

A Blood-Based Test for Epidermal Growth Factor Receptor Mutations in Lung Cancer

□□ Commentary on Kimura et al., p. 3915

Daphne W. Bell and Daniel A. Haber

In this issue of *Clinical Cancer Research*, Kimura et al. (1) report on a blood-based assay to detect epidermal growth factor receptor (EGFR) mutations in patients with advanced non-small cell lung cancer (NSCLC).

The development of effective molecular therapeutic targeting strategies in epithelial cancers requires the ability to sample tumor tissue for markers predictive of drug response and ideally to do so repeatedly and noninvasively during the course of therapy. In chronic myelogenous leukemia, the evolving paradigm of molecularly targeted therapy, sampling of malignant cells is readily feasible through blood or bone marrow analysis (2). Thus, the effectiveness of treatment with imatinib (Gleevec) may be measured by loss of the underlying *BCR-ABL* translocation, whereas the acquisition of secondary mutations associated with imatinib resistance guides the use of second-generation inhibitors.

In NSCLC, activating mutations in the EGFR are associated with dramatic responses to the tyrosine kinase inhibitors (TKI) gefitinib (Iressa) and erlotinib (Tarceva; refs. 3–5). Limited availability of tumor specimens for molecular analyses, however, has seriously hampered genotype-directed clinical trials to date. At initial diagnosis, the increasing use of fine-needle aspirates, which provide minimal amounts of tumor cells for analysis, has confounded the ability to immediately identify patients likely to respond to EGFR-TKIs. The likely accumulation of additional genetic lesions as a tumor progresses through multiple courses of chemotherapy requires the ability to serially analyze tumor cells for molecular markers throughout the disease course. Most tumor-based diagnostic tests for EGFR mutations currently use direct nucleotide sequencing of exons 18 to 21, which encompass the vast majority of drug-sensitizing mutations. Although sequencing permits the identification of all mutations in an unbiased fashion, it is a relatively insensitive technique, particularly for heterozygous mutations. In general, a heterozygous mutation has to be present in at least ~60% of cells (or ~30% of alleles) for reliable detection by sequencing. Consequently, EGFR testing by this method is generally restricted to patients with adequate tumor tissue for pathologic review to evaluate cellular heterogeneity. Thus, patients diagnosed by fine-needle biopsy,

as well as those with surgical specimens consisting of <60% tumor cells, are generally ineligible for testing.

In this issue of *Clinical Cancer Research*, Kimura et al. (1) describe a blood-based assay to detect EGFR mutations in patients presenting with advanced NSCLC. Free tumor-derived DNA in plasma may originate in necrotic cells sloughed from the primary tumor or possibly from lysis of circulating tumor cells. The investigators focus on two recurrent activating EGFR mutations associated with TKI susceptibility, which comprise approximately two thirds of reported variants (6), the L858R missense mutation and the in-frame deletion del E746-A750, and use an exquisitely sensitive allele-specific detection system (Scorpion Amplified Refractory Mutation System; see Fig. 1) capable of identifying these two mutant EGFR alleles within a background of 10,000 wild-type alleles. The two mutations were detected in plasma DNA from 13 of 27 (48%) NSCLC patients, a relatively high frequency but one which is consistent with the increased prevalence of EGFR-mutant NSCLC in Japan and selection criteria, including clinical markers of EGFR-mutant tumors (adenocarcinoma histology, female sex, and absence of smoking history; refs. 5, 7). In cases analyzed by both nucleotide sequencing of the primary tumor and Scorpion Amplified Refractory Mutation System assay of plasma, 8 of 11 (72%) cases were concordant. In two cases, a mutation detected in the primary tumor was not evident in plasma, whereas in a third case a mutation found in plasma had not been observed in the original NSCLC. These discrepancies may relate to technical differences in assay sensitivity, to quality of tumor-based sequencing analysis, or to variation in the amounts of circulating tumor-derived DNA in plasma.

Importantly, Kimura et al. show that the plasma-derived EGFR genotype is predictive of subsequent clinical response to an EGFR-TKI. Mutations were present in the plasma DNA from 77.8% of responsive cases compared with 33.3% of those with stable or progressive disease ($P = 0.046$). NSCLC patients with EGFR mutations identified in their plasma had a significantly longer median progression-free survival (200 days versus 46 days; $P = 0.005$) as well as a trend toward longer median overall survival (611 days versus 232 days; $P = 0.078$) than those without detectable mutations. These findings are consistent with the majority of studies, particularly those conducted in Asia, where the increased EGFR mutation frequency allows for more statistically significant analyses of this NSCLC subset (8–11).

