# Prevalence and prognostic value of c-erbB2 expression in non-small cell lung cancer (NSCLC)

O. Turken[1], E. Kunter[2], H. Cermik[3], T. Isitmangil[4], G. Kandemir[1], M. Yaylaci[1], A. Ozturk[1]

*[1]Department of Medical Oncology, e-mail: oturken@hotmail.com, [2]Department of Chest Diseases, and [4]Department of Thoracic Surgery, GATA Haydarpasa Training Hospital, 81020, Istanbul, Turkey; [3]Department of Pathology, GATA Camlica Chest Diseases Hospital, 81020, Istanbul, Turkey*

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The c-erbB2 oncoprotein is highly expressed in approximately one third of non-small cell lung cancer (NSCLC) patients. We determined the status of c-erbB2 expression in our patients with NSCLC and investigated its correlation with disease stage, histological type and response to treatment. Eighty-four patients were examined for the expression of c-erbB2 by immunohistochemistry using a polyclonal antibody. The scoring criteria of Clinical Trial Assay (CTA) were used to evaluate staining (0 to +3). c-erbB2 overexpression was determined in 35% of the cases. Tumors from higher stage disease (stage IIIb-IV) were more often c-erbB2 positive in adenocarcinoma (ADC) (p=0.04). In addition, there was an association between c-erbB2 score and disease stage in ADC patients (p=0.03). Our study did not demonstrate an association of c-erbB2 overexpression with response to chemotherapy. We conclude that c-erbB2 overexpression may be a prognostic marker for evaluating tumor progression in NSCLC patients but further studies must be performed with larger patient populations.

*Key words: Non-small, lung, cancer, c-erbB2.*

Lung cancer is among the most common malignancies in the world and the leading cause of cancer death from neoplastic diseases in the western world. In USA, in the year 2000, approximately 165 000 new cases were diagnosed and 160 000 patients died of the disease [6]. In developing countries, the death rate from lung cancer continues to accelerate. These changes may be due to the difference in smoking habits and cigarette tar levels in developed and developing countries [19].

Lung cancer cells have a number of molecular genetic lesions, which appear necessary to transform normal bronchial epithelium to an overt lung cancer. Of the three major classes of human cancer genes, the protooncogenes and tumour supressor genes are involved in lung carcinogenesis, whereas evidence implicating DNA repair genes is not yet conclusive. The oncoprotein c-erbB2, a 185 kd protein with tyrosine kinase activity belonging to the epidermal growth factor receptor (EGFR) family, is overexpressed in a number of human carcinomas such as breast, ovary, colon, stomach, testis and lung cancer [11,24]. c-erbB2 is expressed in approximately one third of NSCLC cases, especially in adenocarcinomas (ADC). The importance of its expression for the prognosis in NSCLC is still controversial [5, 10,20,21].

The aim of our study was to determine the status of c-erbB2 expression in our patients with NSCLC and to investigate its correlation with disease stage, histological type and response to chemotherapy.

## Material and methods

Study population consisted of 84 patients with a median age of 61 (range: 47 to 72). The histopathological diagnosis was established with bronchoscopic biopsy or surgical resection in each case according to the World Health Organisation (WHO) guidelines [30]. The clinical staging was done according to TNM criteria of the UICC [17]. Table 1 summarizes the clinicopathological characteristics of our cases. Of 84 patients, 30 patients with a clinical staging less than stage IIIb underwent thoracotomy for resection of NSCLC

