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The effect of EGFR overexpression in inoperable non-small cell lung cancer (NSCLC) patients receiving cisplatin-vinorelbine combination

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ABSTRACT

The epidermal growth factor receptor (EGFR; Her1, erb B1) is overexpressed in many tumors including NSCLC and has been associated with poorer outcome. We included 39 newly diagnosed patients with locally advanced and metastatic NSCLC in the period from January 2001 to April 2002. EGFR protein in serum was analyzed in all patients with a cut off value of 180 fmol/ml. All patients received cisplatinum (75 mg/m^2) on day 1 and vinorelbine (25 mg/m^2) on day 1 and day 8 every 3 weeks for a maximum of six cycles until progression or refusal of treatment. Sixteen (41.0%) showed a level below 180 fmol/ml (no overexpression) and were assigned to ARM 1 and 23 patients (59.0%) showed a level higher than the cut-off value (overexpression) and were assigned to ARM 2. The overall response rate to chemotherapy was 53.8%. Median time to disease progression was 3.5 months. The response rate was 62.5% for ARM 1 versus 47.8% for ARM 2 (p=0.31). Median time to disease progression was 7.8 months in ARM 1 versus 3.9 months in ARM 2 (p=0.039). The median survival was 15 months in ARM 1 versus 10 months in ARM 2 (p >0.05). Results showed that 59% of the cases showed overexpression for EGFR protein. This overexpression can be a poor prognostic factor in NSCLC and those cases can be candidates for newly developed targeted therapy. [Turk J Cancer 2006;36(1):11-18].

KEY WORDS:

EGFR protein, NSCLC, chemotherapy, prognosis, vinorelbine, bronchogenic carcinoma

INTRODUCTION

Approximately 18% of all cancer related deaths are due to lung cancer (1). Non-small cell lung cancer (NSCLC) exhibits a variable clinical phenotype, even in those patients with an apparently limited disease who undergo surgical resection.

Numerous reports suggested that a variety of tumor cell biological markers predict survival (2). The Epidermal growth factor receptor (EGFR; Her1; erb B1) is highly expressed in NSCLC and associated with advanced tumor stage, resistance to standard therapies and poor patient prognosis and can be considered as a predictive biological marker for survival (3-5). Activation of the EGFR signaling pathway has many effects including increased proliferation and angiogenesis and decreased apoptosis. These effects are mediated by a complex series of signaling mechanisms, such as engagement of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K) pathways (6).

No method of analysis of EGFR is consistently employed in all laboratories, making the comparison of results of different studies difficult. Methods include evaluation of EGFR at the DNA, RNA and protein levels as well as at the level of receptor activation in situ (7). EGFR protein may be quantitated by western analysis which measures

the total receptor protein in tumor specimens, regardless of the expressing cell type and cellular localization of the receptor (8).

Enzyme immunoassay (EIA) has been used also to analyze the shedded extracellular domain of EGFR into the circulation that is proportional to the intensity of the expressed receptor protein on the cell membrane. EIA - being a noninvasive technique- can be practiced whenever biopsy samples are difficult to obtain (9,10).

Among NSCLC, squamous cell carcinoma was found to be the most frequent subtype overexpressing EGFR (p=0.0121) and an absence of EGFR expression was correlated with a longer survival (p=0.024) (11). This finding was further confirmed in other trials (12).

Chemotherapy for advanced NSCLC used to be considered ineffective or excessively toxic. However, meta-analyses have demonstrated that chemotherapy results in a small, but sensible improvement in survival in patients with advanced disease in comparison to best supportive care (13-15).

During the last decade, several new chemotherapy agents with activity in NSCLC have become available including taxanes, vinorelbine, gemcitabine, topoisomerase inhibitors and pemetrexed.

Vinorelbine in combination with cisplatinum was the first novel agent regimen to produce a statistically significant survival benefit over vindesin/cisplatinum, a combination that was standard at the time (16). The efficacy of this combination in the advanced setting was further explored and confirmed by the Southwest Oncology Group (SWOG) NSCLC to become the new SWOG standard regimen (17). We have conducted the current study to evaluate the response to vinorelbine/cisplatinum regimen in Egyptian patients with locally advanced and metastatic NSCLC and to investigate the impact of EGFR overexpression on response and survival by the use of this regimen.

