CIMAvax-EGF®

EXECUTIVE SUMMARY

- 2014 -

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Product: CIMAvax-EGF®

Global Summary:

CIMAvax-EGF® is a novel product for advanced lung cancer treatment. It is a therapeutic vaccine inducing an immune response by antibodies against the epidermal growth factor (EGF); an own antigen, which is the main ligand of the epidermal growth factor receptor (EGFR). This antigenic system, known as EGF/EGF-R takes part in the proliferation, angiogenesis and metastases processes. The anti-EGF antibodies induced by vaccination with CIMAvax-EGF® prevent this union, thus blocking the activation of the cellular proliferation mechanism derived from the interaction between the (EGF) ligand and the (EGF-R) receptor. The CIMAvax-EGF® vaccine is a biotechnological product composed by an antigen (conjugate rhEGF-rP64K) and an adjuvant (Montanide ISA51VG). The humanized recombinant EGF is obtained from the *Saccharomyces cerevesiae* yeast, the P64K recombinant protein is a membrane protein of the *Neisseria Meningitides* obtained by recombinant way from the *E. coli* and the Montanide ISA 51 VG adjuvant (Seppic, France). The rP64K carrier protein confers to it the capability for breaking the immune tolerance towards an own antigen, like the EGF, (immunogenicity) and the Montanide ISA51VG oily adjuvant increases this immunogenic capability.

The non-clinical studies evaluated different carrier proteins and adjuvants, and showed that Cimavax-EGF® is immunogenic, has antitumor capability and is safe.

The clinical experience with the CIMAvax -EGF® vaccine started in 1995. Since then up to date (2013), 5 Pilot Assays (Phase I/II) have been concluded in Cuba as well as 2 randomized Phase II assays (1 in Cuba and one in Canada/UK).

Currently, the following studies are ongoing on efficacy-confirming, efficacy and safety on non-small-cells lung cancer (NSCLC):

- Phase III, after the first-line chemotherapy in Cuba,
- Phase II/III, intercurrent with chemotherapy in Cuba,
- Phase /III, after first-line chemotherapy and second-line in Europe.
- Phase /III in China.
- Phase IV post registration, for its extension to the primary health care level in Cuba.

Also ongoing is a Phase II study in patients carrying castration-resistant prostate cancer (CRPC) in Cuba.

More than 2000 patients have received CIMAvax -EGF®, without notifications of related serious adverse events (SEA) having emerged. There are evidences supporting its clinical benefit in terms of an increase in survival and an improvement of the quality of life in patients with advanced NSCLC.

CIMAvax-EGF® has sanitary registration in Cuba and Peru since 2008 and in Paraguay since 2012 for treatment of patients carrying non-small-cells lung cancer in advanced stages (IIIb/IV).

I. Introduction

1.1 Lung cancer. The problem's magnitude

Within neoplasias, lung cancer is number one in incidence and mortality by cancer. Among lung malignancies, non-small-cells tumors prevail, representing between 75 and 80 % of total lung tumors. Approximately, between 50 and 75% of the patients receive a diagnosis of incurable metastatic illness: stage III (loco-regional advanced illness) or stage IV (at- distance metastatic illness).

The survival median of the patients diagnosed with NSCLC in stages III and IV is of approximately 11 months (9-13 months range) after the diagnose, even in those patients who receive all the available onco-specific conventional lines of therapy. Chemotherapy, in particular, moderately increases the survival median in comparison with the supporting treatment in patients with at-distance metastatic illness.

Non-small-cells lung cancer remains currently a non-satisfied medical need. The increasingly deepening understanding of the cellular signaling mechanisms and their essential role in tumor genesis, plus the development of new technologies such as monoclonal antibodies and recombinant proteins, have propitiated the development of new modalities in cancer therapy with promising results.

The emergence of biological therapies and therapies aimed at specific targets, with cytostatic, and not cytotoxic effect on tumors, as well as their impact in the reduction of tumor progression and the possible effect of their rational combination with chemotherapy and radiotherapy, tend to change the concept about cancer being an incurable illness, many times of a short duration, propitiating instead the conception of cancer as a chronic disease.

The CIMAvax-EGF® vaccine is a therapeutic modality, which action mechanism lays in stimulating the immune system of the patient receiving the vaccination, by inducing the formation of specific anti-EGF antibodies as the starting point of this immunization, thus breaking the immune tolerance against an own protein. This becomes possible because the vaccine contains an EGF chemical conjugate with the P64K protein, derived from the highly immunogenic *Neisseria Meningitides* (conjugate rhEGF-rP64K), adjuvated with Montanide ISA 51 VG, an enhancer of the antibody response (immune response) specific for the EGF[González G., 2003].

These specific anti-EGF antibodies generated by the own patient, cause an immunologic deprivation or "castration" of this EGF protein, reducing its concentration in serum, thus significantly debilitating the probability that the EGF links to its receptor located at the external membrane of the tumor cell. [González G., 2007].

By this alternative way, the link of the EGF ligand to its receptor becomes unfit, phosphorylation gets prevented, stopping and incapacitating it for unleashing the intracellular signalization. [González G., 2007]. Once the EGFR becomes stopped, the intracellular signal gets extinguished, therefore preventing the start of the cellular proliferation mechanisms depending from the ligand-receptor link, the angiogenesis and metastatic mechanisms weaken, promoting the tumor cell apoptosis [González G., 1996].

In clinical terms, this inhibition of the before mentioned carcinogenic processes, dependent from the activation of the EGFR signalization cascade, translates into a prolonged illness stabilization, and to an increase in survival[González G., 2007; García B, 2008; Neninger E., 2008].

CIMAvax-EGF® also associates to an improvement in the quality of life and a wide safety profile defined by a very low incidence of adverse reactions, of light to moderate intensity [González G., 2007; García B, 2008; Neninger E., 2008].

II. Physical-chemical and pharmaceutical properties. Formulation

2.1 Active principle

The antigen of the CIMAvax-EGF® vaccine is obtained from the chemical conjugation of biological raw materials: the rhEGF recombinant human protein and the rP64K, recombinant protein. Both biological raw materials (BRM) are produced at the Centro de Ingeniería Genética y Biotecnología (CIGB) and acquired by the Centro de Inmunología Molecular for manufacturing the CIMAvax-EGF® antigen. The EGF humanized recombinant comes from the *Saccharomyces cerevesiae* yeast, and is a polypeptide molecule of 53 aminoacids, 6,054 Da of molecular weight. The rP64K protein is a membrane protein of the *Neisseria meningitides* obtained by recombinant way from the *Escherichia coli*, acting as a carrier for increasing the immunologic response against the own EGF molecule.

Both proteins (rhEGF and rP64K) conjugate between themselves through glutaraldehyide as the linking reactive. The protein mixture obtained becomes purified through an Ultrafiltration / Diafiltration process with a polyester-sulfonic membrane of 50 kDa, obtaining the Active Pharmaceutical Ingredient (API).

The purified chemical conjugate rhEGF-rP64K is a heterogeneous product consisting of a complex mixture of multiple molecular species, similar to other conjugated vaccines existing in the market and under clinical research.

The antigen's molecular characterization has been performed using different techniques: SDS-PAGE/Western blot, gel-filtration chromatography, peptides map, mass spectrometry and aminoacids analysis.

