

Clinical Trials

Treatment of NSCLC Patients with an EGF-Based Cancer Vaccine

Report of a Phase I Trial

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KEY WORDS

epidermal growth factor, epidermal growth factor receptor, EGF vaccine, non-small cell lung cancer, immunotherapy, clinical trial

ABBREVIATIONS

ATP	adenosine triphosphate
ASST	aspartate amino transferase
CXCR4	chemokine receptor 4
CT	computer tomography
ELISA	enzyme linked immunosorbent assay
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
GAR	good antibody response
GMP	good manufacturing practices
NSCLC	non-small cell lung cancer
PAR	poor antibody response
TGFβ	transforming growth factor β
WHO	world health organization

ABSTRACT

Epidermal Growth Factor (EGF) promotes tumor cell proliferation and survival upon binding to its receptor. We have developed a new active specific immunotherapy based on EGF deprivation. In the present paper, we show the results of a Phase I trial in 43 patients with advanced non-small cell lung cancer (NSCLC) who received the EGF vaccine. Patients who had already received first line therapy were randomized to receive a single or double dose of the EGF vaccine, weekly for four weeks and monthly thereafter. No significant toxicity was seen after vaccination. Adverse events consisted primarily of fever, chills, nausea, vomiting and flushing. Fifteen patients (39%) developed a good antibody response (GAR) against EGF. The geometric mean of the antibody titer was higher in the double dose group. EGF concentration was quantified in serum. An inverse correlation between anti-EGF antibody titers and EGF concentration was seen after immunization. Vaccinated patients achieved median survival times of 8.23 months from randomization. Patients who received the double dose of treatment showed a trend toward increased survival in comparison with patients who received the single dose. GAR and patients in whom the serum EGF decreased below the 168 pg/ml cut-off point had a significantly better survival when compared to poor responders or patients in which the EGF levels were not considerably reduced. Our results confirm the immunogenicity of the EGF vaccine in the treatment of patients with advanced stage NSCLC. Antibody titers and serum EGF levels appear to correlate with patient survival.

INTRODUCTION

Lung cancer is a leading cause of cancer death worldwide. The vast majority of patients present with unresectable disease and chemotherapies provide a small survival benefit compared with supportive care.¹

Approximately 75–85% of non-small cell lung cancers (NSCLC) overexpress the Epidermal Growth Factor Receptor (EGFR) and its ligands, i.e., Epidermal Growth Factor (EGF) and Transforming Growth Factor α (TGF- α).² Overexpression of EGFR has been implicated in the process of malignant transformation by promoting cell proliferation, cell survival and motility.³

EGF is a potent growth factor that is believed to enhance the proliferation of cancer cells by both paracrine and autocrine mechanisms. EGF transduces signalling through EGFR upon binding to the cell surface (receptor) ultimately resulting in the stimulation of cell proliferation.⁴ EGF is expressed as a high molecular weight membrane-associated precursor glycoprotein at the cell surface, with eight additional EGF-like repeats.⁵ It has been proposed that shedding of the ectodomain of the EGFR ligands is the way cells regulate the equilibrium between cell-associated and diffusible forms of these growth factors.⁵

In the 1960's, Stanley Cohen identified the mature EGF molecule from mouse submaxillary gland extract as a stimulator of eyelid opening and incisor eruption.⁶ Subsequently, EGF has been implicated in a number of developmental events including palate and skin differentiation, growth of hair follicles, lung maturation, gut and liver growth, neuronal differentiation and tumour development.⁶ Particularly, for normal human bronchial epithelial cells and lung cancer cells, EGF is a key growth factor. Previously, Ehrhardt et al. proposed that the c-myc oncogene and EGF were directly related and cooperated with one another during formation of bronchioloalveolar adenocarcinomas in the lung.⁷

Moreover, in NSCLC that expresses a particularly aggressive metastatic phenotype, EGF has been implicated in the process of invasion and metastasis. It has been described that EGF is capable of stimulating the conversion of matrix-metalloproteinase 9 (MMP-9) from a latent to an active form in human NSCLC cell lines.⁸