MATERIALS AND METHODS

In the period from January 2001 to April 2002, thirty-nine patients with locally advanced (stage IIIB) and metastatic (stage IV) NSCLC were enrolled. All patients were chemonaive and signed an informed consent prior to starting treatment. Duration of treatment was based on tumor response. Assessment was carried out every two cycles. Patients with complete/partial response or stable disease continued until disease progression, unacceptable toxicity, or refusal for a maximum of 6 cycles. Blood samples were obtained from the patients on the day of enrollment for EIA assay of serum EGFR protein. The control normal level for EGFR protein was determined during two previous studies on breast and lung cancers where serum samples from 80 healthy individuals were analyzed for EGFR protein (her-1) and it was found to be between 7-162 fmol/ml with a mean value ± 2 SD of 103±76 fmol/ml (9,18). In our study, using a cut-off level of 180 fmol/ml, patients were divided into two arms; Arm 1 for patients with levels lower than 180 fmol/ml (no overexpression) and Arm 2 for levels higher than 180 fmol/ml (overexpression).

Inclusion Criteria

Patients with histologically or cytologically confirmed NSCLC and with stage IV or selected stage IIIB disease were eligible. Stage IIIB patients had to have a positive pleural effusion or multiple ipsilateral lung nodules.

Other eligibility criteria included the following: bidimensionally measurable or assessable disease, age between 18-70 years old, performance status (PS) less than 3 according to ECOG scale, neutrophil count more than or equal to $1.5x10^6$/L, platelet count more than or equal to 10^8/L, hemoglobin more than or equal to 90 g/L, serum creatinine less than or equal to 1.5 mg/dl or a calculated creatinine clearance more than or equal to 60 ml/min, bilirubin level less than or equal to 2.0 mg/dl, AST less than or equal to twice the institutional upper limit of normal, or less than or equal to four times the institutional upper limit of normal if the patient had liver metastases. Patients were not previously treated with chemotherapy or radiotherapy. All patients gave a written informed consent.

Exclusion Criteria

Patients with brain metastasis, poor performance status (ECOG scale more than 2), grade II peripheral neuropathy or more and pregnant women were all excluded.

Dosage

All patients were started on vinorelbine/cisplatinum combination. Vinorelbine was given 25 mg/m^2 on days 1&8 while cisplatinum was given 75 mg/m^2 on day1. Treatment was given every three weeks provided hemato-

logical recovery. Dose reduction by 25% was done if grade III or IV toxicity was encountered.

Study Assessment

The pretreatment assessment included history, complete physical examination and assessment of performance status according to ECOG scale.

Laboratory investigations included complete blood count (CBC), liver function tests (LFTs), and kidney function tests (KFTs), serum electrolytes and serum EGFR by EIA. Radiological assessment included computed tomography (CT) of the chest and abdominal ultrasound. Cranial CT was done if clinically indicated. Bone scan was performed in cases of bone pain, clinical suspicion or elevated serum alkaline phosphatase level.

Prior to each cycle, complete physical examination, CBC, LFTs and KFTs were routinely performed. Evaluation was carried out every two cycles for a maximum of six cycles then on three monthly bases after the end of treatment.

Evaluation of Response and Toxicity

In patients with measurable disease, the maximum perpendicular diameters for each lesion were recorded. These diameters were then multiplied to estimate the cross sectional area and summed if more than one lesion was present.

Complete response (CR) was defined as the disappearance of all known disease, confirmed by subsequent measurements at least 28 days apart. Partial response (PR) was defined as a decrease in the sum of product of the perpendicular diameters of all lesions by 50% confirmed by subsequent measurements at least 28 days apart. Progressive disease (PD) was defined by ≥25% increase in the cross sectional area of one or more lesions or the appearance of new lesions. All other outcome was classified as stable disease (SD). Toxicity grading was assessed according to the National Cancer Institute common toxicity criteria (NCI-CTC) (19). Criteria for removal of patients from the trial included disease progression, unacceptable toxicity or patient refusal.

