Clinical Cancer Research

A Phase III Clinical Trial of the Epidermal Growth Factor Vaccine CIMAvax-EGF as Switch Maintenance Therapy in Advanced Non–Small Cell Lung Cancer Patients

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Abstract

Purpose: EGFR is a well-validated target for patients with non–small cell lung cancer (NSCLC). CIMAvax-EGF is a therapeutic cancer vaccine composed of human recombinant EGF conjugated to a carrier protein and Montanide ISA51 as adjuvant. The vaccine is intended to induce antibodies against self EGFs that block EGF–EGFR interaction.

Experimental Design: To evaluate overall survival, safety, immunogenicity, and EGF concentration in serum after CIMAvax-EGF, a randomized phase III trial was done in patients with advanced NSCLC. Four to 6 weeks after first-line chemotherapy, 405 patients with stage IIIB/IV NSCLC were randomly assigned to a vaccine group, which received CIMAvax-EGF or a control group, treated with best supportive care.

Results: Long-term vaccination was very safe. Most frequent adverse reactions were grade 1 or 2 injection-site pain, fever, vomiting, and headache. Vaccination induced anti-EGF antibodies and decreased serum EGF concentration. In the safety population, median survival time (MST) was 10.83 months in the vaccine arm versus 8.86 months in the control arm. These differences were not significant according the standard log rank (HR, 0.82; $P = 0.100$), but according a weighted log rank ($P = 0.04$) that was applied once the nonproportionality of the HR was verified. Survival benefit was significant (HR, 0.77; $P = 0.036$) in the per-protocol setting (patients receiving at least four vaccine doses): MST was 12.43 months for the vaccine arm versus 9.43 months for the control arm. MST was higher (14.66 months) for vaccinated patients with high EGF concentration at baseline.

Conclusions: Switch maintenance with CIMAvax-EGF was well tolerated and significantly increased MST of patients that completed induction vaccination. Baseline EGF concentration predicted survival benefit. *Clin Cancer Res; 22(15); 3782–90. ©2016 AACR.*

Introduction

Lung cancer remains one of the leading causes of cancer-related deaths in men and women, provoking approximately 1.6 million deceases, 19.4% of the total deaths per year, worldwide (1). In Cuba for instance, lung cancer is the leading cause of death in both genders with more than one-quarter of all cancer deaths. Non–small cell lung cancer (NSCLC) is the most common form of the disease (87%), and approximately 60% of the patients present with advanced disease at diagnosis (2).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

The EGF vaccine consists of a different approach when compared with other active immunotherapies. CIMAVax-EGF is built on the induction of a specific immune response, aiming to sequester EGF, a molecular driver of cancer cell proliferation. The significantly largest benefit of CIMAvax-EGF in the subpopulation of patients with high pretreatment concentration of EGF is differentiating our cancer vaccine when compared with other modalities of active immunotherapy or targeted therapy. CIMAvax-EGF is a very safe drug that could be a feasible intervention for long-term control of those patients with NSCLC with tumors depending on the EGF, capable to mount a rapid and durable response.

Treatment of advanced NSCLC has undergone a rapid evolution along the last 30 years with three major advances: the platinum-based doublets, maintenance and second-line chemotherapy with docetaxel, pemetrexed, or erlotinib, and targeted therapies with small molecules such as gefitinib, erlotinib, afatinib, crizotinib, or ceritinib for tumors with sensitizing mutations (3–6). Nevertheless, 1-year survival rate is approximately 35% for patients with wild-type tumors, whereas 5-year survival rate is still around 5% (2).

EGFR is overexpressed in approximately 40% to 80% of NSCLCs (7). The oncogenic addiction of the malignancies to this signaling pathway triggers its inappropriate activation and promotes the uncontrolled growth, proliferation, and survival of cancer cells (8). Thus, EGFR overexpression is associated with poor prognosis, lower survival, and resistance to therapy (9).

The EGF-based cancer vaccine CIMAvax-EGF is an active immunotherapy, intended to prevent binding of the endogenous EGF to the receptors, by inducing anti-EGF antibodies that clear the growth factor from circulation (10). CIMAvax-EGF is composed by human recombinant EGF coupled to a carrier protein, recombinant P64. The EGF–P64 chemical conjugate is emulsified in Montanide, an oily adjuvant. The carrier protein and the adjuvant are required to break the tolerance against EGF, which is a self-protein. Previous phase I/II clinical trials evidenced the immunogenicity and safety of vaccination in patients with advanced stage NSCLC (11–13). In a randomized phase II trial using CIMAvax-EGF as switch maintenance or second-line therapy versus best supportive care in subjects with stage IIIB or IV NSCLC, a trend toward survival benefit was found. Patients younger than 60 years, those with good antibody response against EGF, and subjects in whom serum EGF concentration ([EGF]) decreased below a preestablished threshold, achieved a significant survival benefit with vaccination. Postimmune serum hampered the binding between EGF and EGFR in a radioreceptor assay and abrogated EGFR phosphorylation (14, 15).