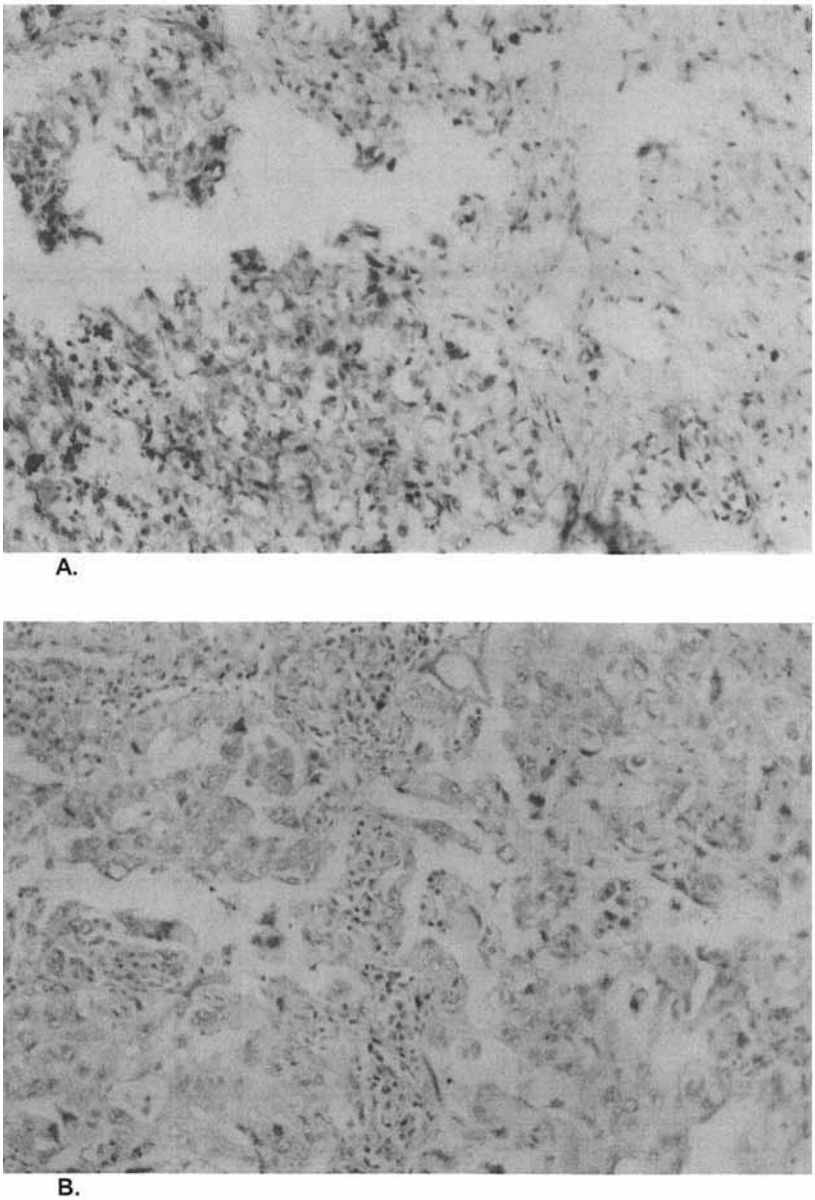


**Figure 1. Examples of c-erbB2 expression immunohistochemically stained by the polyclonal antibody, moderate (A) and strong (B) staining.**

in the department of thoracic surgery. The remaining 54 patients with stage IIIb and stage IV received chemotherapy. c-erbB2 protein overexpression was documented by immunohistochemistry.

*Immunohistochemistry*. Formalin fixed, paraffin embedded blocks from the archive material of the department of pathology were cut (4 $\mu$m), sections were mounted on superfrost slides (Menzel-Glaser, Germany), dewaxed with xylene and hydrated. Antigen retrieval was achieved by pressure cooking in 0.01 M citrate buffer for 18 minutes (min). We used a polyclonal c-erbB2 antibody (DAKO, Germany), which was diluted 1:75 using a background reducing dilution buffer (DAKO). The polyclonal antibody was incubated at room temperature for 15 min. By the conventional labelled-streptavidin-biotin (LSAP kit, DAKO) method with hydrogen peroxide as the reporting enzyme, detection was done. DAB chromogen (DAKO, Germany) was used as the chromogen and then the slides were briefly counterstained with Mayer's haematoxylin and aquaeously mounted. c-erbB2 staining was evaluated according to the Clinical Trial Assay (CTA) criteria, which are the basis for commercial Hercept-test (DAKO) (Fig. 1).

*Chemotherapy*. Fifty-four patients received a chemotherapy regimen of cisplatin 70 to 80 mg/m$^2$ on day 1, etoposide 100 to 120 mg/m$^2$ on days 1, 2 and 3. Patients who received chemotherapy had a Karnofsky performance status of 60 or higher with adequate renal, hepatic, hematologic and cardiac function. Treatment cycles were repeated every 21 days for a maximum of six cycles. Response evaluations were completed after every three cycles of combination chemotherapy. Dose modifications were based on complete blood counts and non-hematological toxicities.