Statistical Analysis

Primary end-points were time to disease progression (TTP) and survival in relation to EGFR status. Secondary end-points were objective response rate (RR) and safety of the chemotherapy combination. Statistical analysis was done using SPSS (Statistical Package for Social Sciences) program. Descriptive statistics was presented as mean±2 standard deviation, and number and percentage (frequency distribution). Comparisons were made by using student's t-test and the chi- square test. Survival and TTP were calculated according to WHO criteria and were recorded in months.

Kaplan–Meier survival curves were used and plotted for EGFR positive and negative patients and the corresponding log-rank tests were performed on the null hypothesis that the risk of death is the same for each factor. Significance level of 0.05 was used through all statistical tests within the study.

Table 1
Patients' characteristics

	n	%
Age		
Mean ± SD	54.3 (±12.9)	
Median	55.5	
Range	26-70 years	
95% CI	49.2-59.6	
Sex		
Male	33	84.6
Female	6	15.4
Performance Status		
0-1	23	58.9
2	16	41.1
Smoking		
No	9	23.1
Yes	30	76.9
Stage		
III	21	53.8
IV	18	46.2
Pathology		
Squamous cell carcinoma	24	61.5
Adenocarcinoma	12	30.7
Others	3	7.8
Grade		
II	19	48.7
III&IV	20	51.3

RESULTS

Patient Characteristics

In the period from January 2001 to April 2002, thirty-nine patients with locally advanced (stage IIIB) and metastatic (stage IV) NSCLC were enrolled. All patients had measurable or assessable disease. Of these, 18 patients (46%) were stage IV and 21 patients (53.9%) were stage IIIB. Their age ranged from 26-70 years with a median age of 55.5 years. Squamous cell carcinoma was the most common pathological subtype, encountered in 24/39 (61.5%) followed by adenocarcinoma 12/39 (30.7%). Patients received a median of 4 cycles (range 3 - 6) of vinorelbine/cisplatinum combination. The demographic data and assay characteristics of the whole group are summarized in table 1.

Out of 39 patients, 23 (58.9%) showed increased serum EGFR protein by EIA more than 180 fmol/ml (Arm 2) and 16 (41.0%) showed a level below the cut-off value (Arm 1).

Both arms were comparable concerning ECOG performance status, disease stage, pathological subtype and pathological grade (Table 2).

Response to Treatment

Response rate was 53.8% (21/39) with two patients achieving CR (both in Arm 1) and nineteen patients achieving PR. The RR was 10/16 (62.5%) in Arm 1 and 11/23 (47.8%) in Arm 2. There was no statistically significant difference between the groups (Table 3). An additional 7.7% of patients achieved SD.

Time to disease progression for the whole group was 5.5 months. Patients in Arm 1 showed statistically significant prolonged TTP with a median of 7.8 months compared to 3.9 months in Arm 2 (p=0.039)

The median survival time for the whole group was 12 months and the one-year overall survival (OS) was 50% (Figure 1). There was no statistically significant difference between the two groups in terms of median survival, 15 months for Arm 1 and 10 months for Arm 2. However, the OS at the end of the trial (21 months) showed statistically significant difference in favor of Arm 1 (33%) versus (0%) in Arm 2 (Figure 2).

Table 2
Patient characteristics with comparison between two arms

	Arm 1 (n=16)		Arm 2 (n=23)		P
	n	%	n	%	
ECOG PS					
I	9	56%	14	60%	0.78
II	7	44%	9	40%	
Stage					
IIIB	6	37.5%	15	65%	0.126
IV	10	62.5%	8	35%	
Pathological subtypes					
SCC	9	57.9%	15	65%	0.52
AC	5	31.3%	7	30.5%	
UC	2	10.8%	1	4.5%	
Pathological grade					
II	10	62.5%	9	39.1%	0.112
III-IV	6	37.5%	14	60.9%	

SCC: Squamous Cell Carcinoma, AC: Adeno-Carcinoma, UC: Undifferentiated Carcinoma