In this article, we show the results of a randomized phase III trial in patients with advanced NSCLC. The primary endpoint was overall survival (OS), whereas safety, immunogenicity, and serum EGF concentration before and after therapy were secondary endpoints.

Patients and Methods

Eligibility criteria

Eligible patients were 18 years or older with histologically or cytological proven stage IIIB/IV NSCLC and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Patients with all histologic NSCLC subtypes and life expectancy of at least 3 months were trial candidates. Other inclusion criteria included hemoglobin values above 90 g/L, leukocytes count $\geq 3.0 \times 10^9$/L, platelets count $\geq 150 \times 10^9$/L, aspartate aminotransferase and alanine aminotransferase up to 2.5 times the upper institutional limit, and creatinine, up to 2 times the upper institutional reference value. All patients received four to six cycles of platinum-based chemotherapy (mostly cisplatin/carboplatin in combination with vinblastine, etoposide, or paclitaxel) and had stable disease or objective response.

The trial protocol, informed consent, investigator brochure, and case report forms were approved by the ethics boards from all participating institutions and by the National Regulatory Agency. Informed consent was obtained from each subject before entering in the study. The study was done in compliance with the principles of Good Clinical Practices (according the International Conference of Harmonization) and the Declaration of Helsinki.

Treatment schedule

Four to 6 weeks after finishing first-line chemotherapy, patients were randomly assigned (2:1) to the vaccine group, which received the EGF cancer vaccine plus best supportive care, or the control group that was treated with best supportive care. Patients in the vaccine arm were given a low dose of cyclophosphamide (200 mg/m^2) intravenously 72 hours before the first immunization. Each vaccine dose was administered at four injection sites (two deltoid and two gluteus regions) every 2 weeks for four doses (induction period) and then monthly.

CIMAvax-EGF was manufactured at the industrial facility of the Centre for Molecular Immunology (Havana, Cuba) in compliance with the Good Manufacturing Practice standards for biopharmaceutical products. The vaccine is composed of human recombinant EGF manufactured in yeast (hu-recEGF), and it is chemically conjugated to the P64K *Neisseria meningitides* recombinant protein (recP64k) manufactured in *Escherichia coli*. The final formulation of the cancer vaccine (0.6 mg hu-recEGF/recP64k) is then mixed in a water-in-oil emulsion with Montanide (Seppic) immediately before injection. At each immunization, patients received 2.4 mg of hu-recEGF/recP64k/Montanide (16).

Patient assessment

Patient assessment was performed at baseline and every 4 weeks, and included physical exam and clinical laboratory tests, as described in the inclusion criteria. In addition, chest radiography, CT scan, and abdominal ultrasound were performed at baseline and every 3 months to assess clinical response according to RECIST, version 1.1.

Toxicity was graded according to the NCI Common Toxicity Criteria (version 3) at each visit. Criteria for discontinuing vaccination included voluntary withdrawal, unmanageable toxicity, or severe worsening of the patient's general conditions. Progressive disease according to RECIST 1.1 was not an interruption criterion.

Measurements of antibody titers

Blood samples were collected at baseline (pretreatment) and every 14 days for 60 days and monthly thereafter. Anti-EGF antibody titers were measured through ELISA as described in

previous studies. Patients were classified in good antibody responders (GAR) if they developed anti-EGF antibody titers equal or higher than 1:4,000 and super-good antibody responders if they reached anti-EGF antibody titers equal or higher than 1:64,000. Those vaccinated patients that did not have titers above 1:4,000 were classified as poor antibodies responders (12–15).

EGF concentration in serum was measured with a commercial ELISA (Quantikine EGF; R&D Systems Inc) in 50% of the enrolled patients. Subjects were classified according to their pretreatment (baseline) serum EGF concentration in high EGF ([EGF] > 870 pg/mL) or low EGF ([EGF] $\leq$ 870 pg/mL). The selected cutoff (870 pg/mL) corresponded to the median EGF concentration for all patients at day 0.

Statistical analysis

The projected sample size was 579, considering that a two-sided log-rank test with 386 vaccinated patients and 193 controls achieves 90% power at a 0.05% significance level to detect an HR of 0.7. The proportion dropping out of the treatment and control groups was anticipated to be 10%. First and second interim analyses were done after 40% and 60% of the patients were enrolled. The trial was stopped before reaching the intended sample size, after the marketing approval of CIMAvax-EGF by the National Regulatory Agency, at the second interim analysis.

Survival time (date of random assignment to date of death or last contact) was estimated by the Kaplan–Meier method in the safety population (patients receiving at least one vaccine dose) and in the per-protocol population as established in the trial protocol. The per-protocol population included those patients that completed four doses of CIMAvax-EGF (induction period). Control patients that did not survive for 6 weeks (time interval needed to complete CIMAvax-EGF induction period) were excluded from the survival comparison. Regarding serum EGF, a prospectively defined subgroup was retrospectively analyzed using the analytically validated test for EGF concentration. The evaluation of the EGF concentration as predictive or prognostic biomarker was classified as exploratory.

