# Clinical Development and Perspectives of CIMAvax EGF, Cuban Vaccine for Non-small-cell Lung Cancer Therapy

**Pedro C Rodríguez, MD, Gryssell Rodríguez, MS, Gisela González, PhD, Agustín Lage, MD, PhD**

## ABSTRACT

**Introduction** CIMAvax EGF is a therapeutic anticancer vaccine developed entirely in Cuba and licensed in Cuba for use in adult patients with stage IIIB/IV non-small-cell lung cancer (NSCLC). The vaccine is based on active immunotherapy by which an individual's immune response is manipulated to release its own effector antibodies (Abs) against the epidermal growth factor (EGF).

**Objective** Review pre-clinical and clinical research conducted during development of CIMAvax EGF, primarily studies published by Cuban investigators in international peer-reviewed scientific journals.

**Methods** An automated search for "vaccine" and "EGF" was conducted in PubMed, resulting in 17 articles published by Cuban authors between January 1, 1994 and September 30, 2009. Main findings were described and discussed, along with unpublished preliminary findings of an initial ongoing phase III clinical trial.

**Results** Articles reviewed describe five phase I/II and one phase II clinical trials conducted in Cuba in 1995–2005. A non-controlled 1995–1996 study resulted in the earliest published scientific evidence of the feasibility of inducing an immune response against autologous EGF in patients with different advanced stage tumors. Subsequent controlled, randomized trials included patients with advanced stage (IIIB/IV) NSCLC. The 2nd and 3rd phase I/II trials differentiated immunized patients as poor antibody responders (PAR) and good antibody responders (GAR), according to their anti-EGF antibody response, and confirmed greater immunogenicity with Montanide ISA 51 adjuvant in the vaccine formulation, as well as the benefits of low-dose cyclophosphamide treatment 72 hours before the first immunization. The 4th phase I/II trial found increased immunogenicity with an increased dose divided in 2 anatomical sites and also established correlation between Ab titers, serum EGF concentration and length of survival. In the first 4 phase I/II trials and the phase II trial, vaccine was administered after chemotherapy (ChTVV schedule). In the 5th phase I/II trial, longer survival and increased immunogenicity were achieved using a VChTV schedule and dividing the vaccine dose in 4 anatomical sites. The phase II clinical trial confirmed results of earlier studies as well as the mild-to-moderate adverse event profile associated with CIMAvax EGF. Longer survival was observed in all vaccinated patients compared to controls, and the difference was significant ($p < 0.05$) in the group aged <60 years.

**Conclusions** CIMAvax EGF's benefits in earlier NSCLC stages and in other tumor locations, as well as in patients unfit for chemotherapy, need to be evaluated. Evidence of the vaccine's safety for chronic use also needs to be systemized.

**Key words:** Epidermal growth factor, EGF receptor, non-small-cell lung carcinoma, lung cancer, vaccine therapy, immunotherapy, cancer vaccines

## INTRODUCTION

In Cuba, cancer is the second leading cause of death and the primary cause of years of potential life lost, making a significant impact on life expectancy at birth. Lung cancer is the malignant disease with highest incidence and also the leading cause of cancer mortality in the country. In 2005–2007, an average of 4234 new lung cancer cases and 4601 deaths from the disease were reported annually, for a crude mortality rate of 54.3 men and 27.3 women per 100,000 population. If present demographic trends and risk factors persist, lung cancer incidence and mortality can be expected to rise significantly in Cuba in the next five years.[1]

Recognizing rising cancer incidence and mortality as a major public health problem, the Cuban Ministry of Public Health has implemented a Comprehensive Cancer Control Program (PICC, its acronym in Spanish), operating across all levels of the national public health system. This program constitutes a new therapeutic approach to the disease with biotechnology serving as a bridge between basic immunology research and public health. In Cuba, biotechnological research and development is conceived as a complete scientific cycle (closed loop), from concept to clinical application and marketing of scientific products. Income from product sales is invested in both sustaining use of immunotherapies in the national public health system and in research and development of new immunotherapeutic modalities.[2,3]

