

Epidermal Growth Factor Receptor Gene Copy Number in Esophageal Cancer and Outcome Prediction to Gefitinib: Does Intratumoral Heterogeneity Matter?

TO THE EDITOR: I read with great interest the recently published study by Janmaat et al,¹ who reported on biologic and clinical factors associated with gefitinib therapy outcome in advanced esophageal cancer. Within a panel of candidate predictive markers, they analyzed epidermal growth factor receptor (EGFR) protein expression by immunohistochemistry (IHC) and *EGFR* gene copy number by chromogenic in situ hybridization (CISH). Twenty-four tumors were evaluated for EGFR expression by IHC and nine were deemed highly positive (3+) for EGFR. High-level EGFR expression was significantly associated with disease control (response + stable disease) in six of these nine cases. In contrast, analysis of *EGFR* copy number was performed only on 16 available tumors, of which four exhibited high EGFR expression. Two of these tumors, both with high EGFR expression, were deemed CISH positive and were eventually associated with a partial response in one of the two cases. The authors concluded that assessment of EGFR level of expression by IHC predicts outcome to gefitinib therapy more effectively than *EGFR* copy number by CISH. Despite the small patient cohort, this study, however, does raise a few points which may help to interpret the lack of predictive value yielded by CISH and as such warrant further discussion.

The report by Hanawa et al,² quoted by Janmaat et al,¹ found that 17 of 18 high-level EGFR expressing tumors had either *EGFR* amplification or low level amplification/polysomy by fluorescent in situ hybridization, thus suggesting that an increased number of gene copies influences the high protein expression in 94% of cases. This figure, among tumors with strong EGFR expression, is much higher than the prevalence that Janmaat et al¹ reported in their study (50%), although the cut offs adopted in both studies to score *EGFR* by in situ hybridization were similar. By mere extrapolation, this discrepancy still persists when analysis of *EGFR* copy number is extended to 1+ and 2+ EGFR staining tumors, questioning the prevalence of increased *EGFR* copy number in esophageal cancer, and the relationship with protein expression.

In several cancer types, a correlation between increasing levels of EGFR protein expression by IHC and increased gene copy number suggests that the addictive effect of gene amplification or chromosome polysomy is a relevant mechanism underlying protein expression. Remarkably, using serial sections, it has been demonstrated that regions with *EGFR* amplification in esophageal cancer coincided with those exhibiting 2+ or 3+ degrees of immunoreactivity by IHC.² In particular, neoplastic cells showing such immunohistochemical features were found to constitute between 10% and 90% of the total cells.² Consistent with previous investigations,³ these findings imply that the proportion of tumor cells displaying *EGFR* gene increased copy number may also vary across a tumor according to the area and

the section being analyzed. As a result, the significant heterogeneity inherent to *EGFR* genetic alterations could contribute to foci with *EGFR* increased copy number being overlooked, and might provide an explanation for the low prevalence of tumors with *EGFR* increased copy number reported by Janmaat et al¹ in their study. Furthermore, these observations might explain the disagreement between the reported rates of strong EGFR protein expression and the rates of increased gene copy number, especially if assays were not performed on serial sections, as could be the case in this study. This also raises the possibility that the results obtained from tumor cross section analysis may not be representative of the whole tumor.

Though several studies evaluated *EGFR* gene status by fluorescent in situ hybridization or CISH in a variety of tumors, there are no definitive recommendations on how to proceed with *EGFR* scoring. Given the real and perceived heterogeneity of EGFR distribution in tumor sections, a score based on the mean number of *EGFR* copies per cell or the mean ratio of gene to chromosome copy, as it is currently recommended with respect to evaluation of *HER2* gene status in breast cancer,⁴ still requires thorough validation. Conversely, an approach based on the percentage of cells displaying specific patterns of *EGFR* and chromosome 7 copy numbers has proven useful in segregating subgroups of non-small-cell lung cancer patients with different outcomes after gefitinib therapy.^{5,6} This kind of approach might hold promise also in evaluating response prediction to tyrosine kinase inhibitors through *EGFR* gene and protein assessments within heterogeneous cancers, such as esophageal cancer. Unless investigators validate their methodology taking into account intratumoral genetic heterogeneity, and unless the studies are conducted on large cohorts of patients, a problem might present itself in evaluating *EGFR* copy number with in situ hybridization techniques.

Nicola Personeni

Gastrointestinal Oncology Unit, Gasthuisberg University Hospital, Leuven, Belgium

REFERENCES

1. Janmaat ML, Gallegos-Ruiz MI, Rodriguez JA, et al: Predictive factors for outcome in a phase II study of gefitinib in second-line treatment of advanced esophageal cancer patients. *J Clin Oncol* 24:1612-1619, 2006
2. Hanawa M, Suzuki S, Dobashi Y, et al: EGFR protein overexpression and gene amplification in squamous cell carcinomas of the esophagus. *Int J Cancer* 118:1173-1180, 2006
3. Owonikoko T, Rees M, Gabbert HE, et al: Intratumoral genetic heterogeneity in Barrett adenocarcinoma. *Am J Clin Pathol* 117:558-566, 2002
4. Ellis IO, Bartlett J, Dowsett M, et al: Best Practice No 176: Updated recommendations for HER2 testing in the UK. *J Clin Pathol* 57:233-237, 2004
5. Cappuzzo F, Hirsch FR, Rossi E, et al: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 97:643-655, 2005
6. Hirsch FR, Varella-Garcia M, McCoy J, et al: Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: A Southwest Oncology Group Study. *J Clin Oncol* 23:6838-6845, 2005

DOI: 10.1200/JCO.2006.07.5622

Author's Disclosures of Potential Conflicts of Interest

The author indicated no potential conflicts of interest.