Survival comparison was done with a standard log-rank test and a weighted log-rank test (Harrington–Fleming), according to the proportionality of the hazards (17). The hazard proportionality was checked graphically in agreement with the methodology proposed by Lambert and colleagues (18). The Pearson correlation coefficient and Fisher exact tests were used to estimate the correlation between anti-EGF titers and [EGF] and to assess the uniform distribution of baseline variables between groups, respectively.

Progression-free survival (PFS) was not a secondary objective as it is not a recommended endpoint for evaluating vaccines efficacy. Because of their immunologic mechanisms of action, cancer vaccines may require considerable time after administration to induce immunity. Therefore, tumors in subjects treated with cancer vaccines may show early progression followed by subsequent response.

The statistical system SPSS (version 15.1) and R (version 2) were used for modeling and verifying the hypothesis in all population sets. This study was registered in the National Public Registry of Clinical Trials; a WHO-validated public registry (http://www.who.int/ictrp/network/rpcec/en, trial number RPCEC00000161)

Results

From July 5, 2006, to January 3, 2012, 1,336 patients were evaluated for eligibility in 19 Cuban clinical research centers. A total of 405 patients with histology or cytology proven NSCLC at stage IIIB and IV were enrolled in the trial: 270 in the vaccine arm and 135 in the control group. A total of 246 patients received at least one vaccine dose (safety population); 219 subjects received 4 doses (per-protocol population), 85 patients received more than 14 CIMAvax-EGF doses (1-year vaccination), 39 patients were vaccinated 26 times or more (2-year vaccination), and 12 subjects received more than 50 vaccine doses (4-year vaccination). Three control patients were vaccinated, as compassionate use, upon trial withdrawal (Supplementary Fig. S1). Control patients who received CIMAvax-EGF were excluded from the analysis.

Twenty-four patients (8.8%) from the vaccine arm did not receive any vaccine dose, while 27 patients (10.9%) who started vaccination did not complete CIMAvax-EGF induction (four doses). The main causes of early dropout were rapid worsening of the performance status, consent withdrawal, uncompensated comorbidities, schedule violations, and rapid unset of death. Eighteen patients, 10 vaccinated (3.7%) and 8 controls (5.9%), died before day 45 (time needed to complete induction vaccination). No significant differences were found between both arms regarding early death.

The two arms were well matched for baseline demographic and tumor variables, such as sex, ethnic origin, age, smoking status, ECOG, disease stage, histology, and response to initial chemotherapy (Table 1).

Most patients did not receive further chemotherapy at progression (in consonance with the national treatment guideline), as the

Table 1. Demographic and baseline characteristics

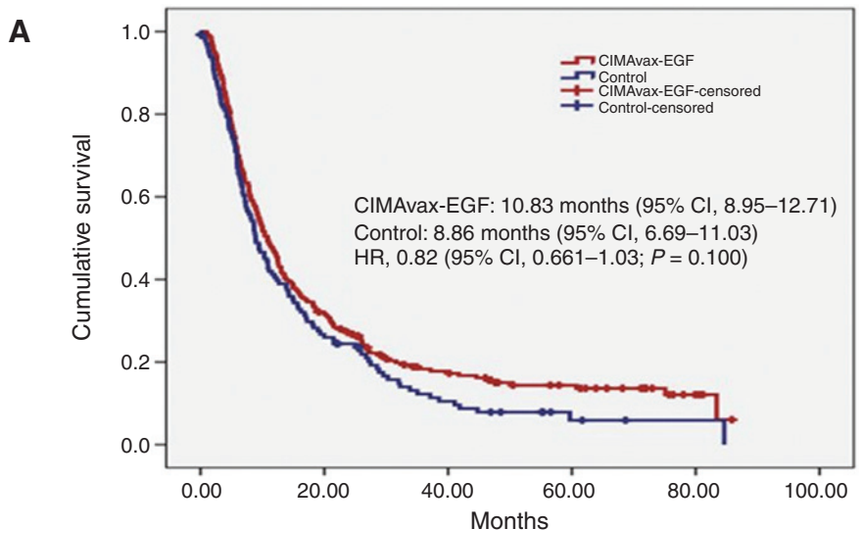
	Vaccine arm ($n = 270$)	Control arm ($n = 135$)
Sex		
Men	178 (65.9%)	86 (63.7%)
Women	92 (34.1%)	49 (36.3%)
Ethnic origin		
White	182 (67.4)	90 (66.7)
African	45 (16.7)	22 (16.3)
Others	43 (15.9)	23 (17.0)
Smoking history		
Current	91 (33.7%)	41 (34.1%)
Pass	140 (51.9%)	71 (52.6%)
Never	39 (14.4%)	23 (17.0%)
ECOG		
0	101 (37.4%)	46 (34.0%)
1	148 (54.8%)	73 (54.1%)
2	17 (7.0%)	12 (8.9%)
Missing	4 (1.4%)	4 (3.0%)
Disease stage		
IIIB	169 (62.3%)	88 (65.2%)
IV	98 (36.3%)	36 (26.6%)
Missing	3 (1.1%)	4 (3.0%)
Tumor histology		
Adenocarcinoma	92 (34.1%)	46 (34.0%)
SCC	142 (52.6%)	73 (54.0%)
NSCLC NOS	36 (13.3)	20 (14.8%)
Response to first-line treatment		
Complete response	28 (10.4%)	10 (7.4%)
Partial response	111 (41.1%)	54 (40.0%)
Stable disease	116 (43.0%)	65 (48.1%)
Progressive disease	15 (5.6%)	6 (4.4%)