All these techniques complement themselves and, although none of them can determine the absolute quantities of each one of the molecular species present, they allow verifying that between lots the relative quantity of each molecular species remains consistent during the manufacturing process. These techniques have shown that the antigen "rhEGF-rP64K conjugate" has two main fractions, one of high molecular weight and another of low molecular weight. The one with low molecular weight is composed by rhEGF monomers and polymers, corresponding to it < 30% of the total chromatogram area. The high molecular weight fraction is composed of rhEGF-rP64k molecular conjugates, constituting ≥ 70% of the total chromatogram area. (Figure 2.1.1)

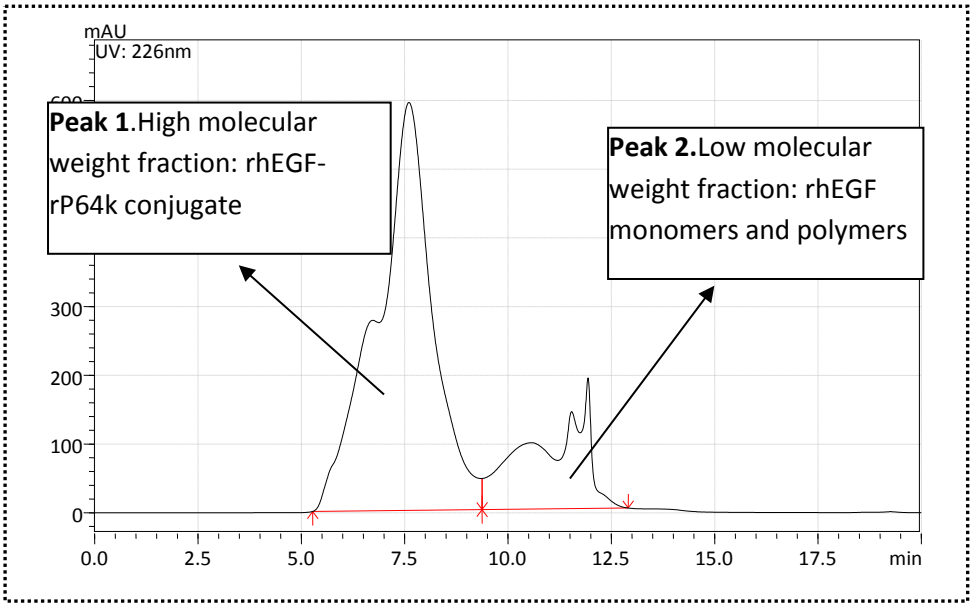


Figure 2.1.1 Gel-filtration type Chromatogram of the CIMAvax-EGF® vaccine's antigen

The quality specifications of the active Pharmaceutical Ingredient (API) of the CIMAvax-EGF® vaccine are: for HPLC-GF area of peak one higher than 70% of the total chromatogram area, this means that more than 70% of the antigen's total mass is composed by conjugated species of rhEGF-rP64k. The specification for protein concentration is 1, 5 - 6 mg/mL and the pH 6, 8 -7, 3. CIMAvax-EGF® vaccine's API is a transparent solution, light yellow in color, which can be stored during 3 months from 2-8 ºC in Stedim pilot (1-5L) disposable, sterile and non-pyrogenic bags

2.2 Formulation of the Pharmaceutical Form, Storage and Handling

The API (rhEGF- rP64K purified conjugate) is formulated with PBS tampon solution until reaching a final protein concentration of 1 mg/mL. The formulated product (at 1 mg/mL total protein concentration) passes through a 0,2µm filter, and later is filled and packaged in 2R bulbs for storage from 2-8ºC during 24 months.

The rhEGF-rP64K conjugate (antigen) is a non-pyrogenic sterile solution with a protein concentration of (1 mg/mL); light yellow in color and transparent, of pH 6, 8-7, 3, dissolved into a sterile phosphate tampon, non-pyrogenic solution. The specification for the chromatography by molecular exclusion for HPLC-FG establishes that the first peak's area should be larger than 70% of the total chromatogram area, guaranteeing that more than 70% of the total mas of the vaccine's antigen are composed by conjugated species of rhEGF-rP64K, warranting a product with biologic activity and clinical efficacy.

The adjuvant of the CIMAvax-EGF® vaccine is Montanide ISA 51 VG, previously used in contraceptive vaccines, vaccines against influenza and poliomyelitis. It consists of a mixture of mineral oil and the Monooleate de Mannide surfactant; with liquid aspect, moderately viscous, light yellow and transparent. Montanide ISA 51 VG is a product from the French company SEPPIC, sold as raw material in 500 mL flasks and acquired by the Centro de Inmunología Molecular (CIM), where is filled into 2R amber bulbs, its final format.

CIMAvax-EGF® is prepared in emulsion form at the time of administering it to the patient, by combining the rhEGF-rP64K conjugate with Montanide ISA 51 VG.

The composition per vial is shown in Table 2.2.1

Table 2.2.1: Quali- quantitative formula of the drug

Each bulb (0,8mL) of rhEGF-rP64K conjugate (antigen of the CIMAvax-EGF® vaccine) contains:

Name of the component	*Quantity*	*Function*	*Quality reference*
rhEGF-rP64K conjugate	0,8 mg	active principle	Manufacturer's own
Sodium chloride (Nacl)	6,4 mg	Tampon solution	USP, Ph. Eur.
Anhydrous dibasic sodium phosphate (Na_2HPO_4)	0,92 mg		USP, Ph. Eur.
Potassium chloride (Kill)	0,16mg		USP, Ph. Eur.
Monobasic potassium phosphate (KH_2PO_4)	0,16 mg		USP, Ph. Eur.
Water for injection	0,8 mL	Vehicle	USP, Ph. Eur.

Each bulb (0.8 mL) of the Montanide ISA 51VG adjuvant contains:

Name of the component	*Quantity per vial*	*Function*	*Quality reference*
Mineral oil	602,48 mg	/	USP
Monooleate de Mannide	77,52 mg	Emulsifier	SEPPIC's internal monograph
Adjuvant	680 mg	adjuvant	Internal monograph

The proportion of the components mixture is 11, 4 %(weight/weight for the Monooleate de Mannide and 88.6% (weight/weight) for the mineral oil.

The rhEGF-rP64K conjugate (antigen) is packed in bulbs, mouth 13 mm ISO-8362-1 Mod. 2R, glass type: White type1, with rubber cap type I, 13 mm, gray color, with silicon A, design 1082, formulation PH4001/45 gray, plastic and aluminum flip off caps 13 mm, blue color and design 5209l.

The adjuvant Montanide ISA 51 VG is packed is bulbs, mouth 13 mm ISO-8362-1 Mod. 2R, glass type: Topaz type1, rubber caps type I, 13 mm, dark gray color, OmniFlex cover, design V9239, formulation FM 259/0. Plastic and aluminum flip off caps, 13 mm, orange color and design 5209.

III. Preclinical Toxicity Studies on the drug

The preclinical toxicological studies proved:

- Immunogenicity of the autologous EGF: Immunization with vaccine preparations of the autologous EGF managed to develop an immune response against this own molecule.
- Anti-tumor activity: *In vivo* models showed anti-tumor effect derived from the vaccination with rhEGF.
- Safety: Toxicity at single dose, at repeated dose and local tolerance revealed no risk for the doses used in humans.
- Selection of the carrier protein: Different proteins were studied, such as cholera toxin, (CTB) [Gonzalez G, Sanchez B, 1996], Tetanic Toxoid (TT), the *Neisseria meningitides* rP64K recombinant protein and several monoclonal antibodies [Gonzalez G, Pardo O.L 1997] in mice and non-human primates. TT and rP64K were selected as the candidate carrier proteins to evaluate in clinical assays with patients with cancer [González G, Crombet T, 1998].
- Selection of the adjuvant: The Freund's complete and incomplete adjuvant, alumina and Montanide ISA 51, were evaluated, the latter being selected for clinical studies. (Gonzalez G, Pardo O.L. 1997)).

3.1 Action mechanisms studies

3.1.1 Immunogenicity studies

The purified murine EGF (mu-EGF) was obtained from the mouse sub-maxillary glands, for studies with mice and human EGF was evaluated in monkeys, as this is a molecule almost identical between the two species. In all cases, immunogenicity of the vaccine preparation was proved.
[González G, Sánchez B, 1996; González G, Pardo O.L, 1997]

In the following table 3.1-1 appears a summary of the immunogenicity studies performed in mice from different stocks.