Although chemotherapies have become an indispensable arsenal for reducing tumor burden and extending survival, their impact on lung cancer is measurable only in months with the added burden of severe adverse reactions. Lung cancer patients generally confront an initial stage of diagnosis and oncological treatment, during which complete or partial remission is achieved, followed by a second stage, during which the disease inexorably progresses toward terminal illness and death. The mechanism of action of immunotherapy products, such as monoclonal antibodies (mAbs) and therapeutic vaccines, is much more selective toward tumor cells and may increase cancer patient survival with improved quality of life.[3–5]

The Epidermal Growth Factor Receptor (EGFR) is a well-known oncogene. Its overactivation can induce malignant transformation of a normal cell, signaling inhibition of apoptosis, cell proliferation, angiogenesis, metastasis and tumor-induced proinflammatory and immunosuppressive processes. The EGFR signaling and transduction pathway can be efficiently interrupted by EGF deprivation, direct specific mAb receptor inhibition, or low molecular weight molecules competing intracellularly with adenosine triphospate (ATP) for the receptor's tyrosine kinase activity site, with negative repercussions on cell proliferation and, consequently, on tumor development.[6,7]

Inducing EGF deprivation by active immunotherapy is an emerging concept developed by Cuban researchers which involves manipulating an individual's immune response to release its own effector antibodies (Abs) against EGF, thereby reducing tumor size or preventing its progression.[8–13]

CIMAvax EGF is a therapeutic anticancer vaccine developed entirely in Cuba. Its active pharmaceutical ingredients (API) are produced by the Center for Genetic Engineering and Biotechnol-

ogy (CIGB, its Spanish acronym); the vaccine is formulated at the Molecular Immunology Center (CIM, its Spanish acronym); and clinical trials are conducted in hospitals meeting professional and technological Good Clinical Practice standards.[14,15] Proof of principle (POP) of this novel therapy's clinical impact was made possible by the integration of Cuban biotechnological development in the public health system and collaboration between research institutes and hospitals.

Since 1995, CIMAvax EGF has undergone five phase I/II and one phase II clinical trials. Results of these investigations led the Government Center for Quality Control of Medicines (CECMED, its Spanish acronym), the Cuban regulatory authority, to license this therapeutic vaccine for use in adult patients with stage IIIB/IV non-small-cell lung cancer (NSCLC). Research results have also been published in various national and international scientific journals.

The objective of this article is to review the pre-clinical and clinical research conducted during development of this novel Cuban therapeutic anticancer vaccine, based primarily on studies published by Cuban investigators in international peer-reviewed scientific journals.

## METHODS

An automated search of the PubMed database (Medline) through WHO's HINARI service was made using the key words "vaccine" and "EGF". General selection criteria were articles published by Cuban authors in peer-reviewed international journals. Seventeen articles, published between January 1, 1994 and September 30, 2009, were found and reviewed for their coverage of concepts, clinical data and essential technological aspects of CIMAvax EGF development and potential impact on NSCLC. The main findings in each article were described and discussed, along with unpublished preliminary findings of an initial ongoing phase III clinical trial.

## RESULTS AND DISCUSSION

### Vaccine Formulation and Clinical Induction of Immune Response against Autologous EGF

When an individual is immunized with autologous EGF (from its own species), no anti-EGF antibody (Ab) response occurs. To prove the feasibility of manipulating an individual's immune response to induce an anti-EGF Ab response, reducing EGF concentration in blood and depriving the tumor of this growth factor,[8] a vaccine formulation was needed that would make EGF recognizable to the immune system, that is, render it immunogenic. Therefore, selection of an adequate immunopotentiator and adjuvant was required.