Abbreviation: NOS, not otherwise specified.

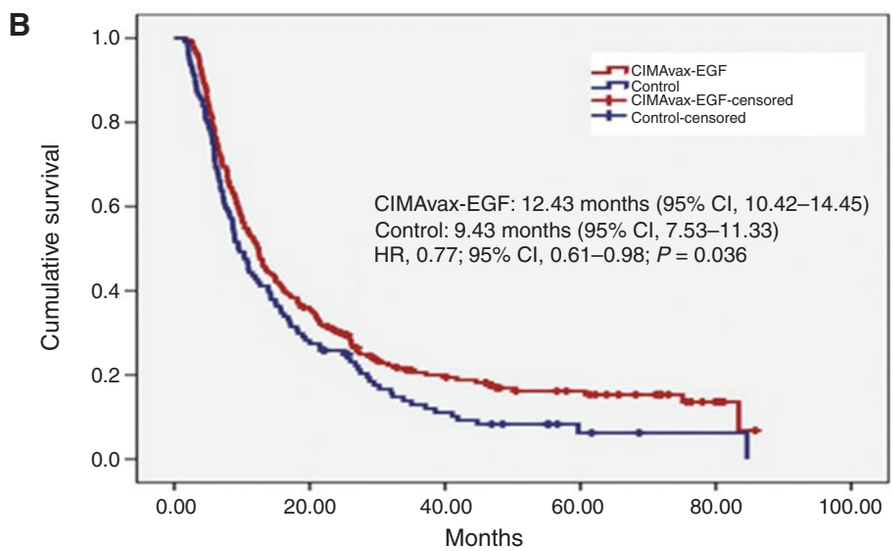
recommended second-line drugs pemetrexed, docetaxel, and erlotinib were not widely available in the country at the time of trial execution. In the vaccine arm, 16 patients (5.9%) received additional chemotherapy, including carboplatin, cisplatin, paclitaxel, etoposide, vinblastine, cyclophosphamide, and docetaxel. In the control group, nine subjects (6.6%) were treated with other chemotherapies comprising paclitaxel, carboplatin, vinblastine, etoposide, vincristine, and docetaxel. Overall, only two patients, one from each arm, received docetaxel, one of the drugs accepted to increase survival after progressive disease.

CIMAvax-EGF efficacy

In the safety population (patients receiving at least one CIMAvax-EGF dose), vaccinated patients had a survival benefit that did



Number at risk	0	6	12	24	36	48	60	72	84
CIMAvax-EGF	246	173	114	64	35	24	19	10	1
Control	132	88	53	30	14	8	3	1	0



Number at risk	0	6	12	24	36	48	60	72	84
CIMAvax-EGF	219	168	112	64	35	24	18	10	1
Control	124	88	53	30	14	8	3	1	0

Figure 1.
A, Kaplan–Meier curve in the safety population. MST for the vaccine arm was 10.83 months (95% CI, 8.95–12.71) versus 8.86 months (95% CI, 6.69–11.03) for the control arm. HR, 0.82 (95% CI, 0.661–1.03; $P = 0.100$).
B, Kaplan–Meier curve in the per-protocol population. MST for the vaccine arm was 12.43 months (95% CI, 10.42–14.45) versus 9.43 months (95% CI, 7.53–11.33) for the control arm. HR, 0.77; 95% CI, 0.61–0.98; $P = 0.036$.

not reach statistical significance according to the standard log-rank test [HR, 0.82; 95% confidence interval (CI), 0.661–1.03; $P = 0.100$]. MST in the vaccine arm was 10.83 months (95% CI, 8.95–12.71), whereas MST in the control group was 8.86 months (95% CI, 6.69–11.03). Five-year survival rate was 14.4% for vaccinated patients and 7.9% for controls. As a delayed separation of the survival curves and a nonproportional HR between the two groups was verified, the Harrington–Fleming (HF) test was applied. The survival difference was significant according to this weighted log-rank test (HF, $P = 0.04$).

