Epidermal Growth Factor Receptor Mutation Testing in Lung Cancer: Searching for the Ideal Method

Commentary on Hoshi et al., p. 4974

William Pao1,2,4 and Marc Ladanyi1,3

Some somatic mutations, especially those in genes encoding tyrosine kinases, are central to the biology of specific cancers. In such instances, these “driver” mutations lead to the production of mutated enzymes, which then serendipitously serve as excellent substrates for targeted therapies. Examples include BCR-ABL—dependent chronic myelogenous and acute lymphoblastic leukemias (1), mutant KIT- and PDGFRA-dependent gastrointestinal stromal tumors (2), mutant PDGFRA-dependent hypereosinophilic syndrome (3), and mutant epidermal growth factor receptor (EGFR)—dependent lung adenocarcinomas (4–6). In each disease, treatment with selective kinase inhibitors [i.e., imatinib mesylate (Gleevec), gefitinib (Iressa), erlotinib (Tarceva)] leads to rapid and durable clinical responses.

As efforts to identify clinically relevant mutations intensify, mutation testing of tumors will become more routine (7). Already, EGFR mutation testing in lung adenocarcinomas is used to help guide treatment decisions and/or to enroll patients on specific arms of clinical trials. However, for this to become a standard of care, clinical mutation detection testing will need to become more reliable, quick, and accurate. These considerations have been driving the development and evaluation of a wide variety of EGFR mutation detection techniques.

Dideoxynucleotide or “Sanger” sequencing of PCR-amplified DNA products is the classic method for the detection of genomic mutations and is still widely employed to uncover “new” mutations. However, this technique has several drawbacks in the clinical setting, where the focus is on the detection of known recurrent mutations with clinical relevance. First, the method involves multiple steps (i.e., DNA extraction, PCR-based amplification, DNA sequencing, and sequence interpretation) and typically requires a few days to obtain a result after tissue acquisition. More importantly, the sensitivity of direct sequencing is suboptimal for clinical tumor samples; mutant DNA needs to comprise ≥25% of the total DNA to be easily detected. In the setting of lung cancer, in which diagnoses are often based on cytologic specimens or in which surgical tumor specimens may contain a high percentage of nonneoplastic cells, mutation detection by direct sequencing could not infrequently lead to “false-negative” results.

Over the past three years, many methods have been proposed for the improved detection of EGFR mutations in lung cancer specimens (Table 1). All assays claim to be more sensitive than direct sequencing, with the ability to detect mutations in samples containing ≤10% mutant alleles. Some techniques save time by eliminating the sequence interpretation step. Some require expensive equipment or proprietary reagents, whereas others could be easily adapted for use in molecular pathology laboratories without the need for additional machines.

In this issue of Clinical Cancer Research, Hoshi and colleagues describe a novel method for EGFR mutation detection based on a technique called SMart Amplification Process (SMAP)—a genotyping strategy that can detect a mutation “within 30 minutes” under isothermal conditions and in a single step (8). Importantly, in this procedure, background misamplification is suppressed by two approaches: (a) by using an asymmetrical primer design, which minimizes potential misamplification, and (b) by including Taq MutS, a mismatch binding protein that recognizes mismatched primer/template pairs and prevents them from being active templates for DNA synthesis. This strong suppression of mispriming and nonspecific amplification permits the detection of mutant sequences even when present in a sample at only 0.1%. It also allows for scoring of any DNA amplification (detected in a real-time PCR machine under isothermal conditions) in the SMAP assay as a positive result. Suppression of nonspecific amplification is not a novel concept (9). The approaches used in the SMAP technique could also be applied to improve other mutation detection techniques. Thus, the significance of the work extends beyond the SMAP assay itself.

The SMAP assay is further accelerated by the use of crude tumor sample DNA prepared in as little as 5 min using a simple lysis in sodium hydroxide. By contrast, most other methods use overnight digestion in proteinase K and then some type of extraction step to isolate DNA. We should note, however, that to expedite sample processing, the use of crude DNA preparations could also be incorporated into other PCR-based EGFR mutation detection methods.

The SMAP assay does present some limitations. First, as illustrated by the article’s Fig. 1, it is a conceptually complex amplification process that may be correspondingly difficult to troubleshoot. Second, because of the need for mutation-specific primers, comprehensive detection of in-frame deletions in EGFR exon 19 is not possible; the authors designed specific primers for seven of the most common types of deletions. This is a drawback common to methods that rely on mutation-specific primers to detect small variable insertions or deletions.
As more such assays are developed, oncologists and molecular diagnostic laboratory directors will need to grapple with some practical questions. First, is there a clinically meaningful difference between an *EGFR* mutation test that returns results in less than an hour versus a few days? Unlike electrolyte or complete blood count testing, clinicians are unlikely to act within a few hours on the results of *EGFR* mutation testing. Second, because most diagnostic labs perform mutation testing in batches in order to use resources and technologist time efficiently, would a rapid assay suited for real-time case-by-case testing be applied outside of a point-of-care setting? Finally, should labs use assays that comprehensively detect all clinically relevant *EGFR* mutations or just the most common ones? These are only some of the logistical questions that will need to be addressed as molecularly tailored therapy becomes the new paradigm in cancer care.

### Acknowledgments

We apologize to the investigators whose methods/papers were not cited due to space constraints.

## References


### Table 1. Methods for detecting *EGFR* mutations in lung cancer specimens

<table>
<thead>
<tr>
<th>Technique</th>
<th>Reference</th>
<th>Sensitivity (% mutant DNA)</th>
<th>Mutations identified</th>
<th>Comprehensive detection of deletions and insertions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sequencing</td>
<td>Multiple</td>
<td>25</td>
<td>Known and new</td>
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<td>10</td>
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<td>(13)</td>
<td>7.5</td>
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<td>(14)</td>
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<tr>
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<td>(15)</td>
<td>5</td>
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<td>MALDI-TOF MS–based genotyping</td>
<td>(16)</td>
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<td>SMAP</td>
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<td></td>
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</table>

Abbreviations: SSCP, single-strand conformation polymorphism; PNA-LNA: peptide nucleic acid–locked nucleic acid; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; ARMS, amplified refractory mutation system; dHPLC, denaturing high performance liquid chromatography.
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