

## RESEARCH COMMUNICATION

# Prognostic Significance of the C-erbB-2 Expression in Turkish Non-Small Cell Lung Cancer Patients

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### Abstract

**Background:** The prognostic value of c-erbB-2 expression in patients with non-small cell lung cancer (NSCLC) remains controversial. The prevalence of c-erbB-2 expression in NSCLC and relation to disease prognosis were therefore investigated. **Methods:** Eighty-nine patients with NSCLC diagnosed at Baskent University, Adana Hospital, Medical Oncology Department, between 2000-2005 were investigated. Expression of c-erbB-2 was evaluated by immunohistochemistry in paraffin-embedded sections. Characteristics of patients, histology and stage of the disease were obtained from clinical records. **Results:** C-erbB-2 expression was detected in 18 patients (20.2%). Median survival of the patients with c-erbB-2 negative was 13 months, as compared to 6 months for c-erbB-2 positive cases ( $p=0.02$ ), the relative risk of death being 1.96 times higher. No correlation was found between c-erbB-2 positivity and stage of the disease or histology of the tumor ( $p>0.05$ ). **Conclusions:** C-erbB-2 positivity may indicate shorter survival and can be regarded as an unfavorable prognostic factor in NSCLC. Immunohistochemistry seems to be a readily applicable, inexpensive methodology for determining c-erbB-2 expression in NSCLCs.

**Key Words:** C-erbB-2 expression - immunohistochemical staining - non-small cell lung cancer

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### Introduction

Non-small cell lung cancer (NSCLC) is one of the most common cancers seen in Turkey (Firat et al., 1998), constituting a leading cause of cancer death in both men and women. Despite of the developments in the cancer care, overall 5-year survival rate of patients with NSCLC is <15% (Jemal et al., 2007). NSCLC has poor prognosis, but the pathological characteristics are not enough to predict disease outcome and molecular prognostic factors are needed to detect patients who carry high risk for disease progression and who are mostly likely to benefit from chemotherapy and other therapies.

C-erbB-2 (also known as HER2/neu) is a member of erbB gene family, located on chromosomal region 17q11.2-q12, and encodes for transmembrane receptor-type tyrosine-protein kinases (Schechter et al., 1984). The tyrosine kinase receptor is a component of IL-6 signaling through the MAP kinase pathway and functions in a manner similar to epidermal growth factor receptor (EGFR). Dimerization of c-erbB-2 with an activated EGFR molecule leads to activation of signal transduction and a cascade of events with subsequent increase in cell proliferation, angiogenesis and metastatic potential, as well as a decrease in apoptosis. C-erbB-2 overexpression was found more often in breast and ovarian, as well as in

lung cancer, especially adenocarcinoma (Hung and Lau, 1999). In vitro, NSCLC that overexpress c-erbB-2 have been found to resistant to chemotherapeutics such as cisplatin, doxorubicin and etoposide (Tsai et al., 1993). Transfection of c-erbB-2 into NSCLC cell lines increases their invasiveness, and metastatic potential in mouse xenograft experiments (Yu et al., 1994).

In different tumor types the level of c-erbB-2 expression in pathological samples is commonly determined by immunohistochemistry (IHC). Because of its relative technical ease and acceptable sensitivity, IHC is commonly used in breast cancer for selecting patients for treatment and determining high risk patients (Pauletti et al., 2000). However, it has not been determined yet whether the IHC criteria used for c-erbB-2 detection in breast cancer is applicable to lung tumors.

Prognostic implications of overexpression of c-erbB-2 in NSCLC are matter of controversy. Some reports have indicated that c-erbB-2 has been associated with tumor aggressiveness and poor prognosis in (NSCLC) (Giatromanolaki et al., 1996; Han et al., 2002; Saad et al., 2004). Other studies have either failed to show this association or demonstrated conflicting results (Pfeiffer et al., 1996; Fu et al., 1999). In this study, we investigated the frequency and prognostic significance of the c-erbB-2 and its effect on survival in NSCLC patients.