Table 3.1-1 Summary of immunogenicity studies in mice

Study	Animal species # animals	Antigen, dose (the reported µg are the equivalent quantity in EGF), # doses & way of administration	Adjuvant	Results
RTI 95-025/1	75 BALB/c 5/ doses	rhEGF-TT: 5 µg - 50µg rhEGF-rhEGF: 5µg- 10 µg rhEGF: 0.25 µg - 50 µg 4 doses, weekly, sc.	CFA/IFA (0.1 mL)	Positive anti-EGF titles obtained for all carriers and at all doses assayed.
RTI 95-033/1	35 BALB/c 5/ doses	rhEGF: 1 µg - 5 µg rhEGF-TT: 1µg - 5 µg 4 doses, weekly, sc.	$Al(OH)_3$ (2 mg/ doses)	Positive anti-EGF titles obtained up to µg for the EGF and up to 2.5 µg for the EGF-TT
RTI 95-042/1	70 NMRI 10/ doses	rhEGF-TT: 10 µg rhEGF-rP64K: 10 µg rhEGF: 10 µg rhEGF-B7: 10 µg rhEGF-t3: 10 µg rhEGF-PA: 10 µg 1 doses, sc.	CFA (0.1 mL)	80% of mice did not develop negative titles when immunized with EGF without conjugating to carrier. Positive titles obtained for all animals immunized with EGF conjugated to carrier protein analyzed, with best results for TT and rP64K.
RTI 96-025/1	20 NMRI 5/ doses	rhEGF-TT: 10 µg rhEGF-rP64K: 10 µg rhEGF: 10 µg 1 doses, sc	$Al(OH)_3$ (2 mg/ doses)	80% negative for EGF non-conjugated. 100 % positive for rhEGF-TT and rhEGF-rP64K.
RTI 97-007/10	40 BALB/c 5/ doses	rhEGF- Montanide ISA 51, 50 µg, im rhEGF- Montanide ISA 51, 50 µg, sc -Weekly doses rhEGF-Alum, 50 µg, im rhEGF-Alum, 50 µg, sc -4 weekly doses	Montanide ISA 51 (2 mg/ doses) $Al(OH)_3$ (2 mg/ dose	Positive titles obtained of anti-EGF antibodies when using both adjuvants. The highest titles were achieved with Montanide ISA 51.

Study	Animal species # animals	Antigen, dose (the reported µg are the equivalent quantity in EGF), # doses & way of administration	Adjuvant	Results
RTI 00-003/7	50 BALB/c, 4/ doses	rhEGF- Montanide ISA51, 50 µg, sc rhEGF- Montanide ISA51, 10 µg, sc rhEGF- Montanide ISA51, 100 µg, sc rhEGF- Montanide ISA51, 200 µg, sc	Montanide ISA 51 (50µl/doses)	It is observed a statistically insignificant tendency to increase the titers if the dose increases, starting from 10ug. There were not toxic effects related to the increase of the dose.
RTI 03-008/2	5 BALB/c , 1 y 2/ doses	rhEGF- Montanide ISA51, 4 µg, im	Montanide ISA 51 (50µl/doses)	The conjugated vaccine induces better primary and secondary antibodies responses when compared to non-conjugated molecular species.
RTI 03-008/4	5 BALB/c , 5 BALB/XID 2/ doses	rhEGF- Montanide ISA51, 4 µg, im	Montanide ISA 51 (50µl/doses)	The EGF vaccine induces antibodies responses in both mice stocks, BALB/c y BALB/XID. Higher titles in BALB/c
RTD 04-012/7[a]	28 BALB/c 2/doses	rhEGF- Montanide ISA51, 4 µg, im Adriamycin, 0,265 mg, iv Cyclophosphamide, 2,65 mg iv	Montanide ISA 51 (50µl/doses)	Induced from spleen colony former cells (SFC) after immunization of BALB/c mice.
RTD 04-012/2	24 BALB/c 2/doses	rhEGF- Montanide ISA51, 4 µg, im Adriamycin, 0,265 mg, iv Cyclophosphamide 2, 65 mg iv o 50mg/Kg. Cisplatin, 0.43mg, iv Vinblastina,0,03 mg,iv	Montanide ISA 51 (50µl/doses)	The anti-EGF SFC peak in spleen comes around 7 days after immunization of BALB/c mice.
RTD 04-012/8	30 BALB/c 2/doses	rhEGF- Montanide ISA51, 4 µg, im rhEGF,40 µg, im rP64K, 40 µg,im Adriamycin, 0,265 mg, iv Cyclophosphamide, 2, 65 mg iv.	Montanide ISA 51 (50µl/doses)	Cyclophosphamide + Adriamycin (CA) before the second dose of the vaccine, increases the number of anti-EGF colony-former cells (SFC). This effect is not observed when using the vaccine alone.
RTD 03-008/6	45 BALB/c, 10 BALB/XID 2/doses	rhEGF- Montanide ISA51, 4 µg, im EGF,40 µg, im rP64K, 40 µg, im Adriamycin, 0,265 mg, iv Cyclophosphamide,2,65 mg,iv	Montanide ISA 51 (50µl/doses)	Cyclophosphamide + Adriamycin (CA) before the second dose of the vaccine, increases the number of SFC in spleen.
RTD 04-012/3	6 BALB/c , 6 BALB/XID 2 /doses	rhEGF- Montanide ISA51, 4 µg, im EGF,40 µg, im P64K, 40 µg,im Adriamycin, 0,265 mg, iv	Montanide ISA 51 (50µl/doses)	Pre-treatment with CA before the first dose of the EGF vaccine reduces the number of anti EGF SFC in spleen.

Study	Animal species # animals	Antigen, dose (the reported µg are the equivalent quantity in EGF), # doses & way of administration	Adjuvant	Results
		Cyclophosphamide, 2, 65 mg iv. (CA)		
RTD 03-008/6[a]	30 BALB/c , 2 /doses	rhEGF- Montanide ISA51, 4 µg, im EGF,40 µg, im P64K, 40 µg,im Adriamycin, 0,265 mg, iv Cyclophosphamide, 2, 65 mg iv.	Montanide ISA 51 (50µl/doses)	The number of anti-EGF colony formers cells (SFC) diminishes at day 24, which associates with an increase of apoptotic bodies.
RTD 04-012/7	39 BALB/c 2 /doses	rhEGF- Montanide ISA51, 4 µg, im EGF,40 µg, im P64K, 40 µg,im Adriamycin, 0,265 mg, iv Cyclophosphamide, 2, 65 mg iv.	Montanide ISA 51 (50µl/doses)	Treatment CA during the EGF immunization affects primarily the lymphocytes. It does not affect an ulterior increase of anti EGF SFC.

Immunogenicity studies in non-human primates

The following table 3.1-2 summarizes the immunogenicity studies performed in non-human primates, using different carrier proteins: tetanic toxoid, t3, CEA, B7 and rP64K.

Table 3.1-2 Summary of the immunogenicity studies in non-human primates

Study	Animal species	Antigen, dose (the reported µg are the equivalent quantity in EGF)	Adjuvant	Results
RTI 94-03	*Macaca fasicularis* 2 Males	rhEGF-TT: 50µg 4doses, weekly, id.	CFA/IFA (0.1 mL)	Positive anti-EGF titles until month 12 with maximum up to 1:200,000
RTI 95-001	*Cercopithecus aethiop* 1 Males/ 1 Female	rhEGF-rhEGF: 50 µg total rhEGF 4 doses, weekly, sc.	CFA/IFA (0.1 mL) $Al(OH)_3$ (2 mg/doses)	Positive Max. 1:100,000 Positive max 1:5,000
RTI 95-002	*Cercopithecus aethiop* 1 Female, 5 Males	rhEGF-TT: 50 µg rhEGF-t3: 50 µg rhEGF: 50 µg 2 doses, weekly, sc.	$Al(OH)_3$ (2 mg/doses)	Positive for rhEGF-TT and rhEGF-t3. Negative for EGF alone (non-conjugated). Maximum at 1 month (1:20,000), diminishing at month 3.
RTI 95-003	*Pantroglodytes chimpanzee* 3 Females	rhEGF-t3: 50 µg rhEGF + t3: 50 µg rhEGF-CEA1: 50 µg 4 doses, weekly, sc.	CFA/IFA (0.1 mL)	All positive. Max rhEGF-t3 1:50,000; rhEGF + t3 1:10,000; rhEGF-CEA1 1:200,000
RTI 95-004	*Cercopithecus aethiop* 4 Females, 2 Males	rhEGF-rP64K: 50 µg: d1,d7,m6 rhEGF-B7: 50 µg: d1, d7 rhEGF-t3: 50 µg: d1, d7, m6 All sc.	$Al(OH)_3$ (2 mg/doses)	Maximum positive: RhEGF-rP64K 1:20,000; rhEGF-B7 1:10,000; rhEGF-t3 1:10,000.

