Non-small cell lung cancer

Diagnostic challenges

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Accurate and validated diagnostic procedures are at the heart of the development of new therapies for all types of cancer. Advances in breast cancer, for example, were made possible by the broad availability of tissue and clinical data. Recognition of the role of human epidermal growth factor receptor 2 (HER2/neu) protein expression and gene amplification was discovered and uncovered by correlating it with clinical outcomes.

Therefore, maintaining clinical registries and banking tumour samples from significant cohorts of patients is the only way to ensure:
1. The identification of statistical distinctions/differences between populations with a better or a worse prognosis.
2. The proposal of hypotheses to confirm possible predictive markers that correlate with clinical outcomes.

Expression patterns of different molecular and signalling pathways are more accurately evaluated in fresh tissue. Messenger RNA is obtained in greater quantity and of better quality with fresh-frozen tissue from a tumour sample. Quantitative reverse transcriptase polymerase chain reaction assays are more representative of actual levels of gene translation and expression in this context. However, tissue microarray studies in non-small cell lung cancer (NSCLC) in the metastatic setting have reported variable and conflicting results with regards to correlating activation of signalling pathways with survival. Similarly, predictive marker studies based on tissue microarrays have often reported dissimilar results.

More robust data were obtained from molecular studies evaluating clonal events. A priori, let us define a clonal event as a mutation, deletion or translocation that is recurring within a tumour and recurring in a significant proportion of patients with NSCLC. As in the case of mRNA studies, material obtained from fresh-frozen tumour tissue will lead to high-quality DNA isolation in sufficient quantity to permit multiple analysis. Similarly, cytogenetic analysis in the context of large cohort evaluation will lead to a more precise and relevant level of interpretation of the demonstrated correlations if a sufficient number of samples can be analyzed completely for all predefined markers. Multivariate analysis should identify clonal anomalies that have significant effects on clinical outcomes, therefore defining prognostic factors. Further studies are then required to estimate if the clonal events lead to an oncogenic “addiction.” An addiction oncogene is the product of a gene that is the site of a clonal event, and that is particularly necessary for the proliferation advantage of cancer cells. Inhibition of the adding oncogene would therefore lead to cell death and should be a potential target of interest.

In the clinical setting, identification of molecular abnormalities should provide information of interest that could lead the physician to propose a therapy (or not) based on the prognostication, and a specific therapy based on predictive markers. In NSCLC, it has been recognized for many years that clinical parameters are excellent prognostic markers: age, stage, performance status and presence or absence of central nervous system (CNS) metastasis. Histologic characterization (squamous vs nonsquamous) also leads clinicians to refine the prognosis, although it is rarely used to establish a prognosis for a particular patient. Moreover, a certain number of clonal molecular events have been associated with specific prognostic features. Of note in NSCLC, recent knowledge of activating mutations in epidermal growth factor receptor (EGFR) and KRAS, a GTPase, have been recognized to influence prognosis. Translocations involving anaplastic lymphoma kinase (ALK) or c-ros oncogene 1, receptor tyrosine kinase (ROS1) have also recently been demonstrated in NSCLC, but their prognostic significance remains controversial.

Clinical specimens in NSCLC patients are rarely as “generous” as those obtained in breast or colorectal cancer patients. In fact, the specific difficulty of obtaining a biopsy from the site of the primary disease or from frequent sites of metastasis (lung, liver, bone, brain, adrenals) limits the quantity of tumour cells that can be processed for analysis.

Molecular analysis is not the first step in diagnostic procedures for a specimen for suspected NSCLC. Histology is obviously done, and is generally followed by immunohistochemistry (IHC) to differentiate squamous cell carcinoma and adenocarcinoma. Recently published guidelines have proposed that a certain number of analyses be conducted to ascertain the histology subtype (p63, thyroid transcription factor 1 [TTF-1], cytokeratin 5/6 [CK5/6]). Considering the need to establish a histologic diagnosis of NSCLC, a large portion of the biopsy specimen (if not the whole specimen), will often be spent on this diagnostic procedure.