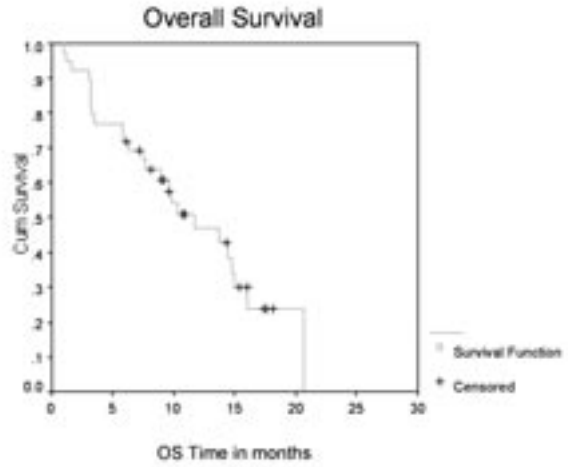

Fig 1. Overall survival of the whole group

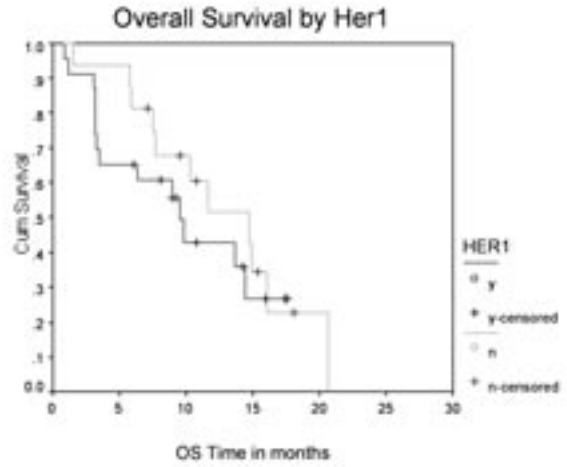

Fig 2. Overall survival in relation to EGFR status

Table 3
Response rate in relation to EGFR expression

Response	Arm 1 (n=16)			Arm 2 (n=23)	
	n	%		n	%
RR	10/16	62.5		11/23	47.8
CR	2	12.5		-	-
PR	8	50		11	47.8
SD	1	6.2		2	8.6
PD	5	31.3		10	43.6
P-value			0.31		

Table 4
Treatment related adverse events

Adverse Events	Total		Grade I / Grade II		Grade III / Grade IV	
	n	%	n	%	n	%
Leukopenia	17	43.5	15	38.5	2	5
Neutropenia	12	30.7	10	25.7	2	5
Anemia	10	25.6	10	25.6	-	-
Neuropathy	6	15.4	6	15.4	-	-
Vomiting	8	20.5	8	20.5	-	-
Diarrhea	6	15.4	6	15.4	-	-
Fatigue	5	12.8	5	12.8	-	-
Elevated liver enzymes	3	7.7	3	7.7	-	-

Treatment Related Toxicities

Treatment was well tolerated. The most frequently reported adverse events were leukopenia (43.5%), anemia (25.6%), vomiting (20.5%) and neuropathy (15.4%) (Table 4).

All treatment related toxicities were grade I and grade II (according to NCI CTC). Grade III and IV toxicities were encountered in two patients with neutropenia; one of them showed neutropenic fever. No treatment-related death was recorded (Table 4).

DISCUSSION

It was reported that among 169,400 new cases of lung cancer diagnosed in 2002 in USA, approximately 154,900 deaths were recorded (20). Unfortunately, only 14% of all lung cancer patients (all stages) will be alive for five or more years after diagnosis (21). Over 70% of lung cancer patients, when diagnosed, have locally advanced or metastatic disease. This situation implies an obvious need for systemic chemotherapy since the majority of NSCLC tumors are inoperable at diagnosis (20).

Our study showed that vinorelbine/cisplatinum combination is an active combination with an overall RR of 54%. The RR achieved in the current study was higher than those reported in other clinical trials which investigated platinum based combination chemotherapy for NSCLC & ranged from 20 to 41% (22-25). This higher RR may be explained by the higher percentage of stage IIIB patients include in this study in comparison to other trials. Yet the role of biological factors that can modulate the response to certain therapies should not be neglected.