Recently, Philips et al. demonstrated that activation of the EGFR by EGF increases Chemokine (C-X-C) receptor 4 (CXCR4) expression and the migratory capacity of NSCLC cells.⁹ CXCR4 is a cell surface receptor that mediates the metastasis of many solid tumors including lung, breast, kidney and prostate.⁹

In the clinical setting, simultaneous expression of EGFR and its ligands in lung tumors and adjacent tissues is associated with lower recurrence rate and overall survival in patients.¹⁰

Several strategies have been developed to disrupt the EGFR associated signal transduction cascade in cancer patients.³ Therapeutic approaches include monoclonal antibodies against the extracellular domain of the receptor and small molecule tyrosine kinase inhibitors, targeting the Adenosine Triphosphate (ATP) binding site of the EGFR intracellular protein.³ Our approach consists of specific active immunotherapy intended to induce an immune response against self-produced EGF.¹¹

The EGF vaccine consists of human recombinant EGF, coupled to a recombinant carrier protein, P64 from the meningitis B bacteria. The vaccine has been adjuvanted with Alum or Montanide to increase the vaccine immunogenicity.¹¹ Since 1998, several pilot trials have been completed to optimize the vaccine formulation in terms of treatment schedule, carrier proteins and adjuvants.^{11,12} Results from these studies have demonstrated that vaccination with EGF is immunogenic and appears to be well-tolerated. Furthermore, those who developed a “good antibody response” appeared to have significantly better survival rates compared with patients who had lesser anti-EGF antibody responses.¹²

Here, we report the results of a Phase I clinical trial designed to evaluate safety, immunogenicity and the impact on serum EGF concentration on single (one 71 µg injection) or double (two 71 µg injections) intramuscular doses of the EGF vaccine adjuvanted with Alum.

MATERIAL AND METHODS

Eligibility criteria. Patients were required to have histologically or cytologically confirmed Stage IIIB or IV NSCLC and have completed first line oncologic therapy at least four weeks prior to participation.

Other eligibility criteria were: age greater than 18 years, a life expectancy of 24 weeks or greater and adequate bone marrow and organ function defined as an absolute neutrophil count $\geq 1,500/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$, creatinine ≤ 1.5 mg/dL, bilirubin ≤ 1.5 times the institutional upper normal limit and AST ≤ 2.5 times the institutional upper normal limit. Patients with known brain metastases were ineligible. EGFR overexpression in primary tumors was not mandatory for enrollment. Approval of the studies by the Institutional Review Boards of the two participating centers was required. The protocol was also approved by the national regulatory authority. All patients provided informed consent before inclusion in the study.

Patient evaluations. Evaluations included physical examination, hematologic and biochemical profile, chest radiography and computed tomography (CT). Laboratory tests were performed every four weeks and CT scans were repeated every three months. Toxicity was graded according to the WHO standard toxicity scale.

EGF vaccine. The EGF vaccine is composed of human recombinant EGF chemically conjugated to a carrier protein, P64K *Neisseria meningitidis* recombinant protein, as previously described.^{11,12}

Aluminum hydroxide (Alum) was used as the adjuvant and EGF-P64K conjugates were mixed after filtration with 2 mg of the adjuvant per dose of EGF vaccine. Adsorption of the conjugates to Alum was achieved by constant stirring at room temperature for one hour under sterile conditions. All procedures were performed according to Good Manufacturing Practices (GMP).