In all studies, with different carrier proteins and employed adjuvants, immune response against rhEGF was obtained. No animal, from the 19 employed in these five studies showed signs of toxicity or evidences of autoimmunity disorders.

Conclusions of the immunogenicity studies

These studies showed the immunogenicity of the autologous EGF in mice and non-human primates (19 totally immunized), as well as the need to conjugate them into carrier proteins to transform them in immunologic ones.

3.1.2 Action mechanisms studies: in vivo antitumor activity

Three studies were performed in mice, administering to them the rhEGF plus AIF protein; in two of the studies, the mice were transplanted with the TAE (Erlich ascitic tumor) and the third one took place in mice, to which lung tumor with urethane was induced.

A study was performed in which one of the groups of animals was treated with the conjugated rhEGF-rP64K vaccine in Montanide ISA 51 to observe the effects of vaccination on the number of pulmonary metastases with the F3II tumor model.

Also studied was the anti-metastatic effect of the vaccination with rhEGF-rP64K, combined with cyclophosphamide as immunosupressors, in a study performed in the tumor model 3LL-D122.

In tumor models: Erlich ascitic tumor, chemo-induced tumor by urethane, F3II and 3LL-D122 the anti-tumor effect of the vaccination was verified.

Table 3.1-3 shows the summary of the studies performed for determining anti-tumor activity.

Table 3.1-3 Summary of anti-tumor activity

Study	Animal species	Antigen, dose (the reported µg are the equivalent quantity in EGF)	Tumor model	Results
RTA 97-007/13	70 NMRI, female, 4 doses, weekly.	60 mice: 50 µg rhEGF /ACF-AIF 10 mice : PBS /ACF-AIF	Ehrlich ascitic tumor(EAT), intra-peritoneal transplant	The immunized animals that developed titles showed a significant increase in survival, compared with non-immunized controls, an increase depending of the Anti-EGF antibodies titles.
RTA 96-013/1	20 Balb C, female, 4 doses, weekly.	10 mice: 50 µg rhEGF / ACF-AIF 10 mice: PBS/ACF-AIF	Ehrlich ascitic tumor(EAT), subcutaneously transplanted	The group of immunized mice showed a slower tumor growth than the control group
RTA 97-007/19	40 Balb C, female, 4 doses, weekly.	20 mice s: 50 µg rhEGF/ACF-AIF 20 mice: PBS/ACF-AIF.	Chemical tumor induced with urethane previous to immunization with rhEGF	The group of immunized mice showed less lung tumor focuses in months 3 and 10 after inducting the tumor.
EXP 004/03/2002	30 Balb C, female	10 mice: 50 µg rhEGF-rP64K/ACF/Montanide ISA51 10 mice: 10 µg TGF-rP64K / ACF/Montanide ISA51 (negative control) 10 mice: PBS/ACF/Montanide ISA51 ALL: 4 doses every 15 days	F3II	The group of mice immunized with the rhEGF-rP64K chemical conjugate showed lower number of metastases tan the negative controls
CENP-04	49 C57BL/6, males	7 groups of 7 mice c/u received different combinations of rhEGF-rP64K / alumina with chemotherapy or anti-CD25	3LL-D122	Reduction in number of metastases in immunized mice, further reduction when combining vaccination with immunosuppression.

3.2 Toxicology

Acute and sub-acute toxicology studies for the EGF vaccine were performed at Nucro Technics, Canada. These experiments proved that there is not an obvious toxicity induced by a single dose equivalent in humans of the vaccine preparation adjuvated in Montanide ISA51; a single dose from 10 to 100 times the equivalent dose in humans without adjuvant, or 14 repeated daily doses of doses equivalent to 10 times the one used in humans without adjuvant.

The effect of single or multiple doses of the vaccine preparation and of the resulting anti-EGF antibodies was studied in 2 assays comparing pregnant female mice immunized with similar controls non-immunized regarding pregnancy and characteristics of the breeding; also on newly-born mice.

No adverse effects were observed for multiple doses of 2500 X (ACF/AIF) or single dose of 30,000 X (Al (OH) 3 adjuvant) equivalent to human hu EGF dose.

A third study assessed the effect of high doses (2500 X, ACF/AIF) in hepatic enzymes and histopathology for diverse tissues and organs. No toxic effects were observed, being particularly relevant that there were not negative effects in the gut cells, which present a high content in rhEGF.

In the immunogenicity studies in mice, different toxicological parameters were also measured. The animals received multiple doses of hu-r-EGF with or without adjuvant (ACF, AIF, Al (OH) 3; Montanide ISA51). From the 19 animals of 3 different species, only one that received hu-r-EGF-hu-r-EGF en Al (OH) 3 presented an adverse effect. At the end of the study the animal presented symptoms of health deterioration and elongation of the left part of the thorax. The animal was treated with vitamins and the symptoms disappeared. This animal was previously used in other study on monoclonal antibodies. There are no conclusions about the cause of this episode, but a retarded effect of the former treatment cannot be discarded. The animal stayed alive for three years after the study started, and gave birth to a healthy offspring. Two monkeys were treated with the EGF vaccine formulated to be used in the Canada assays. None of them showed adverse events during the course of the study and both remained alive and healthy during the whole observation period.

In conclusion, there is no evidence of the EGF vaccine being toxic in mice or monkeys, and the safety profile in animals was satisfactory enough for guaranteeing studies in humans.

The safety profile of the rh*EGF-P64Kvaccine/adjuvated in Montanide* obtained by the manufacturing process at laboratory scale and by the industrial scales process through a toxicity study at repeated doses during 6 months, intramuscular way was evaluated in rats Cenp: SPRD.

Table 3.2-1 shows a summary of the main results of the toxicological studies.

Table 3.2-1 Summary of the toxicological studies in mice and rats performed with the rhEGFrP64K vaccine.

Study	Animal species	Antigen, dose (the reported µg are the equivalent quantity in EGF)	Adjuvant	Results
Acute toxicity (single dose)				
CIMYM-001-TS YMI/9902-1.	30 mice CD: 15 males, 15 females	1X, 10X y 100X equivalent dose in humans of rhEGF-rP64K/alumina	Alumina	Acute toxicity study: No evidences of toxicity at the studied doses (1X, 10X and 100X humans).
Sub-acute toxicity (repeated dose)				
CIMYM-002-TS YMI/9902-2	20 mice CD-1 10 males, 10 females	10X dose in humans of non-adjuvated rhEGF-rP64K 1 daily during 14 days	Without adjuvant	Sub-acute toxicity study: No evidences of toxicity to the studied dose (10X humans).
CIMYM-004-TS YMI/003-1	30 rats CD 15 males and 15 females	15X dose in humans of rhEGF-rP64K /Montanide ISA51 5 doses in 2 weeks	Montanide ISA 51	Sub-acute toxicity study: No evidences of toxicity to the studied dose (15X humans).
RTT 95-030/1	10 BalbC, females	5 mice: 50 µg rhEGF/ACF-AIF; 4 doses 5 mice: PBS/ACF-AIF, 4 doses	ACF / AIF	No effects observed on the tissues studied or in the hepatic enzymes, with the exception of an increase in areas of the terminal segment of the salivary glands of immunized mice.
CEGF1006	120 Rats Cenp:SPRD 60 females y 60 males	1 administration weekly until reaching 9 administrations (days 0, 7, 14, 21, 28, 35, 42, 49, 56). Remaining period: 1 administration each14 days, until reaching 9 administrations (days 70, 84, 98, 112, 126, 140, 154, 168 y 182)	Montanide ISA 51	The EGF-P64K/MONTANIDE vaccine purified by ultrafiltration/dialysis administered by intramuscular way in repeated doses in rats Cenp: SPRPD did not cause alterations at systemic level or in the injection site, without evidencing differences between both vaccines.

