by Schnyder et al. did not include subjects with homocysteine levels higher than 13.5  $\mu$ mol per liter. Studies are needed that will test the efficacy of homocysteine-lowering vitamin regimens containing betaine instead of folate.

Helmut Lange, M.D. Klinikum Links der Weser 28355 Bremen, Germany Harry Suryapranata, M.D.
Isala Klinieken
8011JW Zwolle, the Netherlands

hsuryapranata@diagram-zwolle.nl

- 1. Schnyder G, Roffi M, Pin R, et al. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N Engl J Med 2001;345:1593-600.
- 2. Herrmann HC. Prevention of cardiovascular events after percutaneous coronary intervention. N Engl J Med 2004;350:2708-10.

## EGFR Mutations and Sensitivity to Gefitinib

TO THE EDITOR: The important study by Dr. Lynch and colleagues (May 20 issue)1 suggests that specific mutations in the epidermal growth factor receptor (EGFR) characterize a subgroup of non-smallcell lung cancers that may be highly responsive to gefitinib therapy. Do these mutations predict a greater sensitivity to chemotherapy as well? The overall objective response rate to first-line combination chemotherapy for metastatic non-small-cell lung cancer is about 20 percent.<sup>2</sup> Only tumors from a small cohort of patients who had a response to gefitinib were studied for the specific mutations, but all patients except one had also received prior chemotherapy. Although the authors describe Patient 6 as "representative" of the cohort, the percentage of other patients who previously had a response to chemotherapy is not reported. If the rate of response to first-line chemotherapy was high for the other patients in the cohort who had a response to gefitinib, the specific mutations may be predictive of either chemotherapy or gefitinib sensitivity, thus identifying a distinct subgroup of patients with non-smallcell lung cancer.

Steven M. Sorscher, M.D.

Marshfield Clinic Wausau, WI 54401 sorscher.steven@marshfieldclinic.org

- 1. 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.
- **2.** Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non–small-cell lung cancer. N Engl J Med 2002;346:92-8.

TO THE EDITOR: Lynch et al. and Paez et al. 1 report that mutations in the EGFR kinase domain in lung cancers are associated with responsiveness to gefitinib. We performed a mutational analysis of the EGFR kinase region on tumor tissue from nine pa-

tients with an event-free survival of more than 24 weeks in our phase 2 trial of gefitinib in patients with glioblastoma.2 No mutations affecting the amino acid sequence in the kinase region were detected. However, our experience with EGFR immunolocalization in brain and lung tumors indicates that the cytoplasmic and membranous localization of wild-type EGFR and the constitutively active mutant EGFRvIII in brain tumors as compared with only membranous localization in lung tumors supports additional differences in the biology of EGFR between these tumor systems (McLendon R: personal communication). In summary, EGFR in glioblastoma did not have mutations in the kinase region, and any activity of gefitinib in glioblastoma would occur through an alternative mechanism reflective of important pathophysiological differences between glioblastomas and lung carcinomas.

Jeremy N. Rich, M.D. B.K. Ahmed Rasheed, Ph.D. Hai Yan, M.D., Ph.D. Duke University Medical Center

Duke University Medical Center Durham, NC 27710 rich0001@mc.duke.edu

- 1. 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.
- 2. Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. J Clin Oncol 2004;22:133-42.

TO THE EDITOR: Lynch et al. elegantly demonstrate the presence of gain-of-function mutations of EGFR in patients with non–small-cell lung cancer who had a response to gefitinib. However, the authors do not mention whether there were correlations between mutational findings and the results of immunohistochemical studies or fluorescence in situ hybridization (FISH), the most commonly used techniques for detecting EGFR. In fact, we observed that responsive cases had heterogeneous results of FISH