In addition, OS was evaluated in the per-protocol population as established in the trial protocol. The median survival in the vaccine arm (patients completing four vaccine doses) was 12.43 months (95% CI, 10.42–14.45) versus 9.43 months (95% CI, 7.53–11.33) in the control arm (patients surviving for at least 6 weeks). Five-year survival rate was 16.62% for those vaccinated patients who received 4 vaccine doses versus 6.2% for nonvaccinated patients. Survival differences in the per-protocol population were significant according to the standard, unweighted log-rank test: (HR, 0.77; 95% CI, 0.61–0.98; $P = 0.036$; Fig. 1B).

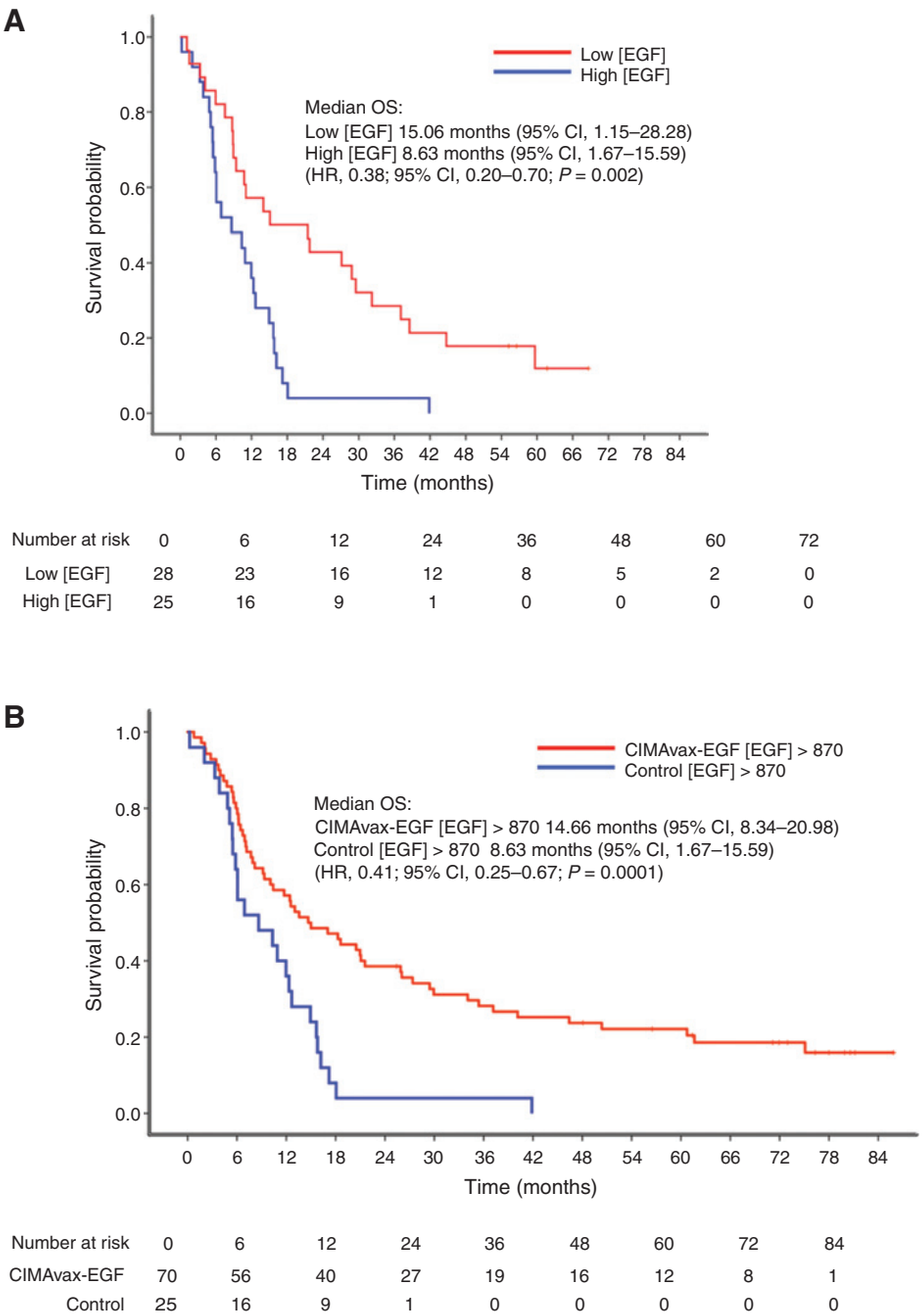


Figure 2.
A, Kaplan–Meier curve in nonvaccinated patients (control arm). MST for patients with low [EGF] was 15.06 months (95% CI, 1.15–28.28) versus 8.63 months (95% CI, 1.67–15.59) for patients with high [EGF] at day 0. HR, 0.38 (95% CI, 0.20–0.70) $P = 0.002$. **B,** Kaplan–Meier curve in patients with high [EGF] at day 0. MST for vaccinated patients was 14.66 months (95% CI, 8.34–20.98) versus 8.63 months (95% CI, 1.67–15.59) for controls. HR, 0.41; 95% CI, 0.25–0.67; $P = 0.0001$.