Table 1 **Patient demographic characteristics according to treatment group**

Sex	Single Dose (71 µg EGF) (n = 21)	Double Dose (142 µg EGF) (n = 22)
Males	18 (86%)	17 (77%)
Females	3 (14%)	5 (23%)
Age		
Mean (Range)	59.6 (46-73)	(54.7) (35-69)
Race		
White	16 (76%)	13 (59%)
African	5 (24%)	9 (41%)
Tumor histology		
Adenocarcinoma	9 (43%)	6 (30%)
Squamous carcinoma	6 (29%)	8 (36%)
Other	6 (29%)	8 (36%)
Tumor stage		
Stage III	9 (43%)	15 (68%)
Stage IV	12 (57%)	7 (32%)
Previous therapy		
Surgery	5 (24%)	1 (4.5%)
Radiotherapy	13 (62%)	13 (59%)
Chemotherapy	18 (86%)	15 (68%)
Performance Status (WHO)		
0	4 (19%)	2 (9%)
1	14 (67%)	17 (77%)
2	3 (14%)	2 (9%)
3	1 (4.5%)	

Treatment. Eligible patients were randomly assigned to groups designated to receive single (71 µg) or double doses (142 µg) of the EGF vaccine. The vaccine was administered intramuscularly in one or both upper limbs, based on treatment group assignment (single or double dose group). The vaccination schedule consisted of four weekly doses of the vaccine followed by monthly reimmunizations. The vaccination schedule could be interrupted or discontinued for any of the following reasons: severe or very severe, related adverse events, worsening patient condition, lost to follow up, or after personal request.

Measurement of antibody titers. Blood samples were collected every 15 days for 60 days and then monthly thereafter. Anti-EGF antibody titers against hu-EGF were measured through an enzyme linked immunosorbent assay (ELISA) as previously described.^{11,12} Anti-EGF antibody titer was defined as the maximum seric dilution, which gave absorbance measurements higher than the blank plus three times the standard deviation value.

Anti-EGF antibody response was considered positive (seroconversion) when anti-EGF antibody titers were at least twice their preimmunization value at any time during the study. Patients were additionally classified as “Good Antibody Responders” (GAR) if anti-EGF antibody response reached titers equal to or higher than 1:4000 and were at least four times the preimmunization value at any time during the study. Patients were considered “Poor Antibody Responders” (PAR) if titers did not meet these values.

Measurement of EGF concentration in serum. EGF concentration ([EGF]) in serum was measured using a commercially available kit (Quantikine Human EGF Kit; R&D Systems, Minneapolis, MN, USA). Briefly, the assay employs the quantitative sandwich enzyme immunoassay technique, in which an anti-EGF monoclonal antibody is precoated onto a microplate. After adding the standard calibration curve and the patients’ samples, an enzyme-linked polyclonal antibody specific for EGF is added to the wells. After a washing step, a substrate solution is added to the wells and the intensity of the color is measured. The minimum detectable level of EGF in serum is 78 pg/ml.

Table 2 EGF vaccine-related adverse events according to treatment group

	Single Dose (71 µg EGF) (n = 21)		Double Dose (142 µg EGF) (n = 22)	
	No. Patients	Percentage (%)	No. Patients	Percentage (%)
Fever	7	33	9	41
Chills	11	52	9	41
Nausea	9	42	10	46
Vomiting	7	33	10	46
Flushing	2	9.5	3	14
Tremors	8	38	11	50
Anorexia	8	38	7	32
Cramps	4	19	4	18
Headache	4	19	8	36
Dyspnoea	5	24	5	23
Pain	11	52	10	46
Paresthesias	3	14	1	4.5
Hypertension	2	9.5	3	14

Statistical methods. Survival time was calculated from patients' randomization. Survival data were analyzed using the Kaplan-Meier method and the log-rank test. Pearson's correlation coefficient was used to estimate the correlation between the anti-EGF antibody titers and the EGF concentration in patients' sera.

RESULTS

Forty-three stage IIIB/IV patients from two hospitals were included in the trial, from June 2000 to July 2003. Twenty-one patients received the single dose of the vaccine while 22 patients were immunized with the double dose. All patients were bearing tumors classified as non-small cell lung cancer and had finished first line oncospecific therapy at least four weeks before entering the trial. Twenty-one patients had an associated disease, for instance diabetes mellitus, hypertension, or peptic ulcer. The demographic characteristics of patients are described in Table 1. Thirty-seven patients completed four doses of the vaccine (induction phase), while the median number of vaccine doses was eight.

