

by Schnyder et al. did not include subjects with homocysteine levels higher than 13.5 μmol 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

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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-small-cell 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.² 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-small-cell lung cancer.

Steven M. Sorscher, M.D.

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

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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
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.
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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

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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.³ 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 cases.

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

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