Serum EGF at baseline might be a prognostic and predictive factor of vaccine efficacy

Serum EGF was quantified in 188 patients. Mean and median EGF concentrations were 1,195 pg/mL and 873 pg/mL, respectively. There were no differences in the serum EGF levels between vaccinated and control patients. Mean and median EGF concentrations were 1,194 pg/mL and 930 pg/mL for the vaccine arm and 1,197 pg/mL and 820 pg/mL for controls ($P = 0.98$).

Median EGF concentration (870 pg/mL) was established as a cutoff to classify patients in high or low [EGF] at enrollment, as prespecified in the protocol. Survival according to [EGF] at baseline was evaluated in control and vaccinated patients to preliminarily assess the prognostic and predictive value of the referred biomarker.

In the control group, patients with high [EGF] had a worse survival as compared with patients with low [EGF] (HR, 0.38; 95% CI, 0.20–0.70; $P = 0.002$; Fig. 2A). Median OS was 8.63 months (95% CI, 1.15–28.28) for controls with high [EGF] versus 15.06 months (95% CI, 1.67–15.59) for subjects with low [EGF]. According to this analysis, high EGF levels may be a poor prognostic factor, whereas low EGF may be a good prognostic factor for NSCLC patients. The association between EGF levels and prognosis remained significant when other prognostic variables (gender, smoking history, performance status, and staging) were included in the multivariate analysis. In the multivariate analysis, the most significant variables were EGF concentration and ECOG.

On the contrary, patients with serum [EGF] > 870 pg/mL had a better survival as compared with controls with the same EGF serum levels if vaccinated with CIMAvax-EGF (HR, 0.41; 95% CI, 0.25–0.67; $P = 0.0001$). MST for vaccinated patients was 14.66 months (95% CI, 8.34–20.98) versus 8.63 months (95% CI, 1.67–15.59) for nonvaccinated patients. Five-year survival rate was 23% for vaccinated patients while no controls were alive after 60 months. According to this retrospective analysis (predefined in the protocol), [EGF] above the 870 pg/mL threshold, could be a predictive biomarker of CIMAvax-EGF efficacy (Fig. 2B). The interaction between EGF levels and treatment was checked and was statistically significant ($P < 0.0001$).

Vaccination with CIMAvax-EGF induced anti-EGF antibodies and decreased EGF concentration in sera

Anti-EGF antibody titers were evaluated in 112 patients (40% of subjects enrolled in the vaccine arm). Eighty-nine patients (79.4%) were classified as good responders, whereas 24 patients (21.4%) were categorized as super-good responders, as they developed anti-EGF antibody titers above 1:64,000 sera dilution. The percentage of patients reaching the GAR condition after one, two, three, or four vaccine doses was 0, 7%, 39%, and 56%, respectively. Four doses was the minimum number of injections after which 50% of the patients met the GAR status. Patients who met the GAR criterion after the induction period had a significant survival benefit: MST was 14.90 months versus 8.86 months for the controls (HR, 0.638; 95% CI, 0.44–0.92; $P = 0.017$). Overall, the geometric mean of the maximal antibody titer was 1:12,646, whereas the highest antibody titer was 1:1,024,000.

In addition, serum EGF was measured before and after vaccination. A significant inverse correlation was observed (Spearman $r = -0.523$; $P < 0.01$) between the anti-EGF antibody titers and serum EGF concentration in vaccinated patients (Fig. 3). In control patients, there was no association between antibody titers and serum EGF.

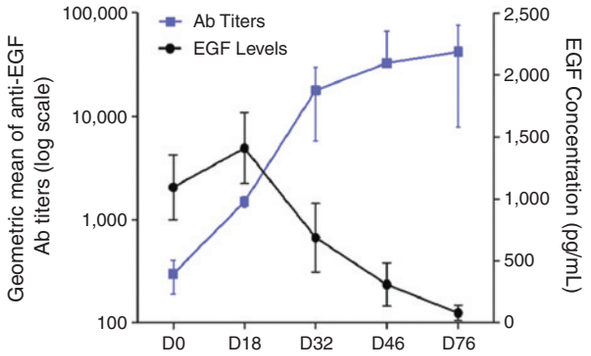


Figure 3.
Kinetics of the anti-EGF antibody titers and serum EGF concentration in vaccinated patients. Blood samples were collected at baseline every 14 days for 60 days and monthly thereafter. Anti-EGF antibody (Ab) titers were measured through an ELISA. EGF concentration in serum was measured with a commercial ELISA (Quantikine; R&D Systems Inc).

A subgroup exploration including the most important demographic and tumor variables was done in the safety population (Fig. 4). In addition to high serum EGF concentration, patients with the largest benefit after vaccination were those bearing squamous cell carcinoma (SCC) histology, smokers, and with stage IV.

