throughput technologies to promote a systems biology approach to pharmacogenomic discovery. Indeed, the repeated failures in validating preliminary positive results using traditional discovery and validation datasets for genomic biomarkers call for a change in our methodological approach. Heterogeneity between cohorts from different studies and small patient numbers represent a major issue, particularly when looking at selected molecularly defined subgroups. Statistical approaches for developing predictive signatures from high-dimensional integrative genomic datasets involving small numbers of patients and samples are needed. To maximize the utility of existing data, innovative approaches such as meta-

analyses of large patient populations accounting for patient and treatment heterogeneity can be applied, thereby reducing the rate of false discoveries and increasing the level of confidence in the observed results. On the other hand, implementing biomarker-driven clinical trials and prospective pharmacogenomic profiling is a priority for current and future research.

In conclusion, new methods and perspectives are warranted to improve our current approach to biomarker validation if we want to move from negative results to successful biomarker discovery.

### References


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