The EGF vaccine, adjuvanted in Alum, was well tolerated. No severe or very severe related adverse events were seen. All adverse events considered "related" to study treatment were classified as Grade 1 (mild) or 2 (moderate) events. These events (frequency presented for single dose; double dose) consisted mostly of fever (33%; 41%), chills (52%; 41%), nausea (42%; 46%), vomiting (33%; 46%), tremors (38%; 50%), anorexia (38%; 32%) and pain (52%; 46%). All toxicities resolved within the first 48 hours post-dose. The frequency of related events was similar in the single and double dose treatment groups except vomiting (33% vs. 46%, respectively), tremors (38% vs. 50%, respectively) and headache (19% vs. 36%, respectively), which were more frequent in patients receiving the double dose. No skin rash was detected. Adverse events, by treatment group, are presented in Table 2. There were no significant changes in any hematologic parameter over time or significant differences between cohorts.

Humoral response against EGF was evaluated in 38 patients; 19 patients in each group. Five patients (two in the single dose and three in the double dose group) did not provide samples and could not be evaluated for immune response to the EGF vaccine. Pre-existing reactivity to EGF, defined as anti-EGF antibody titers higher than 1:100 serum dilution at baseline, was detected in 13 patients (33.3%; five in single dose and eight in double dose); however, this specific response was of low magnitude and the geometric mean of the baseline anti-EGF antibody titer was 1:226 in this subset of patients. The percentage of seroconverters and GARs was estimated by

treatment cohort. Twenty-nine subjects of 38 (76.3%) achieved seroconversion while only 15 subjects (39.5%) met the GAR criterion. There was a trend toward increased seroconversion in patients receiving the double dose of the vaccine (63% vs. 90%, single vs. double dose, respectively, Fisher exact test $p = 0.062$), while no differences were detected between treatments when comparing the proportion of GARs (37% vs. 42%, single vs. double dose, respectively, Fisher exact test $p = 0.5$) (Table 3). The geometric mean of the maximal anti-EGF antibody titer was equivalent to 1:1838 serum dilution for all patients. Although not significantly different, the geometric mean of maximal anti-EGF antibody titers was higher in the patients who received the double dose of treatment (1:1425 vs. 1:2372, single vs. double dose, respectively) (Table 3). Peak anti-EGF antibody titer values were reached by 60 days (median time) for the single dose cohort and after 45 days in the double dose cohort. The predominant immunoglobulin subclasses were IgG1 and IgG3 for both treatment groups.

EGF concentration in serum was determined in 20 patients (ten in the single dose and ten in the double dose cohorts). Mean baseline [EGF] concentration was 1449 pg/ml (227–5000 pg/ml) in the single dose group and 1189 pg/ml (78–5000) in the double dose group. In all but two patients, vaccination resulted in EGF reduction in serum. For the 20 patients, the mean lowest [EGF] was 239 pg/ml, representing a 65% reduction from baseline EGF levels. Patients vaccinated with the single dose achieved the minimum [EGF] (222.8 pg/ml) in serum after 120 days while the median time to reach the lowest EGF serum levels (254 pg/ml) was 60 days for the double dose cohort.

To further characterize patient response, a cut-off point of 168 pg/ml was established to classify patients according to the extent of EGF reduction in serum. This cut-off level was selected since it represents half the mean [EGF] in normal subjects. In 70% of the patients in the double dose arm of the study, [EGF] was reduced to less than 168 pg/ml during the study, while only 30% of the patients treated with the single dose met this reduction (Table 4). No significant differences between the two groups were detected for the mean lowest EGF concentration ever achieved after vaccination or the median time to reach the minimum EGF concentration.

Evaluation of EGF concentration and anti-EGF antibody response over time demonstrated a statistically significant inverse correlation between anti-EGF antibody titers and serum [EGF] according to the Pearson correlation coefficient ($p = 0.05$) (Fig. 1).