Long-term vaccination with CIMAvax-EGF was safe

The safety evaluable population consisted of 246 patients who received at least one dose of CIMAvax-EGF. Adverse events were reported in 78.3% of the safety evaluable population, and 1,200 vaccine-related events were reported in 59.4% of the treated patients. Most frequent related adverse events were injection-site pain (46.6%), fever (36.5%), vomiting (23.3%), and headache (22.5%). Grade 3–related adverse events were seen in 3.6% of the vaccinated patients and consisted of headache (two patients), dyspnea (two patients), injection-site reactions (two patients), eosinophilia (two patients), fever (one patient), chills (one patient), tremors (one patient), and arthralgia (one patient). No patient developed grade 4 adverse events (Table 2 and Supplementary Tables S2 and S3).

Discussion

The treatment of advanced NSCLC has undergone a rapid evolution along the last 30 years: from a dark landscape in the 1980s to the demonstration of survival gain after the combination of platinum doublets, maintenance, and second-line therapy with docetaxel, pemetrexed, and erlotinib for patients with EGFR and anaplastic lymphoma kinase (ALK) wild-type tumors (3–5).

Another big wave of improvement came with targeted therapies with tyrosine kinase inhibitors (TKIs) like gefitinib, erlotinib, and afatinib for tumors carrying EGFR mutations and crizotinib and ceritinib for tumors carrying ALK-activating translocations (19). The median survival in patients with metastatic disease and defined mutations ranged from 23 to 27

Group	n_vaccine	n_control	HR	95%CI_LL	95%CI_UL
Caucasian	182	90	0.792	0.607	1.032
Afro	45	22	1.162	0.658	2.063
Other	43	23	0.876	0.504	1.523
ECOG0	101	46	1.09	0.753	1.578
ECOG1	148	73	0.748	0.554	1.011
ECOG2	17	12	0.985	0.462	2.099
IIIB	169	93	0.948	0.723	1.242
IV	98	38	0.708	0.476	1.053
Smoker	91	41	0.676	0.457	1.001
Ex-smoker	140	71	0.943	0.696	1.277
Nonsmoker	39	23	0.974	0.551	1.722
Adenocarcinoma	92	50	1.216	0.835	1.77
Squamous_cell_carcinoma	142	65	0.715	0.524	0.977
NSCLC (NOS)	36	20	0.684	0.38	1.229
Complete response	28	10	0.711	0.328	1.538
Partial response	111	54	0.934	0.658	1.326
Stable disease	116	65	0.821	0.597	1.13
Age ≤ 65	168	82	0.843	0.635	1.117
Age > 65	102	52	0.906	0.637	1.289
egf < 870	65	28	1.491	0.921	2.413
egf ≥ 870	70	25	0.412	0.25	0.679

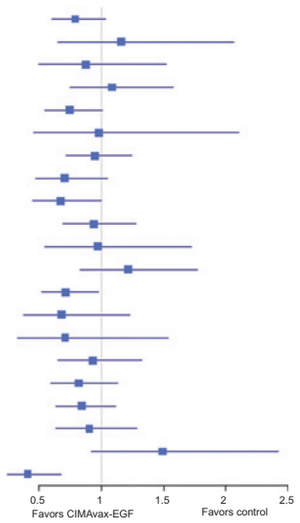


Figure 4.
Cox regression analysis: subgroup analysis considering the most important demographic and tumor variables. NOS, not otherwise specified.

months (20). Unfortunately, the population bearing EGFR or ALK mutations, and thus benefiting from the approved targeted therapies, is small (∼15%–20%; ref. 6).

The next wave of progress is coming from immunotherapy. Long-lasting responses have been reported after the use of the anti-PD1 antibodies (nivolumab, pembrolizumab) and the anti-PD1L antibodies (MPDL3280A, BMS936559, MEDI4736). Moreover, nivolumab has recently demonstrated to increase survival of patients bearing metastatic squamous and adenocarcinoma NSCLC that progressed on or after platinum-based chemotherapy, as compared with docetaxel (21, 22). Pembrolizumab showed remarkable antitumor activity in patients with advanced NSCLC and PD-L1 expression in at least 50% of tumor cells (23). FDA recently approved both checkpoint inhibitors for the treatment of metastatic NSCLC as second-line therapy.

Active immunization might also improve lung cancer survival if the vaccine successfully triggers a strong immune response and if used in the right population. Clinical studies with three vaccine candidates in advanced population, such as tecemotide, TG4010, and belagenpumatucel-L, did not meet their primary endpoints but showed possible benefits in patient subpopulations: for tecemotide, a potential role of vaccination was seen in patients treated with concurrent chemoradiotherapy; for TG4010, the lymphocyte phenotype and concomitance with chemotherapy were reported as potential predictors of outcome; and for belagenpumatucel-L, the number of circulating tumor cells appears to correlate with OS (24–26).

The EGF vaccine consists of a different approach when compared with other active immunotherapies. CIMAvax-EGF is built on the induction of a specific immune response, aiming to sequester EGF, a molecular driver of cancer cell proliferation (11–15). Its mechanism of action is based on the "hormone deprivation theory" that has proven to be effective for sexual hormones–dependent tumors (10). The rationale of CIMAvax-EGF is based on the finding that EGF concentration is higher in NSCLC patients than in normal donors. The preliminary role of [EGF] as a negative prognostic marker for advanced NSCLC reinforces the validity of the "removal" approach (9).

