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The Tissue Is the Issue: Personalized Medicine for Non-Small Cell Lung Cancer

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The development of molecular markers is crucial for improving therapy in advanced non-small cell lung cancer (NSCLC). Here, we review perspectives of biomarker-driven personalized therapy in NSCLC, with particular reference to the detection of *EML4-ALK* gene rearrangements. Different molecular assays, validation, and clinical application to different types of tissue specimens are discussed. *Clin Cancer Res; 16(20); 4909–11. ©2010 AACR.*

In this issue of *Clinical Cancer Research*, Sakairi and colleagues (1) screened endobronchial ultrasound-guided transbronchial needle aspirates (EBUS-TBNA) of hilar and/or mediastinal lymph nodes from non-small cell lung cancer (NSCLC) patients for the presence of *ALK* fusion gene by immunohistochemistry (IHC), followed by confirmation of positive and suspicious cases with fluorescence *in situ* hybridization (FISH) and direct sequencing. The authors showed that IHC-, FISH-, and reverse transcriptase-PCR (RT-PCR)-based assays were feasible in the small specimens obtained.

After many years with only modest improvements in the therapy of NSCLC, we have recently learned about the importance of molecular testing for individualized treatment of patients with advanced NSCLC.

Several recent randomized studies have shown that patients with advanced NSCLC and tumors harboring activating epidermal growth factor receptor (EGFR) mutations have a significantly better outcome when treated with EGFR tyrosine kinase inhibitors than with chemotherapy (2–4), whereas the opposite is the case for patients with EGFR wild-type tumors (2).

The importance of testing for *anaplastic lymphoma kinase* (*ALK*) gene rearrangements was recently shown. The phase I-II study of crizotinib (PF-02341066, Pfizer), an oral inhibitor of ALK and MET (HGF receptor) tyrosine kinases, showed an unprecedented high response rate for patients with advanced NSCLC and *echinoderm microtubule-associated protein-like 4* (*EML4*) and *ALK* gene fusion, despite previous chemotherapy treatment (5).

Although new treatment paradigms for patients with NSCLC are emerging, challenging issues related to the details of biomarker testing remain. What kind of assays should be used for mass screening of molecular targets and what type of tumor specimens are suitable for the proper performance of the assays?

The optimal method for EGFR mutation testing is still under validation. DNA sequencing is considered the "gold standard," but more sensitive PCR-based methods (e.g., DxS Scorpion) are currently in use. However, the road toward personalized medicine will most likely involve mass-mutation testing involving multiple genes, and at least two approaches are currently in use: Sequenom's Mass ARRAY system and the SNaPshot system. Both systems are feasible on formalin-fixed paraffin-embedded (FFPE) tissue samples (6, 7). The SNaPshot technology was recently validated in lung cancer specimens and found to detect mutation samples in which mutant DNA comprises much less of the total DNA than required for direct sequencing (8).

The method for *EML4-ALK* testing in the crizotinib clinical studies was a FISH assay using a break-apart probe (Abbott Molecular Diagnostics), which is currently being validated in prospective clinical trials. Although the FISH assay for molecular testing is well established for other targets, for example, *HER2* in breast cancer, the clinical utility of this assay for new targets is not immediately transferable, and stringent validation is needed. Other assays for EML4-ALK testing have been reported, for example, RT-PCR, sequencing, and IHC with specific antibodies targeting the ALK protein (Fig. 1; refs. 9, 10).

Each diagnostic platform has advantages and disadvantages, which should be considered before mass screening of molecular targets. Standardization efforts are currently ongoing for the *ALK* gene-rearrangement FISH assay. However, the FISH assay is still a highly specialized technique, which requires advanced training and specialized skills. *ALK* amplification and copy number gain are also frequent in NSCLC, the clinical implication of which is not yet known (11).

The RT-PCR of cDNA is also used for screening for *ALK* rearrangement. The technology is well suited for clinical high-throughput assessments; however, the assay requires predefined primers. Although different break-points in *EML4* and *ALK* may be detected, new fusion partners may not be. Although PCR-based assays that are applicable for FFPE tissue have been a challenge, this obstacle seems to be able to be managed by newer methods (9).