The approach of mutant allele-specific amplification from plasma DNA is potentially powerful but also has significant limitations. It is of course restricted to known variants, and a larger panel of assays would be required to capture all reported

Authors' Affiliation: Massachusetts General Hospital Cancer Center and Harvard Medical School, Charlestown, Massachusetts
Received 3/17/06; accepted 3/31/06.

Requests for reprints: Daniel A. Haber, Massachusetts General Hospital Cancer Center, CNY7, 149 13th Street, Charlestown, MA 02129. Phone: 617-726-7805; Fax: 617-724-6919; E-mail: haber@helix.mgh.harvard.edu.

© 2006 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-06-0670

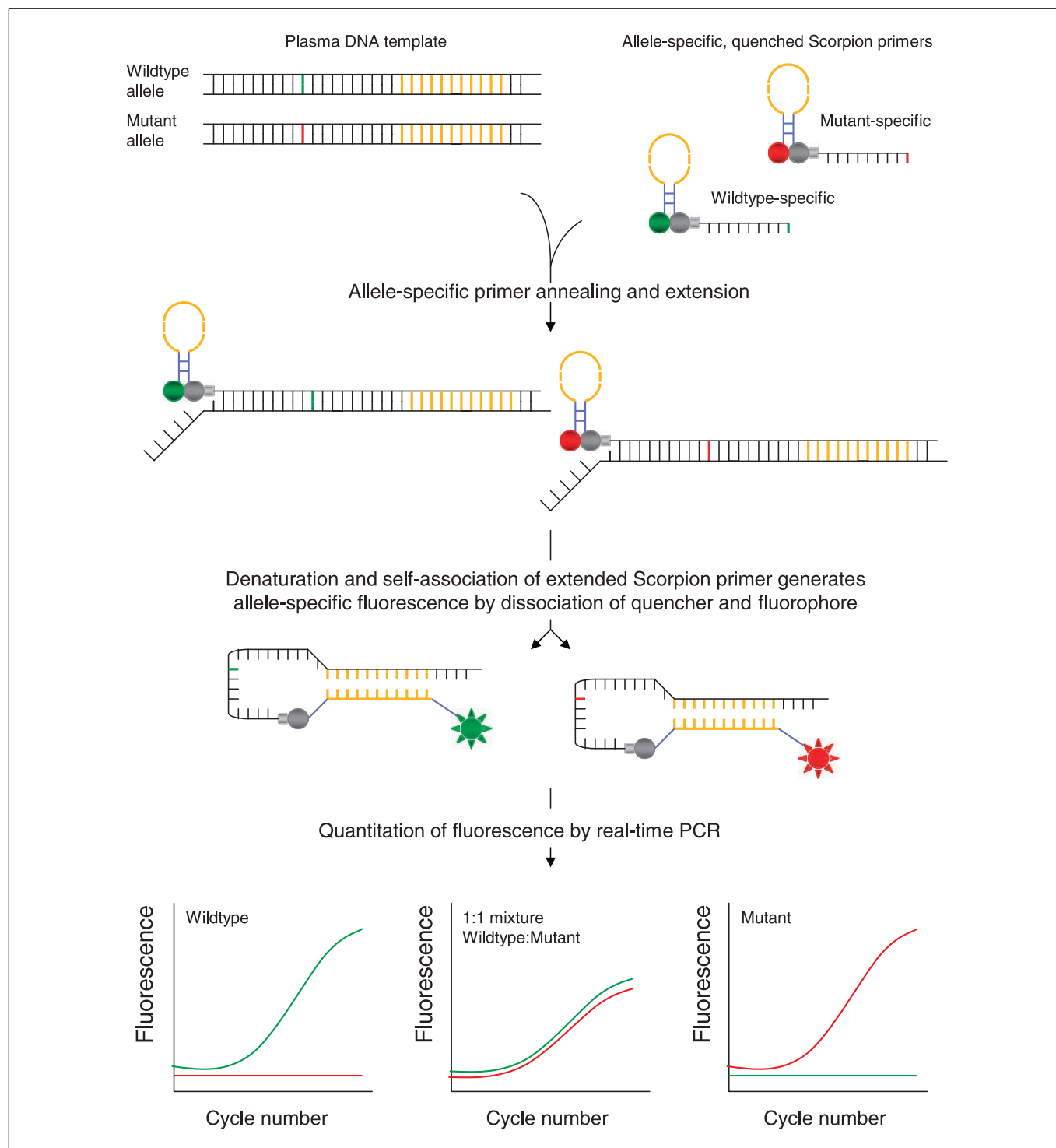


Fig. 1. Allele-specific mutation detection by Scorpion Amplified Refractory Mutation System. Allele-specific Scorpion primers are composed of a specific fluorophore (green circle or red circle), a stem (purple), a gene-specific probe region (orange), a quencher (gray circle), a blocker molecule (gray square), and a primer (black) with a 3'-terminal nucleotide complementary to either the wild-type or the mutant base in the DNA template (green line or red line). Genomic DNA extracted from plasma is amplified by PCR in a reaction containing Scorpion primers specific for wild-type or mutant alleles. Primers anneal to template DNA, and the fluorophore remains quenched. Extension occurs in an allele-specific manner. On denaturation, the probe mediates self-association of the Scorpion primer and, consequently, dissociation of the fluorophore/quencher to generate allele-specific fluorescence. Fluorescence is detected and quantitated by real-time PCR permitting a determination of the genotype of input plasma DNA.

EGFR mutations. Even more important will be the development of assays to detect secondary mutations in EGFR, such as T790M, which has been linked to the acquisition of resistance to the reversible EGFR-TKIs gefitinib and erlotinib (12–14). Second-

generation EGFR inhibitors, such as the irreversible inhibitor HKI-272 (14), are now entering clinical trials, and, as for imatinib-resistant chronic myelogenous leukemia, the ability to monitor for the emergence of this or other drug resistance

mutations may hold the key to long-term treatment of this disease. An important but unresolved question is the sensitivity of the Scorpion Amplified Refractory Mutation System assay in patients with variable tumor burdens. Failure to identify a mutant signal may indicate absence of mutation, presence of a mutation that is not represented in the allele-specific assay, or simply absence of sufficient amounts of circulating DNA in plasma. As such, some correlation of *EGFR* mutant-specific amplification with the total amount of tumor-derived plasma DNA would be important in interpreting a negative result. Additional tests, including the capture and analysis of intact circulating tumor cells (15), may provide further insight and allow a broad range of molecular characterizations.