analysis, but showed cytoplasm-restricted expression of EGFR on immunohistochemical evaluation; conversely, unresponsive cases were negative or displayed a cell-membrane staining pattern (unpublished data). In a similar fashion, gastrointestinal stromal tumor is characterized by activating c-kit mutations,1 but the gene product, the transmembrane tyrosine kinase KIT, is aberrantly expressed in the cytoplasm, whereas other KIT-positive tumors without c-kit mutations show KIT immunoreactivity on cell membranes.2 Successful results with the use of EGFR and KIT inhibitors are primarily related to gene mutations involving exons encoding for juxtamembrane protein domains,3 possibly leading to cytoplasmic internalization of mutated tyrosine kinase. If this theory is confirmed, one can expect therapeutic benefits from the use of antibodies against EGFR rather than small molecules in lung cancer expressing nonmutated EGFR at the membrane level.

Giulio Rossi, M.D. Alessandro Marchioni, M.D.

University of Modena and Reggio Emilia 41100 Modena, Italy rossi.giulio@unimo.it

Lucia Longo, M.D.

Hospital of Faenza 48018 Faenza, Italy

- 1. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998;279:577-80.
- **2.** Gibson PC, Cooper K. CD117 (KIT): a diverse protein with selective applications in surgical pathology. Adv Anat Pathol 2002;9: 65-9.
- **3.** Singer S, Rubin BP, Lux ML, et al. Prognostic value of KIT mutation type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. J Clin Oncol 2002;20:3898-905.

THE AUTHORS REPLY: We agree with Dr. Sorscher that EGFR mutations are likely to identify a discrete genetic subgroup of non–small-cell lung cancers. This subgroup is uniquely sensitive to gefitinib, but our study was not designed to test for altered sensitivity to other chemotherapeutic agents. Gefitinib is currently approved as third-line therapy in patients with non–small-cell lung cancer; most of the patients in our study had previously received chemotherapy and then subsequently received gefitinib alone. Ongoing analyses of specimens from larger clinical trials, which compared chemotherapy with chemotherapy plus EGFR tyrosine kinase inhibitors, should provide insight into any differential response to chemotherapy by tumors harboring EGFR mutations.

In response to Rich et al., the frequent amplification of EGFR in glioblastomas is well recognized, as is the presence of in-frame deletions within the extracellular domain, such as the vIII mutation, resulting in constitutive activation of the receptor.1 Gefitinib did not induce dramatic responses in brain tumors despite these common EGFR alterations,2 which suggests the importance of specific mutations within the kinase domain of EGFR. We had not detected such mutations in four primary glioblastomas and 11 brain-tumor cell lines; the fact that nine glioblastomas that exhibited modest responses to gefitinib also lack EGFR kinase mutations and do not show consistent EGFR amplification or vIII mutations supports the unique drug susceptibility conferred by the kinase mutations we described. We cannot comment on unpublished data about cytoplasmic as compared with membranous localization of EGFR. In addition to altered receptor processing, amplification and overexpression of EGFR itself may well result in stronger signals in both cellular compartments.

In reply to Rossi et al., we note that previous studies have shown no correlation between responsiveness to gefitinib and levels of EGFR expression, as measured by immunohistochemical analysis.<sup>3</sup> In the cases we studied, we did not detect amplification of either wild-type or mutant EGFR alleles. As noted above, EGFR amplification (measured by FISH) is common in glioblastomas but does not appear to be correlated with gefitinib responsiveness. Again, we cannot comment on unpublished data relating to cellular expression patterns of EGFR, but we note that EGFR mutations are within the kinase domain, not the juxtamembrane domain. We agree that the effectiveness of antibodies directed against EGFR needs to be evaluated in mutation-negative

Daniel A. Haber, M.D., Ph.D. Daphne W. Bell, Ph.D. Thomas J. Lynch, M.D.

Massachusetts General Hospital Cancer Center Boston, MA 02115 haber@helix.mgh.harvard.edu

- 1. Frederick L, Wang X-Y, Eley G, James CD. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res 2000;60:1383-7.
- **2.** Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. J Clin Oncol 2004;22:133-42.
- 3. Wakeling AE, Guy SP, Woodburn JR, et al. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res 2002;62:5749-54.