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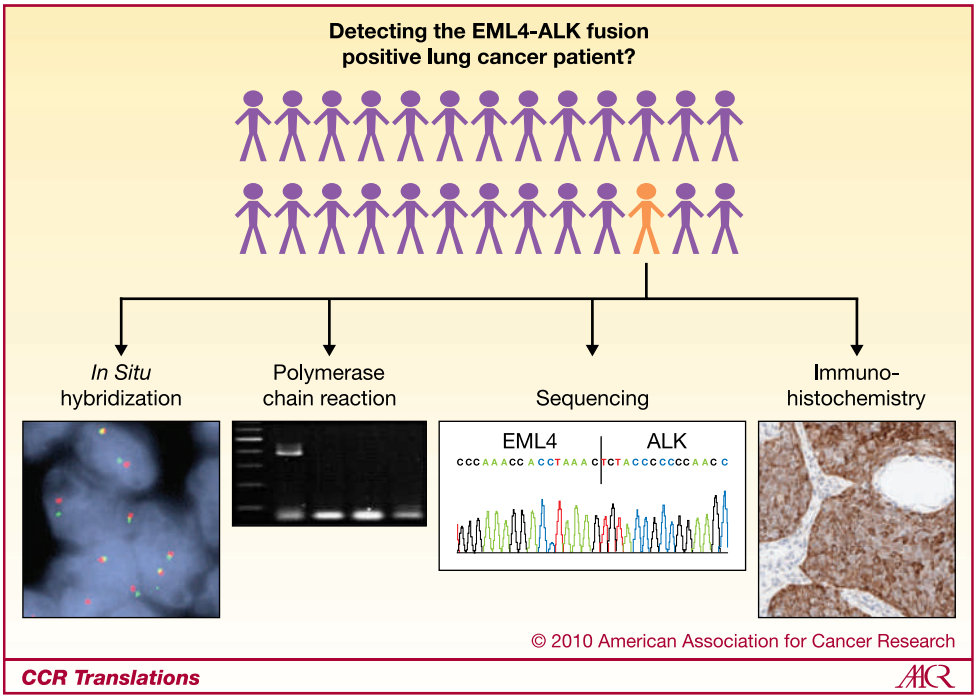

Fig. 1. The most common molecular assays for detection of *ALK* fusion genes, which occur in 3 to 5% of unselected patients with advanced NSCLC. IHC and *in situ* hybridization illustrations modified with permission from Mino-Kenudsen et al. (10). PCR and sequencing illustrations modified with permission from Saikiri et al. (1).

IHC is commonly used for identification of other targets, and novel antibodies targeting the *ALK* rearrangements are developed with encouraging results (10). The advantage of IHC is the capability of *in situ* assessments of the protein, even in small biopsies and cytology specimens.

Tumor heterogeneity is a major challenge for molecular profiling of NSCLC. Histologic and biological heterogeneity are well-known phenomena, which might significantly impact our capability to detect specific molecular targets as well as prediction of sensitivity to specific molecular targeted agents. The heterogeneity of response and outcome associated with specific molecular features is more likely a reflection of biological heterogeneity than technical issues, which might not be captured in small biopsies, fine-needle aspirates, or in tissue microarrays consisting of small selected tissue cores.

Another relevant aspect is whether the molecular profile in metastases is the same as in the primary tumors. Often for patients with advanced NSCLC, the primary diagnosis of NSCLC is based on a small metastatic biopsy, which might not represent the molecular profile of the primary tumor, partly because of the tumor heterogeneity, but also because of biological clonal selection in the metastatic process.

For treatment with a molecular targeted therapy after disease progression, which was the case with the phase I-II study with the ALK inhibitor crizotinib, we rely on the biological characteristics of the diagnostic specimen taken before first-line therapy. However, modification of the tumor's biology during first-line therapy might be significant, and the biological status before second- and/or third-line therapy might not be the same as before first-line therapy. The Biomarker Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) study is unique, clearly showing the feasibility of a biomarker-directed therapy program based on pretreatment molecular profiling (12).

Rapid and robust biomarker assay development is crucial, and stringent validation is necessary to ensure that the presumed predictive biomarker being measured faithfully foretells the clinical benefit of a given drug. The validation process includes the technical robustness of the assay (e.g., stability, reproducibility), as well as a clear distinction between prognostic association (i.e., independent of a given therapy) and predictive association (i.e., outcome related to a specific therapy). Guidelines for clinical biomarker development have been published (13).

For mass high-throughput screening of a molecular target, the validated assay needs to be inexpensive, reliable, intuitive for clinical laboratories, and harmless to the patients. Sakairi and colleagues' study introduces the potential use of IHC as a screening test for detection of the *ALK* fusion gene. The application of the multimodality analysis to the EBUS-TBNAs is important, as many NSCLC patients' diagnosis is based on a small biopsy with a limited amount of tumor tissue or fine-needle aspirations. Future development of plasma biomarkers that are less invasive and amenable to serial sampling may assist with tissue requirements. For further development of biomarker-driven personalized therapies, well-designed prospective validation studies are necessary, preferably including validation of the biomarker in test-positive and test-negative groups,

and the standardization of the definitions of positive and negative tests is crucial.

In summary, after significant success with personalized therapy in other solid tumor types, molecular testing has become important in the management of patients with NSCLC. Hopefully, this paradigm shift will lead to substantial changes in the dismal prognosis for these patients. Although Sakairi and colleagues' study is an encouraging move toward the application of molecular tests in smaller biopsies and cytology specimens, further validations, assay developments, and standardizations are necessary.

The "tissue is the issue" remains an important principle for further progress in personalized medicine, and sufficient tissue for biomarker assessments should be mandatory in future clinical trials.

Disclosure of Potential Conflicts of Interest

F. Hirsch, commercial research grant, OSI Pharmaceuticals, AstraZeneca, Syndax, Merck, Ventan Medical Systems, Inc.; consultant, AstraZeneca, Lilly, Syndax, GlaxoSmithKline, Boehringer Ingelheim, OSI/Genentech/Roche. The other authors disclosed no potential conflicts of interest.

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