IV. Clinical studies and effects in humans

The clinical experience with the CIMAvax-EGF® vaccine started in 1995. Since then, until today (2013), 5 Pilot Assays (Phase I/II) have concluded in Cuba, 2 randomized Phase II assays (1 in Cuba and 1 in Canada/UK) and 1 Phase III clinical assay in Cuba.

Currently, the following studies are ongoing for efficacy confirmation, effectiveness and safety in non-small-cells lung cancer (NSCLC):

- Phase III, after the first-line chemotherapy in Cuba,
- Phase II/III, intercurrent with chemotherapy in Cuba,
- Phase /III, after first-line chemotherapy and second-line in Europe.
- Phase /III in China.
- Phase IV post registration, for its extension to the primary health care level in Cuba.

Also ongoing is a Phase II study in patients carrying castration-resistant prostate cancer (CRPC) in Cuba.

More than 2000 patients have received CIMAvax -EGF®, without notifications of related serious adverse events (SEA) having emerged. There are evidences supporting its clinical benefit in terms of an increase in survival and an improvement of the quality of life in patients with advanced NSCLC.

Table 4.1 summarizes the concluded clinical assays.

Table 4.2 summarizes the ongoing studies.

Table 4.1 Concluded clinical assays with CIMAvax-EGF®.

Clinical assays (Cuba)	No. de patients and indications	Formulation	Treatment scheme	Published results
IIC RD EC019 Phase I/II (Pilot 1)	10 (epithelial tumors)	rhEGF-TT rhEGF-rP64K/ Alumina	After CT 2 doses, days 0 and 7	Annals of Oncology 9: 1-5, 1998.
IIC RD EC025 Phase I/II (Pilot 2)	20 (NSCLC)	rhEGF-rP64K/Montanide rhEGF-rP64K/Alumina	After first-line CT. Induction phase: 5 doses: 1 weekly Consolidation phase: Monthly re immunizations	Annals of Oncology 2003, 14: 461-466.
IIC RD EC033 Phase I/II (Pilot 3)	20 (NSCLC)	rhEGF-rP64K/Montanide rhEGF-rP64K/Alumina	After first-line CT. CPA: 3 days before vaccination. Induction phase: 5 doses: 1 weekly Consolidation phase: Monthly re immunizations	
IIC RD EC041 Phase I/II (Pilot 4)	40 (NSCLC)	rhEGF-rP64K/Alumina Single or double dose	After first-line CT. CPA: 3 days before vaccination Induction phase: 5 doses: 1 weekly Consolidation phase: Monthly re-immunizations	Cancer Biology & Therapy 2006, 5:2, 145-149. Human Vaccines 2007, vol. 3, 1.

Clinical assays (Cuba)	No. de patients and indications	Formulation	Treatment scheme	Published results
IIC RD EC062 Phase I/II (Pilot 5)	20 (NSCLC)	rhEGF-rP64K/ Montanide	VQV Scheme (Vac. –QT-Vac.) CPA: 3 days before vaccination Induction phase: 2 doses: 1 each 14 days. (Days 0 and 14) QTP (Between 4 -6 cycles). CPA: 3 days previous to maintenance phase Consolidation phase: Monthly re-immunizations (until clinical deterioration).	Vaccine. 2008, 26; 26(36):4647–54.
IIC RD EC056 Phase II	80: 40 (vaccine group patients vs. 40 non vaccinated control group patients) (NSCLC)	rhEGF-rP64K/ Montanide	After 1st line CT CPA: 3 days before vaccination Induction phase: 5 doses: 1 each 7 days. Maintenance phase: Monthly re-immunizations (until clinical deterioration). Control Group: Best supporting treatment	Journal of Clinical Oncology. 2008; 26(9):1462-68. Clinical Cancer Research. 2008; 14(3):840-6.
EC Phase II Canada/ United Kingdom	66: 33 vaccine group's patients vs. 33 control group patients (NSCLC)	rEGF-rP64K/ Alumina	After 1st line CT Induction phase: 5 doses: 1 each 7 days.	-

Clinical assays (Cuba)	No. de patients and indications	Formulation	Treatment scheme	Published results
(CIMYM-003-CS)			Maintenance phase: Monthly re-immunizations (until progression).	
IIC RD-EC081 Phase III (Cuba)	405: (270 vaccine group's patients vs. 135 control group patients). Randomization 2:1).	rhEGF-rP64K/ Montanide	After first-line CT. CPA: 3 days before vaccination. Induction phase: 4 doses: 1 each 14 days. Maintenance phase: Monthly re-immunizations (until clinical deterioration). Control group: Best supporting treatment	Closed in monitoring. Results presented in: Journal of Immune Based Therapies and Vaccines 2011, 9:7. 2012 ASCO Annual Meeting abstract No: 2527 Citation: J Clin Oncol 30, 2012 (suppl; acstr 2527)
IIC RD EC 077 Phase II (Cuba)	199 (100 vaccine group patients vs. 99 control group patients	rhEGF-rP64K/ Montanide	Vaccinated group: Received the vaccine-chemotherapy-vaccine scheme (VCV) CPA: 3 days before vaccination. Induction phase: 5 doses: 1 each 14 days. Maintenance phase: Monthly re-immunizations (until the oncologist determined start with CT). Control group: Received only	-

Clinical assays (Cuba)	No. de patients and indications	Formulation	Treatment scheme	Published results
			chemotherapy	

Legend: Alumina: aluminum hydroxide; CPA: Cyclophosphamide; NSCLC: non-small-cells lung cancer; CT: chemotherapy

Table 4.2 Ongoing clinical studies with CIMAvax- EGF®.

Assay/ Phase(country)	Patients	Formulation	Treatment scheme	Status
IIC RD-EC111 Phase II/III (Cuba)	116 (58 vaccine group patients vs. 58 control group patients)	rhEGF-rP64K / Montanide	Scheme VCV (Vac. –CTP-Vac.) CPA: 3 days before vaccination. Induction phase: 2 doses: 1 each 14 days. (Days 0 y 14). CT (Between 4 -6 cycles). CPA: 3 days before vaccination. . Consolidation phase: Monthly re- immunizations (until clinical deterioration). Control group I: (Between 4 -6 cycles of CTP and best supporting treatment.	116 patients included. Closed and ongoing. Information available until Feb 22/ /2013
IIC RD EC120 Phase IV	Inclusion for 2 years all patients with NSCLC receiving onco-specific treatment.	rhEGF-rP64K / Montanide	After first –line CT. Induction phase: 4 doses: 1 each 14 days.	1081 patients included. Closed. Information available until

Assay/ Phase(country)	Patients	Formulation	Treatment scheme	Status
(Cuba)			Maintenance phase: Monthly re-immunizations (until clinical deterioration).	Feb 22. /2013.
Phase I/III China	NA	rhEGF-rP64K / Montanide	NA	Phase I Closed, ongoing
Phase III multinational en 12 European countries	438 (219 vaccine group patients vs. 219 control group patients)	rhEGF-rP64K / Montanide	2 vaccinations before starting the CT (vaccine group) Immunizations in combination with the CT start (cycles 2 and 3) and later a maintenance period	Included: 20 patients.

Legend: CPA: Cyclophosphamide NA: Not available

4.1 Clinical assays phase I/II (IICRDEC019, IICRDEC025, IICRDEC033, IICRDEC41)

Assay IIC RD EC019, phase I/II (Pilot 1): It included 10 patients with tumor of epithelial origin, who receive two doses of the vaccine composed of hu-rec-EGF recombinant protein coupled to tetanic toxoid (hu-r-EGF-TT) (5 patients) or rP64K(protein coming from the *Neisseria meningitides* conjugated with rhEGF (hu-r-EGF-P6K) (5 patients): Both groups used alumina hydroxide as adjuvant. Each dose of the vaccine was administered on days 0 and 14. The main objectives of the study were evaluating safety and immunogenicity of vaccination with own EDF in humans, as well as comparing 2 carrier proteins: TT y rP64k.

