# Prognostic and Predictive Value of Epidermal Growth Factor Receptor Tyrosine Kinase Domain Mutation Status and Gene Copy Number for Adjuvant Chemotherapy in Non-small Cell Lung Cancer

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**Purpose:** Patients with non-small cell lung carcinoma with epidermal growth factor receptor (*EGFR*) mutations may have a more favorable prognosis and greater response to chemotherapy. The effect of *EGFR* mutation and gene copy on patients with early-stage non-small cell lung carcinoma receiving adjuvant chemotherapy has not been reported.

**Patients and Methods:** Tumor samples from NCIC Clinical Trials Group JBR.10, an adjuvant trial of vinorelbine/cisplatin adjuvant chemotherapy [ACT] versus observation (OBS), were analyzed for *EGFR* mutation by multiple sensitive methods and copy number by fluorescent in situ hybridization. Their prognostic and predictive roles were explored in correlation with survival.

**Results:** Mutation results were available in 221 OBS and 215 ACT and fluorescent in situ hybridization results in 159 OBS and 163 ACT patients. Mutations were identified in 43 (27 OBS and 16 ACT) patients (36 sensitizing exon 19 deletions or L858R mutations). Compared with wild-type, sensitizing mutations were not significantly prognostic in OBS patients (hazard ratio [HR]: 0.79, 95% confidence interval [CI]: 0.38–1.63, $p = 0.53$). Although the presence of sensitizing mutations resulted in relatively greater benefit in ACT patients (HR: 0.44, 95% CI: 0.11–1.70, $p = 0.22$) compared with wild-type patients (HR: 0.78, 95% CI: 0.58–1.06, $p = 0.12$), this quantitative difference was not significant (interaction $p = 0.50$). Similarly, high *EGFR* copy was neither significantly prognostic nor predictive, although quantitatively it was associated with greater benefit from ACT.

**Conclusions:** Trends toward longer survival and a greater benefit from chemotherapy were observed in patients with exon 19/21 mutations and high *EGFR* copy, although the differences were not statistically significant. The interpretation of the results was limited by the low *EGFR* mutation rate in this study of mainly white patients.

**Key Words:** Biomarker, Prognostic marker, Predictive marker, Sequencing, FISH, Clinical trial, Correlative science.

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Patients with early-stage non-small cell lung carcinoma (NSCLC) are treated surgically with curative intent; however, 30 to 65% develop recurrence and die of their disease.[1] Recent trials using cisplatin-based doublet chemotherapy and meta-analyses have demonstrated significant survival benefits with postoperative chemotherapy.[2–5] JBR.10, a North American intergroup study led by the NCIC Clinical Trials Group, randomized patients with completely resected stage IB to stage II NSCLC to receive adjuvant chemotherapy with cisplatin/vinorelbine or observation (OBS) alone. Chemotherapy-treated patients derived significant survival benefit (hazard ratio [HR]: 0.70, $p = 0.03$).[5,6] It is recognized that certain clinical and pathologic factors such as stage, age, sex, tumor histology, and differentiation grade are prognostic of outcome in NSCLC.[7] At the molecular level, although a large number of markers have been investigated for their prognostic value,[8] few have been reported to predict a differential effect of adjuvant chemotherapy on survival.[9] Currently, apart from stage, neither prognostic nor predictive markers are used routinely to select patients with NSCLC for adjuvant chemotherapy.

Greater than 60% of NSCLC tumors express epidermal growth factor receptor (EGFR) protein.[10] The discovery that *EGFR* tyrosine kinase (TK) domain mutations are strongly associated with greater sensitivity of NSCLC to EGFR TK

inhibitors (TKIs) and that mutations are more common among patient subgroups who demonstrate response to these drugs suggests that this or other molecular markers may be used to determine patient subgroups most likely to benefit from EGFR TKI therapy.[11,12] Recent studies have shown that patients whose tumors contain *EGFR*-activating mutations on exons 19 and 21 also demonstrate higher response rates to chemotherapy.[13,14] *EGFR* gene copy gain also has been shown to be a predictive marker for response and survival benefit with EGFR TKI therapy[15,16] but has not been shown to be associated with a differential response to chemotherapy.[14–18] We report in this study our analyses of *EGFR* TK domain (TKD) mutation and gene copy number status in patients from the JBR.10 trial and correlate these markers with prognosis in the untreated control arm and survival benefit from chemotherapy.

