RESEARCH HIGHLIGHT

Prognostic molecular assays on the rise for non-small cell lung cancer

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Multigene expression assays have become a popular technique for both basic research and clinical studies. Besides generating hypotheses, this approach is often used in oncology to improve prognostication of patients' outcome based on specific gene expression profiles. Thereby outcome may refer to overall survival, disease-free survival or prediction of response to a defined treatment. Microarray technology has caused enthusiasm for its potential to identify biomarkers for cancer outcome, but at the same time the reproducibility and validity of findings based on such data are often challenged. For women with early stage breast cancer, a commercially available multigene expression assay has been found potentially useful as an option to predict whether certain patients will benefit from chemotherapy (1). Such molecular prognostic profiles can augment, but do not replace classic clinical factors. Similarly, the benefit from adjuvant chemotherapy after surgically resected non-small cell lung cancer (NSCLC) remains controversial. According to the National Comprehensive Cancer Network (NCCN), risk factors for considering adjuvant chemotherapy in completely resected stage IA NSCLC include poor tumor differentiation, vascular invasion, wedge resection, and minimal margins. However since disease relapse rates still reach 30%, efforts towards the identification of additional prognostic parameters and, even better, parameters that may identify patients, who will profit from adjuvant therapy, are constantly ongoing (2). For a certain gene signature to achieve clinical acceptance as a new prognostic factor, it must be more informative than already established factors. In addition, its practicability must be confirmed retrospectively, which is the requirement for its ultimate validation in a prospective study. A previous review assessing the development of gene expression-based prognostic signatures for NSCLC found little evidence that any of the signatures were ready for clinical application (3). This review also showed that many of the studies contained flaws, including small sample sizes, unfocused design, insufficient independent validation and biased reporting (3). Kratz *et al.* developed a quantitative PCR-based assay and recently reported that it reliably identifies patients with early-stage "non-squamous NSCLC" with high risk for mortality after surgical resection (4). Thereby a high-risk gene signature was a stronger predictor of 5 years mortality than standard criteria (sex, age, smoking status, tumor size, disease stage) and even resulted in better risk discrimination than NCCN criteria (4). The methods used to establish this molecular assay are ideal and can be considered a model for such studies. Formalin-fixed, paraffin-embedded (FFPE) tissue was used, which is a very good prerequisite for reproducibility since this is the world-wide most widely used and most standardized method of sample preparation for histopathological examination and in most instances FFPE material is the only available diagnostic tissue. Furthermore, the selection of genes was performed in a training cohort and then independently validated in two large cohorts from different institutions in the USA and China. This confers extra strength since the molecular pathology of lung cancer is known to differ depending on ethnic background (5). A more critical stance must be taken concerning the conclusion that, since such assays stratify patients according to overall survival, they may also identify patients with improved treatment-dependent outcome. Although it is a reasonable assumption that tumors with gene signatures associated with poor prognosis may benefit from adjuvant therapy, this remains yet to be proven. Also, whether such an adjuvant therapy involves an anti-proliferative agent or a different type of treatment is not clear. For instance cytotoxic substances are effective in tumors with a high proliferation fraction. Is there any evidence that a gene signature associated with prognosis also offers information concerning the proliferation fraction in NSCLC? In analogy, studies have revealed that the key biological drivers in nine prognostic gene signatures for breast cancer were proliferation-related genes, in addition to estrogen-receptor signaling and *HER2/NEU* amplification (6). Interestingly, one group suggested that the amount of prognostic information contained in four standard

performed immunohistochemical analyses for breast cancer is similar to that of an mRNA-based 21-gene assay (7). To date no single biomarker exists that can reliably predict the response to treatments with cytotoxic drugs, in contrast to biomarkers that predict responses to molecular-targeted therapeutics. The explanation may be due to the numerous genes that cooperate with each other and are involved in the cytotoxic drug target pathway. Many target genes identified by Kratz *et al.* are involved in cell cycle regulation and apoptosis and may indeed indicate tumors with an altered proliferation and survival fraction (4). This would be an interesting point to address in a subsequent study, especially in connection with cytotoxic agents. The genes *lymphocyte-specific protein tyrosine kinase (LCK)* and *interleukin 11* on the other hand encode for key signaling molecules involved in the selection and maturation of developing T- and B-cells, indicating a possible immunologic component in NSCLC. However it is also known that inflammation plays an important role in a wide variety of diseases that are not primarily disorders of the immune system (e.g., cancer, atherosclerosis and ischemic heart disease). Therefore it is possible that the prognostic outcome attributed to such gene signatures is not directly linked to the primarily addressed NSCLC. It is noteworthy to realize that after the expression levels of several hundred to tens of thousands of genes are quantified by microarray technique, the expression data are grouped and a gene signature involving a relatively small number of genes is generated, correlating with a certain outcome. Despite the information provided, the final choice of target genes is usually not fully comprehensible for third parties. This becomes obvious when different assays designed for the same objective (e.g., overall survival) utilize entirely different target genes. Selecting a precisely defined, homogenous group of patients minimizes inadequate conclusions when interpreting the results of outcome-related analyses. Regarding the inclusion criteria by Kratz *et al.*, the term "non-squamous NSCLC" is somewhat inaccurate and for better comparisons with future studies the terminology suggested by the WHO or proposed international

multidisciplinary classifications should be adopted (4,8). Overall, microarray analyses are continuously being improved technically and statistically and some multigene-based assays are already in use in clinical settings, although further efforts will be necessary to establish general acceptance.

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