Vaccination was safe and immunogenic: 6 out of 10 patients treated achieved serum-conversion of the titles, defined as the double of the EGF antibodies titles, over the titles at basal level. The antibody's response against the TT in the group of patients vaccinated with hu-r-EGF-TT was very high; which was not the case with the antibodies' response in the group of patients vaccinated against rP64K in the group of patients vaccinated with hu-r-EGF-rP64K. In the strategy to avoid any phenomenon related with epitope suppression, rP64K was selected as the carrier for further assays [Gonzalez G, 1998].

Assay IICRD EC-025, Pilot 2: It included 20 patients in stage IIIb-IV of NSCLC, one month after finalizing the first-line CT, randomized to receive one of the two adjuvants: vaccine conjugate in alumina (10 patients) and in Montanide ISA 51 (10 patients). It evaluated safety and immunogenicity with different adjuvants, as well as the effect of 5 doses of the vaccine as induction (day 0, 7, 14, 21 y 51) and re-immunizations when the antibodies titles decrease at least in 50% of the maximum peak values reached during the induction phase. It also studied the relation between antibodies titles and survival.

Assay IICRD EC-033 (Pilot 3): With a design similar to the preceding one, but all patients received a low dose of Cyclophosphamide as an immune-enhancer of the immune response (doses of 200 mg/m^2 of corporal surface), 3 days before the vaccination start.

Results of both assays were analyzed jointly to evaluate immunogenicity, safety and effect of the vaccination with EGF in survival of the different treatment groups.

For the immunogenicity analysis, the percentage of patients capable to develop antibodies titles' levels response was assessed. The patients capable to develop antibodies response (ac) after vaccination were grouped as:

1. Patients that are serum-converters
2. Good responders (GAR)
3. Poor responders (PAR)

According to this classification, the higher percentage of patients that classified as serum-converters and GAR, associated to the patients that used Montanide ISA 51 as adjuvant. Pre-treatment with Cyclophosphamide did not show any improvement regarding the % of serum-conversion, neither in the proportion of patients classified as GAR.

Regarding the antibodies levels in responding patients, the geometrical average of the serum dilutions increased when the adjuvant used was Montanide ISA 51. Cyclophosphamide also improved the Acs titles' levels.

The joint data from both studies indicate that, regarding immunogenicity, percentage of responders and antibodies titles' levels, the best results were achieved when Montanide ISA 51 was used as the adjuvant and Cyclophosphamide in the pre-treatment.

Regarding the kinetics of the antibodies response, the re-immunizations performed when the antibodies titles decreased, did not cause an enhancing effect on the immune response (strong and sustained Acs response). The Acs titles increased after re-immunization, but only to the maximum level previously achieve, decreasing again briefly. This proves that continuous re-immunizations are required for maintaining high anti EGF Acs.

A significant increase in survival (SV) was described for GAR patients (average SV: 12.41 months, SV median: 9.41 months) compared to patients classified as PAR (average SV: 5.47 months, SV median: 4.5 months) and with the historic control group (average SV: 7.41 months, median SV: 5, 67 months). This confirmed the preclinical finding, where a direct relation between anti EGF Acs and survival in mice challenged with tumors was proved. [Gonzalez G, 1996].

In the survival analysis, its increase was also associated to those patients with Ac titles prolonged and sustained in time. This finding presumed that, within the responding patients, the duration of the anti-EGF Ac has an additional relation to survival.

These two Phase I/II clinical assays (IICRDEC025 y IICRDEC033) contributed evidences for selecting Montanide ISA 51 as the adjuvant and the pre-treatment with an immunomodulator dose of Cyclophosphamide 72 hours before the first immunization.

Assay IICRD EC-041 (Pilot 4): Dose escalation: the patients were randomized to receive a single dose of the vaccine conjugate hu-r-EGF-rP64K in alumina as the adjuvant (10 patients) or double dose of the same vaccine and the same vehicle (alumina) in 2 injection sites (10 patients). The vaccination scheme consisted in 5 immunizations with the vaccine (single and double dose) the days 0, 7, 14, 21 and 28, and then monthly re-immunizations. This assay extended the inclusion to 20 more patients (10 patients in each group).

The vaccine resulted more immunogenic in the group of patients who received double dose of the vaccine; also the patients in the double-dose group reduced much more the EGF serum concentrations, than the patients in the single-dose group.

A significant increase in survival was observed in patients classified as GAR (average SV: 17.1 months, SV median: 11.87 months) when comparing to the PAR (average SV: 7.84 months, SV median: 7.07 months) ($p<0.05$).

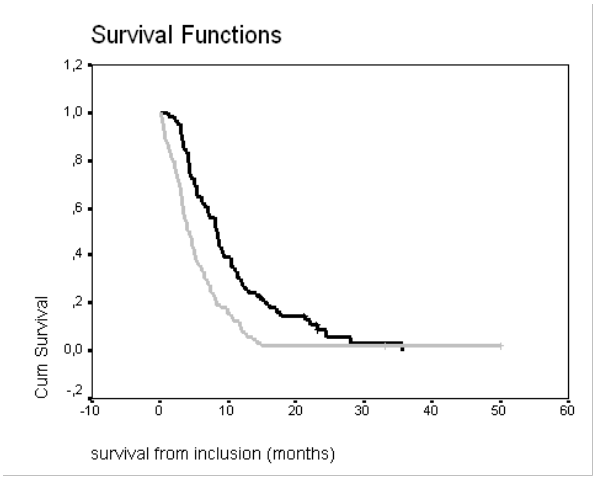
Moreover, an inverse correlation was found between serum levels in patients and the anti-EGF Acs titles. This result fits with the hypothesis that the anti-RGF Acs titles should link to the flowing EGF causing a reduction in the EGF concentration, which means that less or even no EGF is capable to link to the EGFR, thus avoiding the proliferation mechanisms derived from the said link.

As well, a significant increase in survival was directly correlated with the reduction of the serum EGF levels. The patients with serum EGF concentration after vaccination of less than 168 pg./mL showed a significant increase in survival (average SV:15.28 months, SV median: 11.3 months) when comparing to patients with serum concentrations equal or larger than 168 pg./mL (average SV: 5.79

months, SV median: 5 months) ($p<0.05$). This finding suggests that serum EGF concentration could be considered a clinical response biomarker in terms of survival.

This study also described a trend to increase survival in patients who received double doses, respect to the group that received single doses. Estimated survival for patients vaccinated in the pilot studies previously described was of 9.13 months average, 8 months median, significantly higher than a historic control of the participant institutions that resulted in an average SV of 4.85 months and a median of 4.53 months) (Figure 1)

Figure 1: Kaplan Maeyer survival curves of ALL patients that have received EGF vaccine in 4 Phase I/II assays vs. Historic Control.



Source: Gonzalez G et al: Therapeutic Vaccination with Epidermal Growth Factor /EGF) in advanced Lung Cancer: Analysis of Pooled Data from Three Clinical Trials. Human Vaccines, 2007; 3: 1, 8-13.

Assay CIMYM-003-CS: Phase II, conducted in Canada and U.K., including 66 patients in stable illness, after first-line chemotherapy. The patients received 5 doses of hu-r-EGF-rP64K conjugated vaccine using alumina as adjuvant (33 patients) and best supporting treatment the control group (33 patients). The main objectives were immunogenicity and safety. The vaccine resulted immunogenic and safe. There were no reports of severe adverse events, only slight reactions (as described in previous assays), that disappear after medication.

4.2 Clinical assay phase II (IICRDEC056)

Assay IICRD EC-056, Phase II, including 80 patients carrying NSCLC, stages IIIb and IV, after finishing first-line chemotherapy treatment, randomized to receive EGF vaccine (hu-r-EGF/r-P64k/Montanide ISA 51) 40 patients, or supporting treatment only 40 patients. The vaccination scheme consisted in 5 doses on days 0, 7, 14, 21 and 51 as an induction phase, followed by monthly re-immunizations. The main objectives of this study were to evaluate the vaccine immunogenicity and safety as well as to compare survival between both groups.