The median TTP for the whole group was 5.5 months higher than that reported in the ECOG and SWOG trials which was 4 months for both (22,23). The median OS was 12 months and the one year survival was 50%, also longer than that reported by most of the trials which included a third generation chemotherapy (gemcitabine, taxanes and vinorelbine) in combination with cisplatinum which resulted in a survival rate of 33% at one year (20, 22-24).

The adverse events associated with the dosing schedule of vinorelbine/cisplatinum combination used were predictable and controllable, with no toxic deaths. The most frequently reported were leukopenia (43.5%) and anemia (25.6%) which were comparable to that reported in the ECOG and SWOG trials and less than that reported by Sandler et al. (22-24). Non-hematological adverse events included GIT toxicity and neurotoxicity, which were mostly grade I and II toxicities and were comparable to other trials.

EGFR overexpression was encountered in 59% of patients enrolled in the trial, a finding that is consistent with other reports in similar settings (3-5).

In our study, overexpression of EGFR was not found to be associated with advanced stage of disease, pathological subtype, pathological grade or poor performance.

In 1998, Volm et al. (12) reported that patients with squamous cell carcinoma of the lung overexpress EGFR protein more frequently than other subtypes (p=0.01) and such patients suffer a more advanced disease with a poor performance status. This was confirmed a couple of years later by Ohsaki et al. (11). However, our trial failed to further strengthen this finding. It was also found that patients with normal serum levels of EGFR showed higher RR than patients with over-expressed EGFR, the difference was however statistically insignificant unlike that reported by Salomon et al. (3), and Ohsaki et al. (11). It might be related to the small number of patients enrolled in our trial.

The median TTP was significantly longer in patients with Arm 1 versus Arm 2 while the median survival time was not significantly prolonged. The long-term survival (21 months) was significantly longer in Arm 1 compared to Arm 2. These results point to the role of EGFR overexpression as a potential prognostic factor in NSCLC patients and confirm the data previously reported by Ohsaki et al. (11) and Volm et al. (12) correlating EGFR expression and survival.

The overexpression of EGFR has been linked with signaling pathways and has many effects including increased proliferation, angiogenesis, and decreased apoptosis explaining the poor treatment outcome in these patients(6).

Since EGFR is often found in NSCLC cells, it has been the focus of efforts to develop new agents that target the EGFR pathway. Erlotinib and gefitinib inhibit the tyrosine kinase activity of EGFR and have been studied extensively.

In a phase III, multicenter, randomized controlled trial, erlotinib as monotherapy was used for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen. Survival of erlotinib-treated patients was superior to that of placebo-treated patients. The median survival duration of erlotinib-treated patients was 6.67 months, compared with 4.70 months for placebo-treated patients ($p < 0.001$). Erlotinib was also superior to placebo for progression-free survival and a RR of 8.9% versus 0.9% (26).

Both erlotinib and gefitinib received registration approval by the U.S. Food and Drug Administration (FDA) for the second- and third-line treatment of non-small cell lung cancer (NSCLC), but the failure of gefitinib to show a survival advantage over placebo has resulted in a discussion about the registration of gefitinib (27).

CONCLUSION

Our results showed that serum EGFR protein is overexpressed in 59% of Egyptian patients with locally advanced and metastatic NSCLC and its overexpression proved to be a potential bad prognostic factor. Vinorelbine/cisplatinum combination showed to be an active combination in treating Egyptian patients with NSCLC. EGFR-targeted therapy might be considered in cases with EGFR overexpression. Two molecules (gefitinib and erlotinib) have been recently approved to target and inhibit the tyrosine kinase enzyme, which is the protein product of the EGFR gene. By interfering with cell signaling pathways involved in cell proliferation, inhibition of EGFR-associated tyrosine kinase represents a novel approach to the treatment of NSCLC. Many trials are ongoing to couple targeted therapy to chemotherapy whether on concomitant or sequential bases to pave the way for a better chance of survival for this subset of patients.

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