In 1992, preclinical studies were begun to select the most effective vaccine formulation for inducing autologous EGF immunogenicity. In this stage, components of the vaccine formulation were outlined, and the possibility of inducing effective autologous EGF immunogenicity was demonstrated. Different vaccine formulations were tried, and 2 immunopotentiating proteins were selected: tetanus toxoid (TT) and *Neisseria meningitidis* P64k (P64k), both produced in Cuba (Finlay Institute and CIGB, respectively). Two possible adjuvants were also proposed for clinical evaluation: aluminum hydroxide (Superfos, Denmark) and Montanide ISA 51 (Seppic, France).[13–14]

Clinical development of CIMAvax EGF began in 1995 with a phase I/II open-label clinical trial (Pilot 1) at the Medical-Surgical Research Center (CIMEQ, its Spanish acronym) in Havana. A non-randomized design was used in 10 patients with histologically proven primary malignant tumors in different locations, previously treated with first-line chemotherapy (ChTVV therapeutic schedule) (Table 1). The main objective was to evaluate the immunogenicity of the incipient vaccine formulation.[15]

Results of this first clinical trial constituted the earliest published scientific evidence of the feasibility of inducing an immune response against autologous EGF in patients with different advanced stage tumors. Additionally, protein P64k was confirmed as the optimal immunopotentiator for EGF conjugation and the CIMAvax EGF formulation.

### Selection of NSCLC Target Location and Advanced Development of Vaccine Formulation

A key factor in developing the final vaccine formulation was selection of the tumor location in which to introduce this novel therapeutic strategy. NSCLC was selected because of its frequency and because EGFR is overexpressed in tissues during development and progression of lung neoplasms in the following proportions: 62% of all NSCLC tumors, 89% of squamous cell tumors, 41% of adenocarcinomas and 80% of bronchoalveolar tumors. Magnitude of EGFR expression has been reported in the literature as a predictive factor of response to biological therapy in NSCLC patients.[6,8,11,16]

The next two phase I/II clinical trials (Pilot 2 and Pilot 3) were conducted jointly in overlapping periods (1997–1999 and 1998–2001, respectively) at CIMEQ and the National Oncology and Radiobiology Institute (INOR, its Spanish acronym), also in Havana (Table 1). Pilot 2 included 20 advanced stage NSCLC (IIIB/IV) patients; 10 were randomly immunized with EGF/P64k vaccine using aluminum hydroxide as adjuvant (EGF/P64k/AL), and 10 received EGF/P64k adjuvanted with Montanide ISA 51 (EGF/P64k/Montanide ISA 51).[17]

The Pilot 3 study also included 20 advanced stage NSCLC (IIIB/IV) patients who received the same treatments used in Pilot 2 (EGF/P64k/AL and EGF/P64k/Montanide ISA 51) with a similar randomization in 2 groups, except they all received a cyclophosphamide dose (200 mg/m$^2$ of body surface) 72 hours before vaccine treatment onset.[17] Cyclophosphamide is a widely studied anticancer drug. Its immunomodulating effects have significant dose- and therapeutic schedule-related repercussions.[18,19] This pretreatment was introduced to disrupt immunologic tolerance to EGF and to induce immunogenicity toward this human molecule from the first dose.[17]

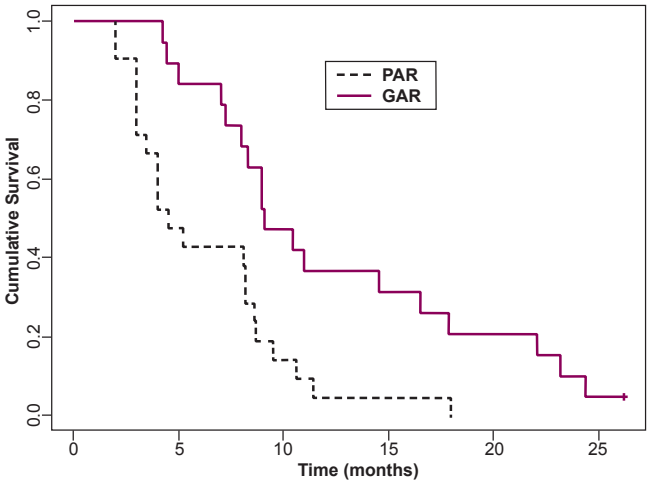
The Pilot 2 and Pilot 3 clinical trials confirmed that vaccination induced a specific anti-EGF Ab response and enabled classification of immunized patients into 2 subpopulations: poor antibody responders (PAR)—those with poor anti-EGF Ab response—and good antibody responders (GAR)—those with an anti-EGF antibody response ≥1:4000 and at least 4 times (4x) their pre-immunization levels. In both studies, survival was longest in the GAR group (mean, 12.41 months; median, 9.1 months), compared to mean, 5.47 months (median, 4.5 months), in PAR patients (Figure 1). The difference in survival was statistically significant ($P < 0.05$). Six-month survival was achieved by 84% of