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## Materials and Methods

### Patients

Eighty-nine patients with NSCLC diagnosed, followed and treated at the Baskent University, Adana Hospital, Medical Oncology Department between 2000-2005 were investigated. Patients' age, sex, histopathological analysis and disease stage were evaluated retrospectively. Patients were staged accordingly to AJCC 1997 TNM classification. The health status of the each patient (alive, death and death date) was noted or inquired by phone call, when required.

### Treatment Regimens

Seventy-one patients received gemcitabine plus cisplatin, 18 patients received vinorelbine plus cisplatin combination. Patients who received chemotherapy had a performance status was between 0 and 2 according to Eastern Cooperative Oncology Group with adequate renal, hepatic, and cardiac function. Treatment cycles were repeated every 21 days for a maximum of six cycles of combination therapy. Dose or interval modifications were carried out based on hematological and non-hematological toxicities. There were 11 patients with stage IA-IIIa disease. Eight of them were operated for curative intent. Four of them received neoadjuvant chemotherapy, 3 of them received adjuvant chemotherapy and four of them received chemotherapy for recurrent disease. According to tumor progression patterns each patient received a standard chemotherapy regimen or radiotherapy on an individual basis at the time of recurrence.

### Immunohistochemistry

Tumor consisting slices were examined. Appropriate slices for IHC staining were selected from the pathology laboratory archives. IHC analysis was performed to paraffin waxes of these selected specimens. Five micron thickness slices prepared from the paraffin blocks. After deparaffinisation, in the 10 mM, PH 6.0 citrate buffer solution 5 minutes in 500 watts, 5 minutes in 400 watts and 5 minutes in 350 watts heat respectively, totally 15 minutes the slices were boiled in microwave oven for antigenic retrieval. Specimens kept in the 3% hydrogen peroxide solution and endogenous peroxides removed. Five minutes ultra V block performed with TP-125-HU. HER2 antibodies (NeoMarkers, Clone e2-4001 + 3B5, Ms-730-R7, ready to use, USA) dropped on the slices and waited for 45 minutes. After 10 minutes wash with tris buffer saline (TBS), biotin (goat antipolyvalant) TP-125-HB applied and TBS wash performed again for 10 minutes. After 15 minutes streptavidin peroxide, slices were washed with TBS again. AEC chromogene (RTU lot: 065020) dropped. Slices closed after opposite staining with Mayer Hemotoxylene. In the light microscopy analyses brown-red coloration in the tumor cytoplasmic membranes considered as c-erbB-2 positive. Unstained membranes considered as negative (-), pale and partial membranous staining in less than 10% of tumor cells considered (1+), pale and complete staining in more than 10% of tumor cells (2+) and strong and complete staining in more than 10% of tumor cells (3+), respectively.

### Statistical Analysis

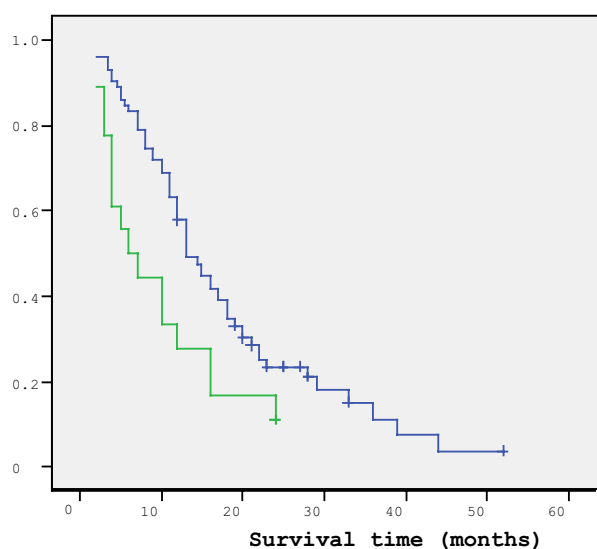
SPSS (Statistical Package for Social Sciences) version 15 used for the evaluation of the results. After descriptive statistical analyses, survival was estimated with Kaplan Meier Method. The differences between survival curves were analyzed according to Log-rank test. Chi-square test is used to investigate the differences between the proportions. The effects of histopathology, c-erbB-2 and stage of the disease on survival are investigated with Cox-Regression Model.  $p < 0.05$  is assumed as statistically meaningful.