*Statistics*. To compare the c-erbB2 expression with clinicopathological parameters, Fisher's exact test was used and a p value 0.05 was considered significant.

## Results

c-erbB2 overexpression was detected in 30 patients (35%). Twenty-two patients (15%) exhibited a moderate staining (+2) and 8 patients strong staining (+3) with immunohistochemistry. There was no correlation between c-erbB2 overexpression and clinicopathological parameters like tumour size (T), grade (G) and histology (Tab. 1). When the patients were separated according to the stage of the disease (stage I–IIIa vs stage IIIb–IV), we found that the tumors from higher stage (IIIb–IV) disease were more often c-erbB2 positive, particularly in ADC cases (p=0.04) (Tab. 1). Additionally, it seemed that there was an association between c-erbB2 score and disease stage, particularly in ADC patients when c-erbB2 score compared with stage of the disease (p=0.03) (Tab. 2).

Of 54 patients to whom chemotherapy were given, 50

**Table 1. c-erbB-2 overexpression according to clinicopathological parameters**

| | c-erbB-2 score | |
|---|---|---|
| | 0–1 | 2–3 |
| Total number (n=84) | 54 (65%) | 30 (35%) |
| Adenocarcinoma (n=30) | 16 (53%) | 14 (47%) |
| SCC (n=54) | 34 (64%) | 20 (36%) |
| Grade I–II | 31 | 19 |
| Grade III–IV | 23 | 11 |
| Stage I–IIIa | 22 (68%) | 10 (32%) |
| Stage IIIb–IV | 28 (53%) | 24 (47%) |
| pT1–2 | 24 | 14 |
| pT3–4 | 30 | 16 |

**Table 2. c-erbB2 score according to disease-stage**

| | Adeno Ca (n=30) | | | SCC (n=54) | | |
|---|---|---|---|---|---|---|
| Stage | I–IIIa | Stage | IIIb–IV | Stage | I–IIIa | Stage IIIb–IV |
| 0–1 | 8 | 8 | | 14 | | 20 |
| 2 | 2 | 6 | | 8 | | 8 |
| 3 | – | 6 | | – | | 4 |

**Table 3. c-erbB2 expression in patients who received chemotherapy**

| | c-erbB2 low (0–1) n(%) | c-erbB2 high (2–3) n(%) |
|---|---|---|
| Partial response (PR) | 8 (25) | 4 (22) |
| Stable disease (SD) | 20 (62) | 12 (66) |
| Progressive disease (PD) | 4 (13) | 2 (12) |

**Table 4. Studies on c-erbB-2 expression in NSCLC**

| Author | No of cases | c-erbB2 expression (%) |
|---|---|---|
| Schneider | 103 | 54 |
| Tateishi | 203 | 17 |
| Volm | 81 | 35 |
| Shi | 120 | 59 |
| Harpole | 150 | 13 |
| Pfeiffer | 186 | 26 |
| Pastorino | 608 | 4 |
| Hsieh | 42 | 50 |
| Kristiansen | 89 | 37 |
| Graziano | 132 | 65 |

patients was eligible for assessing the response. Of these, 18 patients were c-erbB2 positive and 32 patients negative. In general, treatment has been well tolerated. Dose modification because of toxicity was necessary in 4 patients. Two patients experienced grade 4 neutropenia and other 2 patients experienced both neutropenia and thrombocytopenia. A partial remission was obtained in 12 patients (24%). Thirty-two patients had stable disease (64%). In 6 patients (12%), the disease was progressive. Among the patients with c-erbB2 overexpressing tumours, the response rate was 22% (4/18). Eight c-erbB2 negative patients (25%) responded to therapy (8/32) (Tab. 3). No association was observed between c-erbB2 positivity and response to treatment (p=0.94).