Patients' survival was estimated from randomization. The median survival of patients treated with single or double dose of the EGF vaccine was 6.43 and 8.40 months, respectively. Survival, based on immune response, was also estimated. Patients classified as GARs, had a median survival equivalent to 11.87 months, while poor responders had a median survival of 7.07 months ($p = 0.0095$). There was a significant increase in median survival in those subjects in whom serum EGF decreased to below 168 pg/ml (median 11.30 months) in comparison to those patients who did not achieve EGF reductions below 168 pg/mL (median 5 months), ($p = 0.0022$).

DISCUSSION

Despite advances in cancer treatment during the past two decades, the outcome of NSCLC cancer patients is still very disappointing.¹³

The mitogenic activity of EGF on tumor cells, the overexpression of EGFR on many epithelial tumors and the relationship between EGFR expression and bad prognosis, make EGF a very attractive target for the design of new anticancer drugs. Several products targeting EGFR are currently approved, including a monoclonal antibody

targeting the receptor and small molecule tyrosine kinase inhibitors.¹⁴

EGF based specific active immunotherapy is a novel approach to disrupt EGFR signalling. At present, we report the results of a Phase I clinical trial designed to preliminary asses the impact of dose increase in the EGF vaccine safety, immunogenicity and on the EGF concentration in serum. In the previous trials, we evaluated the effect of different carrier proteins and adjuvants on the vaccine properties.^{11,12}

Here we show for the first time, that EGF concentration in sera is dramatically reduced after vaccination and that there is a statistically significant inverse correlation between the anti-EGF antibody response and the EGF concentration in sera. Moreover, we concluded that lowering systemic EGF levels with the EGF cancer vaccine could potentially improve patients' survival.

This trial confirmed that vaccination with EGF-P64K adjuvanted with Alum was well tolerated. The adverse event profile was similar to previously reported results for the EGF vaccine when mixed with other adjuvants or carrier proteins.^{11,12}

An increase in immune response, defined by anti-EGF antibody titers, was reflected in the percentage of patients who seroconverted or had GAR. The proportion of GARs did not increase with increasing dose. In this trial, the frequency of patients who achieved GAR is consistent with that previously reported for Alum and is less than the proportion found when using the oily adjuvant Montanide ISA 51.¹² In general, we concluded that vaccination is still suboptimal, given that only roughly 40% of the patients in both dose groups reached the GAR criterion and further effort to improve vaccine immunogenicity, using better adjuvants, dosages and schedule is mandatory.

The current vaccine formulation and adjuvant method does not allow continuing dose escalation due to technical difficulties associated with the maximum allowed amount of aluminium 3+ (Al³⁺) that can be administered. Accordingly, a new formulation or new adjuvants should be tested to continue optimizing the vaccine conjugate in terms of immunogenicity.

Immunization with the EGF vaccine resulted in reducing serum EGF levels. The inverse correlation between the anti-EGF antibody response and seric EGF supported the hypothesis that vaccination is the cause of the EGF deprivation. On the other hand, we found that the baseline EGF concentration in our patient set (mean 1368 pg/ml) was remarkably elevated in comparison to normal subjects, for which the mean EGF concentration is equivalent to 336 pg/ml (Quantikine Human EGF Kit; R&D Systems, MN, USA). The definitive association between serum EGF and lung cancer progression should be established in a wider and well matched population.

Finally, even though the trial was not designed as a controlled efficacy trial, the overall median survival (8.23 months) of patients vaccinated after first line therapy compared favorably with the historical data for best supportive care and for second or third line therapy in unresectable NSCLC patients.¹⁵

For patients with Stage IIIB/IV incurable disease, only docetaxel, erlotinib and pemetrexed are registered worldwide for second-line