In this phase III study, CIMAvax-EGF was very safe even in patients who received very prolonged vaccination (more than 2 years) and did not show cumulative toxicity.

Immunogenicity assessment was a secondary endpoint of this phase III clinical trial. The protocol projected the evaluation of 40% of the patients for anti-EGF response, as a full characterization of the immunogenicity was done in all previous trials (five exploratory and one phase II trials). This protocol evaluated a different vaccine dose and schedule, as compared with the controlled phase II, that yielded a good antibody response in 53% of the vaccinated patients. After vaccinating with a high-antigen dose at four injection sites (current phase III protocol), 78.8% of the patients had a good response (anti-EGF antibody titers >4,000). Still, only 21.2% of the vaccinated patients achieved a super good response (anti-EGF > 1:64,000). CIMAvax-EGF was not only immunogenic but also reduced the EGF concentration to undetectable levels. There was an inverse correlation between serum EGF and the anti-EGF antibody titers.

Regarding efficacy, patients who received at least four doses of CIMAvax-EGF had a significant survival advantage. For active immunotherapy, where the target is the immune system and not the tumor, it is mandatory to administer a minimum number of vaccine doses to break the tolerance against self-antigens (27, 28).

Moreover, in a *post hoc* analysis in the safety population, CIMAvax-EGF significantly increased OS when a weighted log-rank test was used. The Harrington–Fleming test is very sensitive to detect a delayed effect in the survival curves when the HR is not proportional. The "delayed benefit" and the survival advantage in a population completing the induction period indicate that there is a time lag before CIMAvax-EGF can be effective (17, 28). A rational combination with chemotherapy can allow the vaccine to "buy" that time. The immunogenicity of CIMAvax-EGF has been already demonstrated in combination with platinum doublets.

PFS was not a secondary goal of the study, as endpoints based on tumor assessments (response rate, PFS) may not be appropriate for a late-phase clinical trial for a cancer vaccine. Previously, other vaccines and immunomodulatory antibodies have not shown improvement in PFS: sipuleucel-T and PROSTVAC-VF in prostate cancer and ipilimumab and eltrapuldencel-T in

Table 2. Number of patients with more frequent adverse events by study arm

Adverse events	Vaccine ($n = 246$)	%	Controls ($n = 132$)	%
Injection site reactions	116	46.6	0	0
Fever	91	36.5	10	7.6
Dyspnea	79	31.7	38	28.8
Vomiting	58	23.3	5	3.8
Headache	56	22.5	9	6.8
Nausea	45	18.1	11	8.3
Anorexia	38	15.3	21	15.9
Anemia	35	14.1	13	9.8
Asthenia	29	11.6	13	9.8
Hypertension	25	10.0	4	3
Fatigue	19	7.6	6	4.5
Arthralgia	18	7.2	17	12.9
Hypotension	15	6.0	4	3
Myalgia	15	6.0	2	1.5
Bones pain	13	5.2	14	10.6
Flu-like symptoms	13	5.2	5	3.8
Dry skin	7	2.8	0	0
Mouth dryness	6	2.4	0	0
Bronchospasm	5	2.0	1	0.8
Blurred vision	4	1.6	0	0
Urticarial reactions	3	1.2	0	0

melanoma were associated with no improvement in PFS and response rate, but statistically significant benefit in OS (29).

The incidence of activating EGFR and ALK translocations was not evaluated in the trial, as the TKIs targeting the referred mutations were not accessible. However, theoretically, CIMAvax-EGF would be effective in patients lacking EGFR mutations. EGFR is constitutively activated in tumors with mutations at the intracellular domain, which do not require EGF binding for signal transduction. According to our preliminary data, CIMAvax-EGF is more active in Caucasian, smoker males bearing SCCs. Patients benefiting largely from EGFR TKI are Asian, female, nonsmoker with adenocarcinomas. These demographic characteristics correspond with EGFR-sensitizing mutations at exons 19 and 21. The actual correlation between EGFR mutations and efficacy of CIMAvax-EGF will be addressed in the forthcoming trials.

Particularly, our data suggest that survival gain occurs mainly in patients having high EGF concentration after front-line chemotherapy. MST in this patient population (14.66 months) is comparable with the survival of patients receiving other drugs recommended as continuation or switch maintenance. This result is more relevant, provided that 94% of patients did not receive second-line chemotherapy upon progression. This observation highlights the importance of a predictive biomarker to maximize the therapeutic value of CIMAvax-EGF. A new clinical trial enrolling patients with [EGF] above the 870 pg/mL threshold is already ongoing (30).

In summary, CIMAvax-EGF is a very safe drug that could be a feasible intervention for long-term control of those NSCLC patients with tumors depending on the EGF, capable to mount a rapid and durable response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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