What remains of the sample can then be processed for molecular analysis. There is no formal consensus on which genes should be analyzed. However, most guidelines will suggest tests to propose first-line therapies for patients with EGFR-activating mutations in mNSCLC and for translocations affecting ALK in any line of therapy.

The diagnostic challenge does not lie only with access to samples. Molecular prediction is closely linked with the validation of the test. Validation has to occur at 2 levels: first,
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demonstrating that a test has a high intralaboratory and interlaboratory correlation for both positive and negative cases. Validated test kits and home-brewed laboratory techniques are therefore totally similar if they are used in a laboratory with adequate quality assurance procedures. Both are only as good as the laboratories that use them according to good laboratory practice (GLP).

The second level of validation is the clinical relevance of a test. EGFR mutations can be detected with techniques that have a level of detection of less than 0.1% of the extracted DNA. However, the clinical trials that have reported a positive outcome for patients treated with an EGFR TKI tyrosine kinase inhibitor (TKI) in the first-line setting included those tested with techniques that identify only “classical” EGFR tyrosine kinase domain-activating mutations (exon 19 deletions and exon 21 L858R point mutation) at a level of detection of 0.1 to 1% of extracted DNA. Therefore, it is not known if treatment with an EGFR TKI for patients with a tumour heterogeneity linked to less than 0.1% of cells exhibiting an EGFR classical mutation is associated with a better outcome than if they are treated with standard chemotherapy. Moreover, reporting of nonclassical EGFR mutations cannot be associated with a particular behaviour on EGFR TKI therapy. The method used by the laboratory to evaluate the EGFR gene in NSCLC specimens should then be based on parameters that are clinically relevant.11

Similarly, echinoderm microtubule-associated protein-like 4 (EML4)-ALK translocation was linked initially with a particular histology subtype and clinical course of chemotherapy resistance. A large Phase II trial examined patients with positive fluorescence in situ hybridization (FISH) for multiple translocations involving EML4 and ALK.12 Since the results were presented, several groups have reported NSCLC cases positive for ALK by IHC with a high level of correlation with FISH testing. FISH is sometimes reported as an easy technique to perform. In fact, identifying a translocation with a break-apart probe is easier than classical karyotyping. However, GLP, as well as internal and external validations, require personnel trained specifically in cytogenetics to perform these techniques.

Tests that are used as predictive markers for clinical efficacy, especially positive predictive markers, pose an important clinical challenge. Patients who are erroneously qualified as positive have worse outcomes if they are treated with targeted therapy compared to chemotherapy. It is therefore extremely important that such tests be performed in laboratories qualified for both internal and external validation, and that clinically validated tests are performed to identify the predictive marker of interest.

Recently, important molecular-testing initiatives in NSCLC have been undertaken and reported at key meetings. A multi-institution trial in the US recruited more than 1000 patients to identify mutations in different genes that could be driver mutations and the object of a specific therapy.13 In this trial, a significant mutation was identified in 60% of cases. Moreover, even with limited sample availability, updated techniques that require less DNA were used and formally evaluated in a clinical trial. Data pertaining to correlation with clinical parameters are pending and will be important to associate these technologies to significant clinical outcomes. Similarly, the Memorial Sloan-Kettering Cancer Center is conducting a Lung Cancer Mutation Analysis Project with the objective of identifying a driver mutation in more than 60% of lung adenocarcinomas, to redefine therapy for NSCLC.14

The availability of targeted therapy, and more specifically EGFR TKIs, was the factor that prompted the renewed interest in testing in NSCLC. Two recent meta-analyses evaluating EGFR TKIs in the first- and post-first-line setting demonstrated that no biomarkers can reliably be used after first line to predict clinical outcome.15-16 However, these data will have to be reviewed frequently considering the large number of reports on new clonal events and new techniques to evaluate molecular events in NSCLC.17

References

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