**Table 1: EGF Therapeutic Anticancer Vaccine Phase I/II Trials Conducted in Cuba, 1995–2005**

| Clinical Trial (Date) | Tumor Location | Design | Vaccine Formulation / Schedule | Results | References |
|---|---|---|---|---|---|
| Pilot 1 (1995–1996) | Lung<br>Colon<br>Stomach<br>Prostate | Phase I/II not controlled<br><br>10 patients<br>(5 per arm) | EGF /TT/AL vs EGF/P64k/AL<br><br>CHTVV Schedule | P64k selected as immunomodulator.<br><br>First clinical report of autologous EGF immunogenicity. | Ann Oncol. 1998 Apr; 9(4):431-5 |
| Pilot 2 (1997–1999) | NSCLC Stages IIIB/IV | Phase I/II controlled, randomized<br><br>20 patients<br>(10 per arm) | EGF/P64k/AL vs EGF/P64k/Montanide ISA 51<br><br>CHTVV Schedule | Montanide ISA 51 confirmed as adjuvant.<br><br>PAR and GAR classification of immunized patients. | Ann Oncol. 2003 Mar; 14(3):461–6 |
| Pilot 3 (1998–2001) | NSCLC Stages IIIB/IV | Phase I/II controlled, randomized<br><br>20 patients<br>(10 per arm) | Cyclophosphamide immunomodulation (200 mg/m$^2$ SC) 72 hours before vaccine<br><br>EGF/P64k/AL vs EGF/P64k/Montanide ISA 51<br><br>CHTVV Schedule | Cyclophosphamide immunomodulation pretreatment introduced.<br><br>PAR and GAR classification. | |
| Pilot 4 (2000–2003) | NSCLC Stages IIIB/IV | Phase I/II controlled, randomized<br><br>43 patients<br>(21 and 22 per arm) | EGF/P64k/AL (1 deltoid) vs EGF/P64k/AL (2 deltoids)<br><br>CHTVV Schedule | Increased immunogenicity with double dose divided in 2 deltoids.<br><br>Correlation between Ab titers, serum EGF concentration, and survival. | Cancer Biol Ther. 2006 Feb; 5(2):130–40 |
| Pilot 5 (2001–2005) | NSCLC Stages IIIB/IV | Phase I/II controlled, randomized<br><br>20 patients<br>(10 per arm) | EGF/P64k/Montanide ISA 51 administered in 4 sites (2 deltoids, 2 gluteus)<br><br>VCHTV Schedule | Longer survival than the historical control.<br><br>Increased immunogenicity (sGAR). | J Immunother. 2009; 32(1):92–9. |

NSCLC Non-small-cell lung cancer

**Figure 1: Survival Functions for Good Antibody Responders (GAR) and Poor Antibody Responders (PAR) to EGF Vaccination in 2 Pilot Phase I/II Clinical Trials (pooled data).[17]**




GAR and 38% of PAR. Twelve-month survival was achieved by 37% of GAR and 4% of PAR. These studies also showed that patients with an anti-EGF Ab response ≥60 days survived longer than those whose response lasted <60 days.[17]

Results of the Pilot 2 and Pilot 3 phase I/II clinical trials confirmed greater immunogenicity with Montanide ISA 51 adjuvant (EGF/P64k/Montanide ISA 51) in the vaccine formulation, as well as the benefits of low-dose cyclophosphamide treatment 72 hours before the first immunization.[17,20]

A 4th phase I/II clinical trial (Pilot 4), conducted at the Hermanos Ameijeiras Hospital (HAH) in Havana in 2000–2003, evaluated 2 dose levels of the therapeutic vaccine. Forty-three patients with advanced stage (IIIB/IV) NSCLC were randomized in 2 groups and received 71 µg or 142 µg of EGF (Table 1). The lower dose was applied in one deltoid region and the higher dose distributed between the 2 deltoid regions.[21]