## Discussion

Expression of c-erbB2 varies from low to high levels in NSCLC cell lines [18]. In literature published so far, c-erbB2 expression was reported 4–71% in clinical studies [2, 10, 17, 18, 20, 26, 30, 35]. These varying results may be due to different patient populations, tumor set, technical methods used for c-erbB2 staining and different approaches in the interpretation of the staining. c-erbB2 may show both cytoplasmic and membranous staining patterns. Both have been used in various studies. Pastorino and colleaques used the membranous staining only for the assesment of c-erbB2 expression. Their results were the lowest c-erbB2 expression in literature [20]. It may be partially due to their tumor set which was restricted to stage-I NSCLC. Researchers like Hirashima et al [9] and Cox et al [3] demonstrated that c-erbB2 gene amplification, which controls the overexpression of the protein in the cell membrane, occurs in the homogenously membranous staining regions. Giatromannolaki and colleaques found the membranous staining impossible to evaluate because of invariable cytoplasmic background staining [5]. Using a polyclonal antibody (DAKO), we examined the expression of c-erbB2 in NSCLCs and applied the scoring recommendations of the CTA for evaluating the c-erbB2 expression in our study: circumferential membranous staining in at least 10% or more of the tumor cells was regarded positive and then semiquantitatively scored as minimal (+1), moderate (+2) and strong (+3) staining. Comparing with the older literature, our results may be considered as a high incidence of c-erbB2 positivity in this patient population (Tab. 4).

Suggestion that c-erbB2 expression is associated with the histological type is controversial. Although an association has been found between c-erbB2 expression and histological type by some researchers [10, 20, 21], others could not demonstrate this relationship [3, 13]. Our data shows no association of c-erbB2 overexpressing tumors with histologic type (ADC 33% vs SCC 36%). Tateishi et al found an overexpression in 28% of ADC and 2% of SCC [28]. Pfeiffer et al also showed a high expression of c-erbB2 in 30% of ADC and 14% for SCC [21]. However, in another study by Kristiansen and colleagues, the rates of c-erbB2 expression for ADC and SCC were similar with 36.9% and 37.2%, respectively [13].

Our study demonstrated a correlation of c-erbB2 expression with clinical stage. We found that the tumors from high-

er stage disease (stage IIIb-IV) were more often c-erbB2 positive, particularly in ADC cases (p=0.04). Shi and colleaques reported an overexpression of c-erbB2 in 81% and 87% of stage II-III cases, while stage I cases had an overexpression rate of 50% [27]. Kristiansen and colleaques showed a significant association of c-erbB2 overexpressing tumors with a higher clinical stage [13]. Cox et al also demonstrated that tumors of higher stage were more often Hercep Test positive [3]. But some other researchers found no correlation for c-erbB2 expression with disease stage [14, 21, 22, 32]. It is difficult to explain these discrepancies, they may be due to differences in technical methods used for assessing c-erbB2 positivity and tumour set.

It has been shown that expression of the c-erbB2 protein is associated with an elevated mitotic rate and correlates with poor clinical response to certain chemotherapeutic and antihormonal drugs (5-fluorouracyl, methotrexate, cytoxan and tamoxifen containing regimens) in breast cancer patients [34]. But it has been reported in a Cancer and Leukemia Group B (CALGB) study that dose-intensive chemotherapy including doxorubicine could overcome this chemoresistance [29]. Increased expression of c-erbB2 protein may confer relative chemotherapy resistance and shortened survival in NSCLC patients. Overexpression of c-erbB2 has been reported to affect the sensitivity of certain human cancer cells to cisplatin, presumably by modification of DNA repair activity through interfence with nucleotideexcision repair (NER) mechanism [31]. Clinical trials are currently in progress to explore the role of c-erbB2 on the activity of chemotherapeutic agents like cisplatin. Graziano and colleagues demonstrated that expression of c-erbB2 were not predictive of response to chemotherapy, combined chemotherapy/radiation or for survival in patients with stage-III NSCLC [7]. Our study did not demonstrate an association of c-erbB2 overexpression with response to chemotherapy (p=0.94). In recent years, new strategies have been designed to direct some drugs and therapeutic agents such as monoclonal antibodies (Herceptin) and c-erbB2 specific toxins to c-erbB2 receptors on the tumor cells [1, 4, 7, 8, 12, 15, 16, 23, 33]. Although c-erbB2 protein was found to affect the sensitivity of tumor cells to cisplatin and monoclonal antibodies and c-erbB2 was also shown to have additive and synergistic effect with some chemotherapeutic agents in preclinical studies with tumor cell lines, randomized clinical trials with larger patient numbers are needed. If the prognostic effect of c-erbB2 is confirmed with such trials, this may contribute to the identification of new strategies for treatment of NSCLC.

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