## Results

The median follow-up was 14 months (range, 2.0-52 months). At the end of the follow-up, 75 patients (84%) were dead and 15 patients (16%) were still alive. During the follow-up, 6 bone, 3 brain and 1 surrenal gland metastases occurred.

C-erbB-2 expression was detected in 18 patients (20.2%). Two patients (2.2%) exhibited strong (+3) staining and 13 patients (14.6%) exhibited moderate staining (+2) and 3 patients (3.4%) showed weak staining (+1). Characteristics of C-erbB-2 positive patients were shown in Table 1. Degree of c-erbB-2 positivity was shown in Table 2. No difference was detected between adenocarcinoma and squamous carcinoma in terms of C-erbB-2 positivity ( $p=0.13$ ). There was no statistically difference between c-erbB-2 positivity and clinical stages ( $p=0.61$ ).

Median overall survival of patients with c-erbB-2 negative tumor was 13 months (95% CI: 10-15 months) and median survival of patients with c-erbB-2 positive tumor (95% CI: 2-10 months) was 6 months ( $p=0.02$ ). Survival curves are shown in Figure 1. Although the median survival of patients with squamous carcinoma was longer than those of other types, the difference was not statistical significant ( $p > 0.05$ ). There was no statistical difference in clinical staging according to histopathological type ( $p=0.22$ ) (Table 3).



**Figure 1. Cumulative Survival Curves for Patients with c-erbB2 Positive and Negative Tumors**

**Table 1. Immunohistochemical Staining for C-erbB-2 According to Clinical Characteristics**

Features	Total	Positive
Total	89	18 (20%)
Sex		
Male	84	16 (19%)
Female	5	2 (40%)
Stage		
Stage I-IIIa	11	2 (18%)
Stage IIIB-IV	78	16 (21%)
Histopathology		
Adenocarcinoma	45	7 (16%)
Squamous carcinoma	32	6 (19%)
NOS	12	5 (42%)

**Table 2. Degree of C-erbB-2 Positivity**

C-erbB-2	Number	C-erbB-2	Number
Negative	71 (79.8)	1 (+)	3 (3.4)
2 (++)	13 (14.6)	3 (+++)	2 (2.2)

**Table 3. Stages According to Histopathology**

Stage	Adenocarcinoma	Epidermoid carcinoma	NOS
I-IIIa	5 (11)	6 (19)	0 (0)
IIIB-IV	40 (89)	26 (81)	12 (100)
Total	45(100)	32(100)	12(100)

The effect of c-erbB-2 and clinical stage on the survival was independently statistically significant according to cox-regression analysis. Death risk of the patients with c-erbB-2 (+) were 1.96 fold higher than c-erbB-2 (-) patients (95% CI: 1.08-3.54) ( $p=0.026$ ). Death risk in advanced stage patients (stage IIIB-IV) were four fold higher than early stage patients (stage IA-IIIa) (95% CI: 1.6-10.3,  $p=0.001$ ). Histopathological types were not effecting the survival ( $p=0.29$ ).

## Discussion

C-erbB-2 positivity in NSCLC patients was shown at first by Kern et al (1994). Since then many investigators showed different rate of c-erbB-2 expression in NSCLC (Tateishi et al., 1991; Turken et al., 2003; Nakamura et al., 2005). The average percentage of c-erbB-2 overexpression in non-small cell lung carcinoma is 31% (ranges from 18%-55%) (Hirsch et al., 2002). In our study, by using immunohistochemistry c-erbB-2 overexpression was detected in 18 out of 89 tumors (20.2%). The difference in the results is probably caused by the different methods including flow cytometry, IHC and Western blot. Besides the methodologies, the cut-off points for c-erbB-2 positivity ranges 5% to 10% in different studies (Onn et al., 2004; Fijolek et al., 2006). In our study cut of point was 10%. Patients with c-erbB-2 1+ to 3+ positive by IHC staining were evaluated as c-erbB-2 positive. C-erbB-2 commonly shows a cytoplasmic and more or less pronounced membranous staining pattern, which may lead variability in the interpretation of c-erbB-2 expression (Kristiansen et al., 2001). Frequency of HER2 staining differs in subtypes of non-small cell lung cancer. In some studies, adenocarcinoma, shows much more positive staining with c-erbB-2 when compared to squamous or large cell carcinomas (Hirsch et al., 2002; Fijolek et al., 2006) However, in our study no difference was observed

between c-erbB-2 expression and tumor types.