This assay confirmed the vaccine's safety profile described in the pilot studies (Table 4.2-1). There were no reports of severe or serious adverse events related to the use of CIMAvax-EGF®, the most frequent being fever, cephalalgia, asthenia and chills[Neninger E, 2008].

Table 4.2-1 Adverse events related to immunization (Clinical assay phase II)

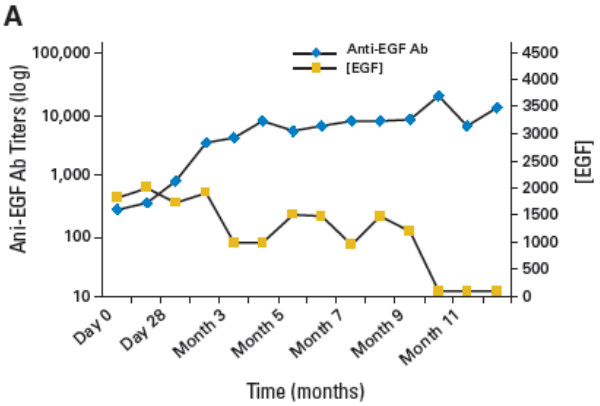
Table 2. EGF Vaccine-Related Adverse Events According to Treatment Group

	Vaccine (n = 40)		Control (n = 40)	
Event	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

NOTE. No grade 3 or 4 treatment-related adverse events were detected according to National Cancer Institute Common Toxicity Criteria version 3.0.
Abbreviation: EGF, epidermal growth factor.

Source: Neninger E et al: Phase II Randomized Controlled Trial of an Epidermal Growth Factor Vaccine in Advanced Non-Small-Cell-Lung Cancer. Journal of Clinical Oncology 2008; vol. 26, No 9

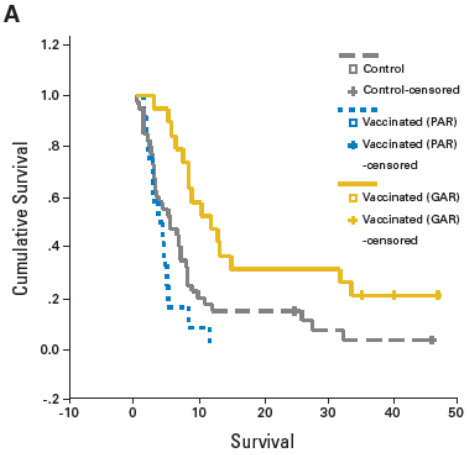
At the same time, were corroborated the correlations between the increase of the anti-EFG Acs titles with the serum reduction of the EGF, as well as between the increase of the Anti-EGF Acs titles and the higher patients' survival (p<0.0001), (Graph 4.2-1). [Neninger E, 2008].



Graph 4.2-1 Behavior of the Anti-EGF antibodies and EGF concentration in sera of the vaccinated patients

Source: Neninger E et al: Phase II Randomized Controlled Trial of an Epidermal Growth Factor Vaccine in Advanced Non-Small-Cell-Lung Cancer. Journal of Clinical Oncology 2008; vol. 26, No 9

For patients classified as GAR (Graph 4.2-2 con n= 20) an average SV was observed of 19.47 months, median of 11.7 months; for the PAR (n= 18) it was of 4.97 months, median of 3.6 months and in the control group (n= 37) the average SV was 8.52 months and the median 5.33 months (p < 0.05). [Neninger E, 2008].

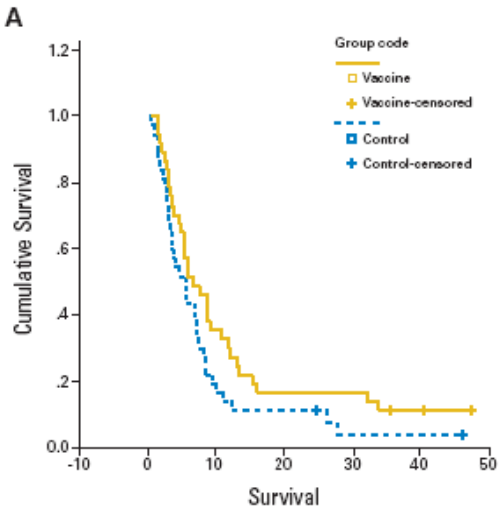


Graph 4.2-2 Behavior of survival in relation to the Anti EGF Antibodies titles

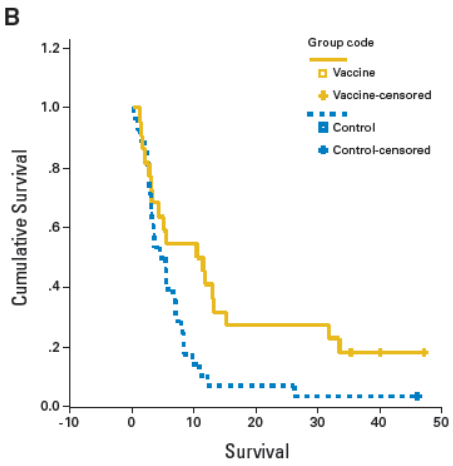
Source: Neninger E. et al: Phase II Randomized Controlled Trial of an Epidermal Growth Factor Vaccine in Advanced Non-Small-Cell-Lung Cancer. Journal of Clinical Oncology 2008; vol. 26, No 9

Furthermore it was proved that there is a trend to increase in survival of all vaccinated patients when compared with non-vaccinated controls. (Graph 4.2-3.) The group of vaccinated patients reached an average survival of 12.73 months and a median of 6.47 months, meanwhile the control group reached an average of 8.52 months and a median of 5.33 months, log Ramk test p = 0.124.

This trend became a significant difference when patients with ages of 60 or less years were considered. [Neninger E, 2008].



Graph 4.2-3 Behavior of all vaccinated patients' survival vs. controls. Source: Neninger E et al: Phase II Randomized Controlled Trial of an Epidermal Growth Factor Vaccine in Advanced Non-Small-Cell-Lung Cancer. Journal of Clinical Oncology 2008; vol. 26, No 9



Graph 4.2-3 Behavior of survival in the age group of less than 60 years
Source: Neninger E et al: Phase II Randomized Controlled Trial of an Epidermal Growth Factor Vaccine in Advanced Non-Small-Cell-Lung Cancer. Journal of Clinical Oncology 2008; vol. 26, No 9

Assay IICRD EC-062 (Pilot 5): 20 patients included for evaluating immunogenicity, safety and therapeutic effect in an optimization scheme different from the rest of the assessed schemes: increase of the doses (4 single doses in 4 different vaccination sites), time interval between vaccination of 14 days and combination between vaccine and chemotherapy; 2 vaccine doses were administered, followed by the first-line chemotherapy, and later vaccinations continued with a monthly frequency. This last element was based in the criterion that vaccination previous to chemotherapy may favor the emergence of specific memory cells by the particular antigen, which will preferentially recover in the post-CT lymphocytic recovery.