The Pilot 4 study established a correlation between vaccine dose, anti-EGF Ab titers, EGF serum concentrations, and patient survival. Survival in treated patients (mean, 9.83 months; median, 8 months) significantly exceeded ($p < 0.05$) the historical control (mean, 6.2 months; median, 4.1 months) while positively correlating with GAR titers (≥1/4000) and lower serum EGF concentrations (<168 pg/ml). This clinical trial demonstrated for the first time that EGF serum concentration levels decrease when Ab titers rise.[21]

**Return to Preclinical Research: Manipulating Immunopharmacological Variables and Fine Tuning the Therapeutic Schedule**

The immunopharmacology of cancer vaccines is not yet fully understood, and there is scant data on immunopharmacological determinants influencing therapeutic anticancer vaccines.[22–28] Many variables must be evaluated, such as therapeutic schedules, administration route, dose, dosing interval, and optimal combination with already established therapies, among others.

This process is even more complex when attempting to fine tune the therapy schedule of a vaccine based on a circulating self-molecule, such as autologous EGF, and induce an Ab response that suppresses it from circulation, thus depriving its receptor.

For CIMAvax EGF vaccine, more efficient strategies for inducing Ab response (priming) were needed, as well as for long-term response maintenance through reimmunization (boosting).[25–27] Investigators therefore returned to preclinical research using murine biomodels, defined as GAR (BALB/c mice) or PAR (C57BL6 mice), depending on their genetic background and antibody response when challenged with the vaccine. The animals were immunized with CIMAvax EGF vaccine (EGF/P64k/Montanide ISA 51), and some immunopharmacological variables (dose, number of immunizations, dosing interval) were manipulated during both the response induction phase (priming) and the reinforcement or reimmunization (boosting) phase, aimed at inducing an early, robust and prolonged anti-EGF Ab response, potentiating its active immune deprivation.[29]

For priming, fractioning an apparently low dose (4 µg) into 4 parts administered by intramuscular injection in different anatomical sites increased maximal Ab titer levels and extended the duration of vaccine response. Shortening the interval between booster doses reduced persistence of anti-EGF Ab titers, whereas repeated boosting converted PAR status to GAR.

Results of this study led to the conclusion that the vaccine should be administered in a high but fractioned dose in multiple anatomical sites (such as the 2 deltoid and 2 gluteal regions), thereby bringing the EGF vaccine closer to regional lymph nodes and synergizing the immune response.[29]

**Return to Clinical Trials: Combining Therapeutic Vaccination (V) and Chemotherapy (ChT)**

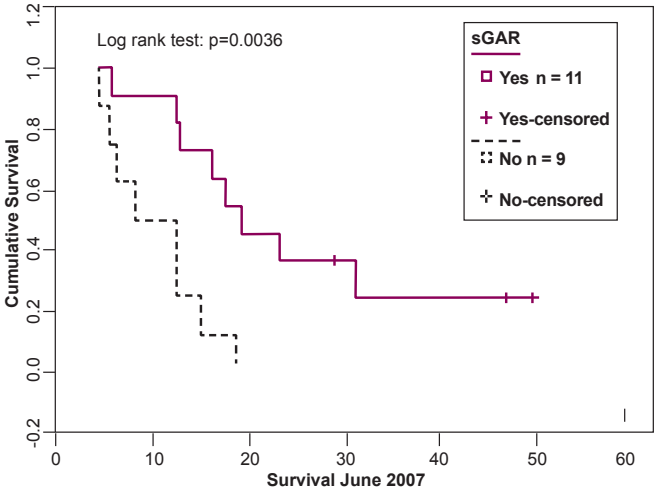
Tumor biology is the result of cell genome interaction with environment; in addition to mutual influence, this interaction has repercussions in both reconfiguration of cell metabolism and evolutionary selection of more efficient tumor mechanisms. The immune system is one of the critical elements involved in shaping cell phenotype.[30–34]