In summary, blood-based molecular diagnostics are a new and exciting approach to monitoring genetic lesions in tumor cells, possibly circumventing the requirement for serial biopsies of inaccessible solid tumors. Although previous studies of genetic and epigenetic markers in free plasma DNA have shown the potential value of this source of tumor-derived material (16), the report by Kimura et al. links plasma DNA-based mutational analysis to a therapeutic choice relating to the administration of TKIs in NSCLC. This approach and related strategies for blood-based analysis in solid tumors come at a most opportune time, as novel molecularly targeted agents enter the clinical arena, setting the stage for genotype-directed therapy of epithelial cancers.

References

1. Kimura H, Kasahara K, Kawaishi M, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small cell lung cancer. *Clin Cancer Res* 2006;13:3915–21.
2. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood* 2005;105:2640–53.
3. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–39.
4. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497–500.
5. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306–11.
6. Chan SK, Gullick WJ, Hill ME. Mutations of the epidermal growth factor receptor in non-small cell lung cancer—search and destroy. *Eur J Cancer* 2006;42:17–23.
7. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–46.
8. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005;23:2513–20.
9. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005;23:2493–501.
10. Haneda H, Sasaki H, Lindeman N, et al. A correlation between EGFR gene mutation status and bronchioloalveolar carcinoma features in Japanese patients with adenocarcinoma. *Jpn J Clin Oncol* 2006;36:69–75.
11. Taron M, Ichinose Y, Rosell R, et al. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clin Cancer Res* 2005;11:5878–85.
12. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;2:e73.
13. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–92.
14. Kwak EL, Sordella R, Bell DW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci U S A* 2005;102:7665–70.
15. Mocellin S, Keilholz U, Rossi CR, Nitti D. Circulating tumor cells: the ‘leukemic phase’ of solid cancers. *Trends Mol Med* 2006; doi:10.1016/j.molmed.2006.01.006.
16. Taback B, Hoon DS. Circulating nucleic acids and proteomics of plasma/serum: clinical utility. *Ann N Y Acad Sci* 2004;1022:1–8.