## METHODS

### Patients and Tissue

This molecular correlative study was approved by the Research Ethics Board of the University Health Network. JBR.10 compared the effect of adjuvant vinorelbine/cisplatin to OBS alone in 482 patients with completely resected T2N0, T1-2N1 NSCLC.[5] Patients provided written consent for the study and for *RAS* mutational analyses (*RAS* mutation status was a stratification parameter) and 445 patients consented to tumor banking for future studies (Supplementary Figure 1S, http://links.lww.com/JTO/A40). These included snap-frozen tissue with or without formalin-fixed, paraffin-embedded (FFPE) blocks from 171 patients, FFPE blocks only from 161 patients, and 10 unstained slides only from 119 patients. Tissue microarrays (TMAs) were constructed from 332 cases with FFPE blocks.

### Analysis for *EGFR* TKD Mutations

The methods for isolation of genomic DNA and analysis of mutations on *EGFR* exons 19 and 21 have been reported previously.[15] For samples that were estimated by histology to have less than 50% tumor cellularity, we performed macrodissection of unstained sections to enrich for tumor DNA. DNA samples were analyzed first by direct sequencing, and positive results were confirmed by repeat sequencing of an independent polymerase chain reaction product. Negative cases were screened secondarily using the higher sensitivity fragment length analysis method to detect exon 19 deletion and L858R mutations.[19] Newly identified deletion/mutations were confirmed by the Scorpion Amplified Refractory Mutation System (DxS, ARMS, Manchester, UK). Samples were classified as failed or indeterminate if repeated analyses failed or were unable to confirm mutations on exons 19 or 21.[15]

### *EGFR* Gene Copy Analysis

*EGFR* gene copy number was evaluated by fluorescent in situ hybridization (FISH), as described previously using the Vysis LSI EGFR probe labeled with Spectrum Orange, and the CEP7 chromosome 7 centromere (7p11.1 through q11.1) probe labeled with Spectrum Green.[16] This was con-

ducted using TMA constructed from 332 cases with tumor FFPE blocks. In 18 cases for which TMA cores showed heterogeneity with the presence of both high and low copy number cores, we repeated the analysis using full sections. To validate TMA results further, full section analyses were also conducted on 23 randomly chosen cases with *EGFR* amplification and 20 cases with low copy number (trisomy and low polysomy). Six FISH categories were used to define *EGFR* FISH status.[15] Tumors with high *EGFR* gene copy ("High *EGFR* copy") included high polysomy or amplification, and all other categories were classified as "Low *EGFR* copy."

### Statistical Analyses

Based on a prespecified statistical analysis plan, exploratory analyses were performed to characterize relationships between marker levels and baseline clinical characteristics and survival.[4] Chi square or Fisher's exact test was used to test association between marker levels and baseline factors; Kaplan-Meier product-limit method and the log-rank test were used to estimate and test overall survival distributions and their difference between marker levels and treatment arms, and multivariate Cox regression models were used to verify the prognostic and predictive effects of markers on survival while adjusting for baseline factors and potential prognostic factors including sex, age, performance status, stage, histology, smoking status, baseline anemia, type of resection, serum lactate dehydrogenase, p53 mutation status, and p53 immunohistochemistry. All reported *p* values are two sided, and a level of 5% ($p = 0.05$) was considered statistically significant. To be consistent with our other mutational analysis reports, survival analyses are based on the original survival analysis of the trial.

## RESULTS

### *EGFR* TKD Mutation

Altogether, 445 patients who consented to future molecular studies had DNA available for mutation analysis. Among these, failed or indeterminate results were obtained in only nine patients. Baseline stratification and potential prognostic factors for 436 patients with and 46 patients without *EGFR* mutation results are shown in Supplemental Table 1S (http://links.lww.com/JTO/A41). More patients with mutation data were ever smokers (94.5% versus 84.8%, $p = 0.027$); there were no other significant differences between the populations. In patients with mutation results, the survival benefit from chemotherapy was similar to that of the overall study (HR: 0.77, 95% confidence interval [CI]: 0.57–1.03, $p = 0.08$).

Forty-five mutations were found in samples from 43 patients (9.9%), with two tumors having two separate mutations (Del E746_750, I744V; G735S, L858R). Female patients were more likely to have mutations (13.4% versus 8.0%, $p = 0.07$), as were lifetime never smokers (40% versus 8.3%, $p < 0.001$) and patients with adenocarcinoma (13.9% versus 4.9% for squamous and 7.0% for other histologies, $p = 0.01$) (Table 1). Each case of squamous cell carcinoma with mutation was reviewed histologically, and the diagnosis was confirmed for all. Among nonwhite ethnicity patients,