The complexity of mechanisms involved in malignant transformation of cells lends itself to a combination therapy approach aimed at, among other things, simultaneously controlling tumor immunoevasion and dissemination. In this context, immunotherapies emerge as an approach that ensures greater specificity and limited associated toxicity. Immunotherapies have been included in standard cytotoxic therapeutic regimens since they were first developed. Passive mAb immunotherapy, in particular, has confirmed the potential of these combinations.[35–39] In this context, it was hypothesized that potentially autoreactive lymphocyte clones, capable of generating an autologous anti-EGF antibody response, reemerge from chemotherapy-induced maximum lymphocyte depletion (lymphopenia nadir), amplified with homeostatic advantage.[40,41]

Based on these concepts and preclinical research results, a therapeutic schedule combining vaccine, chemotherapy and more vaccine (VChTV) was proposed. This schedule was evaluated in the 5th phase I/II clinical trial (Pilot 5) begun in 2001 in the Hermanos Ameijeiras Hospital (HAH) in Havana, using the CIMAvax EGF (EGF/P64k/Montanide ISA 51) formulation in 20 stage IIIB/IV NSCLC patients. This study corroborated the correlation between an increase in anti-EGF Ab titers and a decrease in serum EGF concentration. Correlation between antibody titers and EGF/EGFR binding inhibition capacity was also shown, and GAR patient sera immunodominance against loop B of the EGF molecule was established.[42]

Another important finding in the Pilot 5 clinical trial was the differentiation of a new subpopulation of patients with anti-EGF antibody titers ≥1:64000, defined as super good antibody responders (sGAR). A correlation between Ab response and survival was also observed; sGAR patients survived significantly longer than patients classified as only GAR (Figure 2). This clinical finding tends to confirm the hypothesis that potentially autoreactive clones reemerge and amplify, possibly favoring active immunization with the VChTV therapeutic schedule.[42]

**Figure 2: Survival Functions of Super Good Antibody Responders (sGAR) Compared with Good Antibody Responders (GAR) to CIMAvax EGF Vaccine, Pilot Phase I/II Clinical Trial.[42]**




These findings have led to a new line of preclinical research for evaluating the specific influence of each active immunotherapy combination with standard chemotherapy regimens, defining the immunopharmacological variables considered for rational design of combined therapeutic schedules, and establishing the specific impact of these combinations on T and B lymphocyte populations.[43]

**Phase II Clinical Trial: Proof of Principle and Licensing for NSCLC Therapy**

Parallel to evaluation of the VChTV schedule, a 1st phase II controlled clinical trial was initiated in December 2001 to evaluate the survival effect of CIMAvax EGF (EGF/P64k/Montanide ISA 51) immunization in advanced stage (IIIB/IV) NSCLC patients previously treated with first-line chemotherapy (ChTVV).[44] This trial included 80 patients randomized 1:1 and was conducted in 4 Havana hospitals (HAH, CIMEQ, INOR and the Benéfico Jurídico Hospital) and 5 provincial hospitals (III Congreso Hospital in Pinar del Río, Celestino Hernández Hospital in Villa Clara, María Curie Hospital in Camagüey, Vladimir Ilich Lenin Hospital in Holguín, and Saturnino Lora Hospital in Santiago de Cuba).

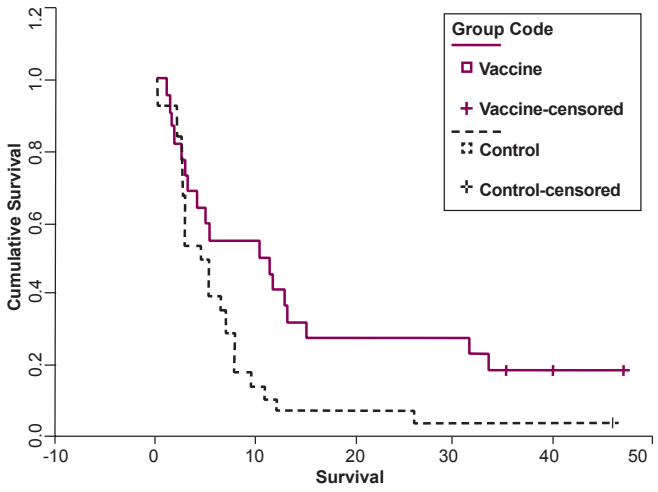
In this phase II clinical trial, the safety profile observed in earlier phase I/II vaccine studies was confirmed. No grade 3 or 4 treatment-related adverse events (AE) were detected according to National Cancer Institute Common Toxicity Criteria version 3.0. The most frequent AE were fever, headache, chills and pain at the injection site (Table 2). Correlations between higher anti-EGF Ab titers and lower serum EGF concentrations, and between higher anti-EGF Ab titers and longer patient survival were also confirmed.