Table 3 Humoral immune response against EGF by treatment group

	Single Dose (71 µg EGF) (n = 19)	Double Dose (142 µg EGF) (n = 19)	All Patients (n = 38)
Seroconversion (n/percentage of patients)	12 (63.2%)	17 (89.5%)	29 (76.3%)
Good antibody response (n/percentage of patients)	7 (36.8%)	8 (42.1%)	23 (39.4%)
Maximum anti-EGF antibody titers (Geometric mean)	1:1425 (seric dilution)	1:2372 (seric dilution)	1:1838

Table 4 EGF concentration in serum by treatment group

	Single Dose (71 µg EGF) (n = 10)	Double Dose (142 µg EGF) (n = 10)	All Patients (n = 20)
[EGF] < 168 pg/ml (n/ percentage of patients)	3 (30%)	7 (70%)	10 (50%)
Mean minimal [EGF] (pg/ml)	222.86	254.80	238.83

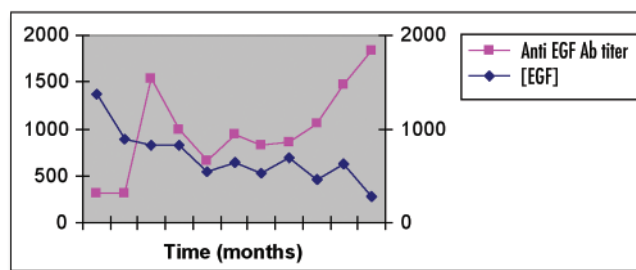


Figure 1. Relation between anti-EGF antibody titers and EGF concentration in serum in vaccinated patients (20 patients).

chemotherapy. Docetaxel and pemetrexed are associated with significant toxicity, (i.e., Grade 3/4 neutropenia and febrile neutropenia).¹⁵ Docetaxel improved survival when compared to best supportive care, with a median survival time of seven months. Pemetrexed was granted accelerated approval on the basis of a surrogate endpoint (response rate) reasonably likely to predict survival. A multicenter, randomized phase III trial showed that the median survival time was 8.3 months in the pemetrexed arm and 7.9 months in the docetaxel arm. The study did not show an overall superiority of pemetrexed over docetaxel.^{16,17}

Two small molecule oral EGFR inhibitors have been approved by the FDA. Iressa (gefitinib) is currently approved for third line use after 2nd-line chemotherapies,¹⁸ while Tarceva (erlotinib) received FDA approval after first or second line chemotherapy.¹⁹ Iressa failed to demonstrate a survival benefit¹⁸ and erlotinib showed a modest survival increase compared to placebo (6.7 vs. 4.7 months).¹⁹ Recently, it has been published that EGFR gene mutations in the receptor tyrosine kinase domain are associated with a higher response rate to these small molecule inhibitors.^{20,21} Whether the EGF vaccine

will be more effective in patients bearing these mutations or without K ras mutation, which predict EGFR inhibitor resistance,²² remains to be determined in pharmacogenomic studies.

As in all previous trials, we found that good responders have a significant increase in survival (median 11.87 months) as compared to poor responders. Moreover, we found a relation between EGF levels in serum and survival, since patients whose serum EGF concentration reached less than half the mean [EGF] in the normal population, had a significantly better survival as compared to patients whose [EGF] exceeded 168 pg/ml.

Recently, a phase IIB trial of a BLP25 liposome vaccine showed very encouraging results in stage IIIB and IV NSCLC patients. Even though the survival difference between the vaccinated and the control arm did not reach statistical significance, the vaccine resulted in 4.4 months survival advantage as compared with the control subjects. Whether the EGF cancer vaccine will result in a significant improvement in survival is being tested in a randomized, phase II trial, where advanced NSCLC patients are receiving the EGF vaccine or best supportive care, after finishing first line chemotherapy.²³

In summary, our results confirm the immunogenicity of the EGF vaccine. Anti-EGF antibody titers and EGF levels in serum appear to correlate with patient survival. Even though increasing the EGF vaccine dose improved anti-EGF antibody titers in this study, further dose escalation may achieve greater clinical impact. These results support the evaluation of active specific immunotherapy with EGF vaccines as an alternative to second line chemotherapy in NSCLC patients.

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