Now's the time to find biomarkers on purpose

Over the past several decades, a tremendous amount of information has been gained regarding the somatic molecular alterations that are involved in the carcinogenesis process for many types of cancers. Knowledge of these alterations has led to the development and utilization of targeted therapeutic agents that are directed at the cancer cells with these alterations. In a subset of breast cancers, ERBB2 (also known as human epidermal growth factor receptor 2) is amplified, which has prognostic significance as women with tumors containing ERBB2 amplification have decreased survival [1]. Trastuzumab is a humanized mAb directed against the extracellular portion of ERBB2 and has shown a survival benefit in treating ERBB2-positive breast cancers [2]. In non-small-cell lung cancer, epidermal growth factor receptor (EGFR) copy number is a predictor for survival benefit from treatment with the EGFR tyrosine kinase inhibitor, erlotinib [3]. However, despite the presence of the molecular alterations being targeted, many cancers do not exhibit favorable responses to the targeted agents being administered. Recent efforts have looked across a drug pathway to identify biomarkers that are predictive for drug response. For example, testing for v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutational status is now recommended for colorectal cancer patients receiving the EGFR inhibitors, cetuximab and panitumumab, because no clinical benefit is observed in patients with tumors exhibiting activating KRAS mutations [4]. Although, non-small-cell lung cancer patients with activating mutations in EGFR show responsiveness to the EGFR inhibitor, gefitinib [5], a second EGFR mutation can yield resistance in tumors that were initially sensitive to gefitinib therapy [6]. Even though utilization of these biomarkers has improved the guidance of therapeutic options, additional biomarkers of drug response need to be discovered to better individualize treatment options for each cancer patient.

Since the completion of the human genome project, genome-wide profiling technologies have matured enough so that they can be utilized in clinical cancer research as an unbiased approach toward identifying putative molecular biomarkers for the trait of interest, be it drug response, tumor recurrence or some other clinical phenotype. In the area of cancer drug sensitivity, many studies have been published in which genome-wide profiling technologies were used to carry out retrospective analysis on already-acquired tissue to identify biomarkers of drug response [7]. The problem with these types of studies is that the clinical trial generally was not powered to identify molecular biomarkers from the use of genome-wide profiling technologies. With just a few exceptions, such as the MammaPrint® test and Oncotype DX™ assay used in breast cancer, most of the putative biomarkers studies have yet to make a clinical impact, either due to an inability of the biomarkers to be validated in additional studies or through a lack of effort to carry out the necessary validation studies to replicate the findings in a prospective clinical cohort.

In this issue, Tan et al. [8] describe the results from a study using gene expression profiling to identify biomarkers of response to erlotinib in non-small-cell lung carcinoma patients. Erlotinib, an EGFR tyrosine kinase inhibitor, has been shown to prolong survival in non-small-cell lung cancer patients [3, 9]. However, identifying biomarkers that predict sensitivity to erlotinib should facilitate the selection of patients most likely to respond to treatment. What sets this study apart from other studies of this ilk is that it utilizes tumor samples from the phase II Marker Identification Trial (MERIT; BO18279), a clinical trial that was prospectively powered to identify candidate genes that are differentially expressed between patients who did and who did not receive a clinical benefit from erlotinib [8]. Microarray-based gene expression profiling was carried out on clinical biopsy samples and differences in expression were assessed between patients receiving clinical benefit from erlotinib and those receiving no clinical benefit. Unfortunately, the results from this analysis indicate that no genes are significantly differentially expressed on the basis of response to erlotinib in non-small-cell lung cancers.

Although the lack of positive results from this study are disappointing, the study was able to demonstrate the feasibility of carrying out prospective tissue-based biomarker analyses in non-small-cell lung cancer. The inability to identify putative biomarkers of erlotinib response may lie in the heterogeneity of the tumors being profiled. The cancer samples profiled in this study fell into three molecular subclasses, which may override subtle drug response signatures. Perhaps, future studies focusing on identifying drug response signatures in a single molecular subclass will provide more positive results. In addition, heterogeneity of the clinical tissues could have affected the gene expression profiles, in which case new techniques for tumor sampling could alleviate this problem.

Even though gene expression profiling was unable to identify biomarkers of erlotinib response, other genome-wide profiling techniques, such as single nucleotide polymorphism profiling, array comparative genomic hybridization or genome-wide sequencing, could identify biomarkers at the DNA level, which would be less variable than expression patterns.

Does the fact that genome-wide expression profiling failed to identify biomarkers of erlotinib response mean that such biomarkers do not exist? No study will rule out the presence of weak but real predictors of drug activity. However, this study...
can rule out the presence of high-penetrant biomarkers (i.e. relative risk >4) because of the attention to study size. This study has also proven the feasibility of using genome-wide expression profiling in a prospective study, which in the future may lead to more rapid implementation of the results in the clinical. Studies need to be conducted to determine the optimal design for using genome-wide profiling to identify putative biomarkers of drug response. To date, most biomarkers of drug response identified through genome-wide profiling have occurred through retrospective analysis of available tissue. To really progress this field, realistic planning for biomarker discovery and validation in clinical trials needs to be conducted. We, as clinical scientists, need to progress from only using convenient clinical cohorts to identify biomarkers to actually planning and following through with prospective clinical trials whose aims are to discover and/or validate putative biomarkers of drug response. To initiate a study without a realistic plan for discovery and validation reflects a lack of serious desire to find robust clinical predictors. In order to achieve this goal, funding bodies should demand that adequately planned and powered biomarker discovery and validation studies are carried out in order to prove the value of these expensive technologies through translating these findings into clinical use. Until this becomes more commonplace, the genomic revolution will be focused on manuscript generation and investigator career development, leaving the benefit to patients nothing more than an unrealized dream.

**funding**

National Institutes of Health Pharmacogenetics Research Network (U01 GM63340); Lineberger Comprehensive Cancer Center (P30 CA016086); National Cancer Institute (Gastrointestinal Specialized Programs of Research Excellence grant P50 CA106991).

---

**references**