**Table 2. EGF Vaccine-Related Adverse Events by Arm, Phase II Clinical Trial**

| Event | Vaccine (n=40) | | Control (n=40) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Fever | 10 | 25 | 3 | 7.5 |
| Chills | 7 | 18 | 0 | 0 |
| Nausea | 4 | 10 | 3 | 7.5 |
| Vomiting | 4 | 10 | 1 | 2.5 |
| Tremor | 7 | 18 | 0 | 0 |
| Headache | 10 | 25 | 4 | 10 |
| Arthralgia | 5 | 13 | 0 | 0 |
| Asthenia | 8 | 20 | 7 | 18 |
| Injection-site pain | 5 | 13 | 0 | 0 |
| Acneiform rash | 1 | 2.5 | 0 | 0 |

Mean survival was 19.47 months (median, 11.7 months) in GAR patients (n=20), 4.97 months (median, 3.6 months) in PARs (n=18), and 8.52 months (median, 5.33 months) in the control group (n=37). Longer survival was observed in all vaccinated patients compared to randomized unvaccinated controls, and the difference was significant ($p < 0.05$) in the group aged <60 years (mean,18.53 months; median, 11.47 months in those vaccinated compared to mean, 7.55 months; median, 5.33 months in controls) (Figure 3).[44]

**Figure 3: Survival Functions for Patients Aged <60 Years, Phase II Clinical Trial.[44]**




Vaccinated: n=22
Control: n=28
Log Rank test: p=0.0124

Laboratory results associated with the CIMAvax EGF mechanism of action confirmed the EGF/EGFR binding inhibition capacity of vaccinated patient sera, and the capacity of vaccinated patient sera to inhibit EGFR phosphorylation was established for the first time. Additionally, survival was better among vaccinated patients whose sera inhibited EGFR phosphorylation, and was significantly better in patients whose sera preferentially recognized loop B of the EGF molecule.[45]

This phase II clinical trial contributed to the proof of principle of the therapeutic vaccination's clinical effect by demonstrating increased survival of CIMAvax EGF-vaccinated patients compared to the control group, and by demonstrating the feasibility of manipulating an individual's immune response to release its own effector Abs against EGF as a tumor growth factor, thereby reducing tumor size or preventing its progression.

Evidence obtained from the 5 phase I/II clinical trials (Table 1) and the results of this phase II study led CECMED, the Cuban regulatory authority, to license CIMAvax EGF as a therapeutic vaccine indicated for adult advanced stage (IIIB/IV) NSCLC patients.[46]

**Technological Development: Production and Quality Control Systems**

Technological development of CIMAvax EGF therapeutic anticancer vaccine involved inducing autologous EGF immunogenicity by conjugating it with other molecules acting as immunopotentiators (P64k), selecting the most appropriate adjuvant (Montanide ISA 51) and increasing the quantity with each formulation, beginning with laboratory amounts used in preclinical studies and vaccination of the first patients, through scaling up to supply the ongoing phase III clinical trial in Cuba and other clinical trials abroad, as well as health system demand for use in treating patients.

The EGF used in the vaccine is a recombinant human growth factor (hu-rEGF). The P64k protein is also recombinant; both are produced by CIGB as active pharmaceutical ingredients and supplied to CIM, where they are chemically conjugated and the final vaccine formulation is prepared.

Parallel to the production process, physicochemical assays have been conducted to characterize the product at different stages of development, from POP to the current vaccine formulation.[47] Steps have been taken at each stage to guarantee comparability between different batches and different stages of development. Good Manufacturing Practices (GMP) have been observed throughout the production and quality control process, which is audited and inspected by regulatory agencies from Cuba and other countries.