The level of c-erbB-2 expression in pathological samples is commonly determined by IHC. Fluorescence in situ hybridization (FISH) is another method to evaluate c-erbB-2 expression. Chromosome duplication or gene amplification of c-erbB-2 is determined by FISH or polymerase chain reaction. Pauletti et al. showed that FISH is better in detection of c-erbB-2 overexpression in patients with breast cancer (Pauletti et al., 2000). In NSCLC the optimal technique for showing c-erbB-2 overexpression has not been determined yet. In NSCLC c-erbB-2 overexpression is more likely caused by gene amplification rather than chromosomal duplication (Hirsch et al., 2000). C-erbB-2 expression and relation with treatment outcome in locally advanced lung carcinoma were investigated by both methodologies. The c-erbB-2 -FISH results were marginally correlated with IHC results (Kuyuma et al., 2008). Only c-erbB-2- FISH, not c-erbB-2-IHC, result was determined as an independent poor prognostic factor for cisplatin-based chemotherapy and survival. In our study we used IHC to detect c-erbB-2 overexpression. FISH results are more useful for c-erbB-2 status in breast cancer however, until clarifying which method is the better the IHC seems to be the extensively available, easy and less expensive methodology for determining the HER2 expression in non-small cell lung cancer.

Trastuzumab, monoclonal antibody that binds to HER2, was originally developed for use against breast cancer. The major breast cancer trials of trastuzumab used  $\geq 2+$  staining by IHC as an inclusion criteria. In some phase II trials of trastuzumab enrolled lung cancer patients with 2+ or 3+c-erbB-2 expression however the others included patients whose tumor were c-erbB-2 positive 1+ to 3+ by IHC staining (Azoli et al., 2002). From the results of those trials whether and to what degree c-erbB-2 overexpression will be a prerequisite for the effective treatment of trastuzumab remains unclear.

Nakamura et al. published a metaanalysis to evaluate prognostic significance of c-erbB-2 overexpression in NSCLC. Among the 44 articles 24 studies were excluded from final analysis owing to lack of survival rate. Effects of c-erbB-2 expression on survival of patients with NSCLC were shown in remaining 20 studies. Among them, significant survival difference related to the c-erbB-2 overexpression was shown in 10 studies (Nakamura et al., 2005). However in rest of the studies no difference was observed. In our study we detected significant decrease in survival in patients with c-erbB-2 positive NSCLC. Also risk of death was increased 1.96 fold in patients with c-erbB-2 positive tumor.

Shi et al reported c-erbB-2 overexpression rate of 81% and 87% in stage II-III NSCLC however that of 50% in stage I (Shi et al., 1992). Turken et al. (Turken et al., 2003) found higher c-erbB-2 expression for higher stages (stage IIIB and IV). Very low rate of C-erbB-2 positivity (4%) was detected in another study including patients with in stage I NSCLC (Shi et al., 1992). However some other researchers found no correlation for c-erbB-2 expression with disease stage (Pfeiffer et al., 1996; Lopez-Guerrero et al., 1999; Reinmuth et al., 2000). In our study tumor

burden and c-erbB-2 was not correlated. Discrepancy between the results may be a result of different criteria for staining, staining techniques and clinicopathological characteristics of the tumors.

Up to date many studies were reported c-erbB-2 expression in NSCLC, owing to diversity in the results, prognostic implication of c-erbB-2 status has not been clearly determined. Our study showed c-erbB-2 overexpression was a negative prognostic factor for patient's survival regardless of tumor characteristics. IHC seems to be a commonly available methodology for detecting c-erbB-2 status. Any degree of positive IHC result for c-erbB-2 can be acceptable until the standard has been determined.

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