Conducting assays to evaluate vaccine immunogenicity has been particularly difficult. This type of assay is carried out *in vivo*. Autologous human EGF is foreign to other species. To overcome this challenge, NMRI mice were used. The genetic background of this outbred line of mice (no parental mating) makes it an adequate biomodel for representing the genetic variability of open human populations. Due to this particularity, NMRI mice only produce anti-EGF Ab responses when immunized with the whole vaccine formulation (EGF/P64k/Montanide ISA 51) and do not respond when immunized with the EGF molecule alone, that is, outside the vaccine formulation that makes it immunogenic.[47]

**Ongoing Phase III Clinical Trial**

A phase III clinical trial has been underway since June 2006 at 18 clinical research sites throughout the country. The same ChTVV therapeutic schedule used in the phase II clinical trial is being used, but the vaccine is being distributed in 4 injection sites. This study is planned to recruit 579 advanced stage (IIIB/IV) NSCLC patients, with a 1:2 randomization (1 control patient for each 2 treated patients). Results will be evaluated in 2 patient strata: aged >60 years (n= 381) and aged ≤60 years (n=198).

Preliminary results from 160 patients show numerical differences in 24-month survival rates. Graphic survival analysis in both strata and by protocol shows a trend towards delayed separation of the curves over time in favor of vaccinated patients compared to unvaccinated, as expected in survival evaluation of patients treated with therapeutic vaccines.[48–50] Although statistical significance of these differences has not yet been confirmed, they suggest possible benefit for patients treated with CIMAvax EGF vaccine.

**Future Challenges**

Even though the survival benefit of therapeutic immunization with CIMAvax EGF in advanced stage NSCLC patients has been demonstrated, this evidence was obtained in a clinical trial context with predefined inclusion and exclusion criteria, and specialized oncology service standards of care. The next stage in CIMAvax EGF vaccine development is the transition from specialized services to primary health care. To meet this objective, systematizing evidence of the vaccine's safety for chronic use needs to begin now.

Today's oncology patients are the object of a therapeutic paradigm shift through which the sequential impact of immunotherapy combined with surgery, chemotherapy and radiotherapy tends to prolong survival with an ethically acceptable quality of life. [3,4,48–50] Cancer is beginning to be approached as a 2-stage disease: the first stage begins with diagnosis, followed by very aggressive toxic treatment in a hospital setting, aimed at maximum reduction of tumor burden; the second stage begins when chemotherapy potential has been exhausted, progression is slow and continuous, prognosis depends on tumor progression speed, and the disease behaves like a chronic non-communicable condition requiring permanent care. It is in this second stage, when, due to their low toxicity, immunotherapies could be administered chronically in a primary care setting.

For CIMAvax EGF, meeting this challenge implies a transition from establishing proof of principle in clinical trials to making an impact on population health by prolonging survival of the approximately 4234 new NSCLC cases reported annually in Cuba and consequently reducing the number of deaths in the same period.

At the same time, the vaccine's effect needs to be evaluated in earlier NSCLC stages and in patients unfit for chemotherapy. Predictors are needed to indicate which NSCLC patient subpopulation may or may not respond to this immunotherapy. Moreover, benefits of administering CIMAvax EGF in other tumor locations—such as prostate carcinoma, in which EGFR has a central role in the resistance mechanisms to androgenic blocking—need to be evaluated.[51]

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## THE AUTHORS

**Pedro C Rodríguez Rodríguez** (Corresponding author: camilo@cim.sld.cu), physician and pharmacologist. Assistant professor and clinical investigator, Molecular Immunology Center (CIM), Havana, Cuba.

**Gryssell Rodríguez Martínez**, biologist with a master's degree in biotechnology. Project manager, Molecular Immunology Center (CIM), Havana, Cuba.

**Gisela González Marinello**, chemist with a doctorate in biology. Project manager, Molecular Immunology Center (CIM), Havana, Cuba.

**Agustín Lage Dávila**, physician with a doctorate in biochemistry. Full professor and director general, Molecular Immunology Center (CIM), Havana, Cuba.

*Submitted: October 28, 2009*
*Approved for publication: December 11, 2009*