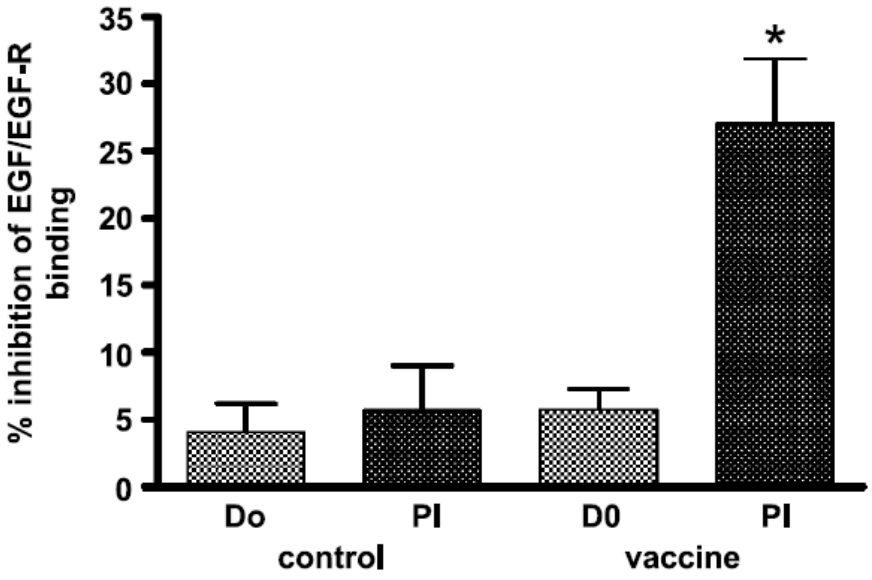
The results of this assay proved a significant increase of the vaccination immunogenicity with this treatment scheme: 95% of the patients classified as GAR and maximum titles were observed up to 20 times the values previously reached. Due to the high anti-EGF ac titles, an additional classification of immune response was considered denominated sGAR (super Good Responding), defined for those patients reaching anti-EGF ac titles of 1:64,000 or higher. In this study, 55% of the patients classified as sGAR; meanwhile in the previous Phase II assay only 2, 8% reached such condition.

The association between the Acs response and survival was again acknowledged: the sGAR patients survived significantly more than the GAR patients. The EGF serum concentration decreased to 78 pcg/mL (the kit's basal detection figure) in all patients vaccinated in this VCT scheme. [Neninger E, 2009].

4.2.1 Functional action-mechanisms studies

The evaluation studies on functional mechanisms show that serum from immunized patients are capable of inhibiting the phosphorylation of the EGF receptor (EGFR), and this inhibition capability is proportional to the anti-EGF Ac titles. The patients' serum coming from the control group does not achieve inhibition of the EGF phosphorylation.

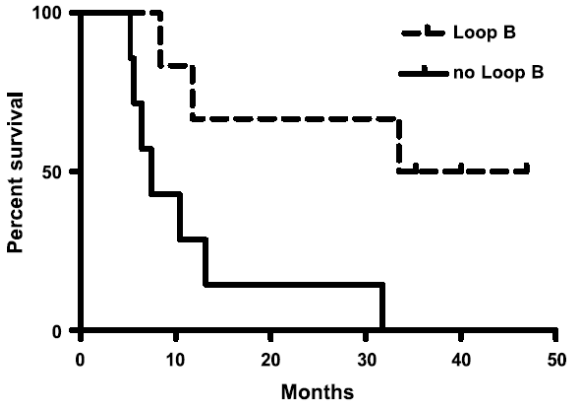
The capability to inhibit the union between the EGF and the EGFR was assessed in a competence assay (*in vitro* competence assay), which proved that serum from vaccinated patients inhibit the union between the EGF and the EGFR; however, serum from control group patients did not achieve that (Graph 4.2-4). This inhibition capability is proportional to the antibody titles and to the patients' Survival [B Garcia, 2008].



Graph 4.2-4 EGF/ EGFR inhibition capability of immunized patients' sera

The preferential recognition of the union area between the EGF and the EGFR known as immune-dominance against several EGF epitopes was also studied. Those patients whose sera recognize preferentially the Loop B, epitope of the EGF molecule (site of the central union of the EGF molecule

to the EGFR), survive significantly more than those who do not recognize the said epitope (Graph 4.2-6). [B Garcia, 2009]



Graph 4.2-6. Survival of patients immunized whose sera recognized preferentially the loop B of the EGF molecule.

This phase II clinical assay contributed to the concept test (POC) of the therapeutic vaccination's clinical effect, under the referred vacunal optimization conditions.

The results of the five pilot clinical assays and the Phase II controlled assay, were the base for conferring to CIMAvax-EGF® the conditioned sanitary registration as a therapeutic vaccine indicated for adult patients carrying NSCLC in advanced stages (IIIB/IV).

4.3 Phase III clinical assay (IICRDEC081)

Phase III clinical assay, multicenter, open, controlled and randomized, aimed at confirming its therapeutic efficacy. Currently closed for inclusion, it is under monitoring. It included 405 patients: 242 vaccinated and 109 controls. Randomization is 2:1 in favor of the vaccinated group which follows an immunization scheme of 4 doses each 14 days (induction), followed by monthly re-immunizations until significant deterioration of the general condition (PS=3) or unmanageable toxicity. The vaccine is subdivided in four anatomic sites, for a dose of 2.4 mg per each immunization. [Rodríguez PC, 2011]

Following, an updated summary of the main data:

4.3.1 Demographic and basal characteristics of the patients

The general characteristics of the patients included are balanced. (Table 4.3.1)

Table 4.3-1 Demographic and basal characteristic of the patients (Phase III Clinical assay)

Demographic and tumor characteristics		Groups under study	
		Vaccine Group (n = 270)	Control Group (n = 135)
Age (median and range)		61 (34- 81)	61 (42- 82)
Skin color	White	69.7	71.6
	Black	17.1	12.7
	Other	13.2	15.7
Sex	Feminine	33.5%	35.7%
	Masculine	66.5%	64.3%
Clinical stage	IIIB	62.2%	73%
	IV	36,8%	27%
Histological type	Adenocarcinoma	42.2%	39.4 %
	Non-Adenocarcinoma	57.8%	60.6%
ECOG	0	39.8%	32.7%
	1	55.8%	58.7%
	2	4.4%	8.6%
Smokers	Smokers	25.9%	33.3%
	Ex smokers	51.1%	45.2%
	No smokers	23%	21.5%

4.3.2 Analysis of survival by treatment intention

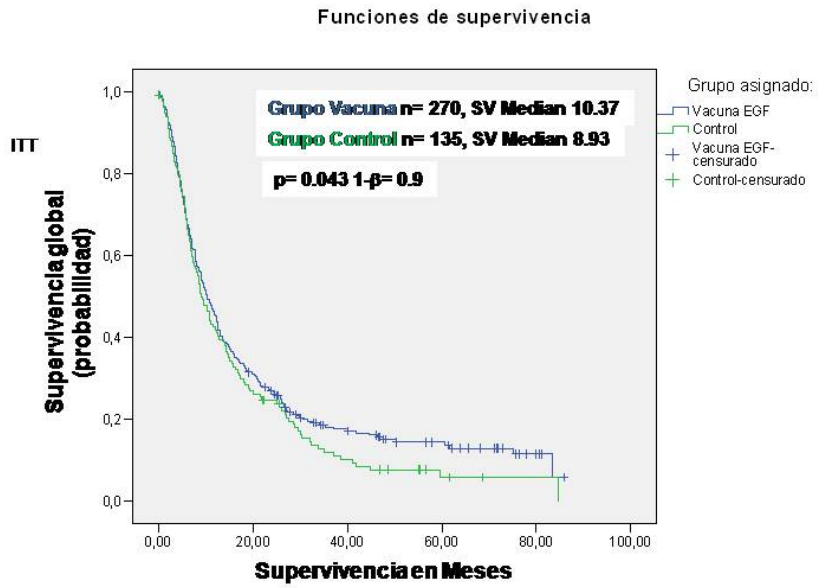
For the efficacy analysis of the therapeutic vaccine by treatment intention (ITT), were considered 405 patients (270 vaccinated and 135 controls). The survival median of the vaccinated group surpasses in 1.44 months the control group (10.37 months vs. 8.93 months respectively) (p= 0.043). (Graph 4.3-1 and Table 4.3-2)

Table 4.3-2 Survival by treatment intention (ITT)

Group	Median (months)	p
Control (n= 135)	8,93	0.043

Vaccine (n= 270)	10,37	

Graph 4.3-1 Survival analysis by ITT.



Grupo	0	6	12	24	36	48	60	72	84
Vacuna	270(100)	184(68	123(46	71(27)	37(18)	26(15)	23(14)	17(13)	1(6)
Control	135(100)	91(68)	56(42)	33(25)	14(12)	9(8)	9(8)	3(6)	3(6)

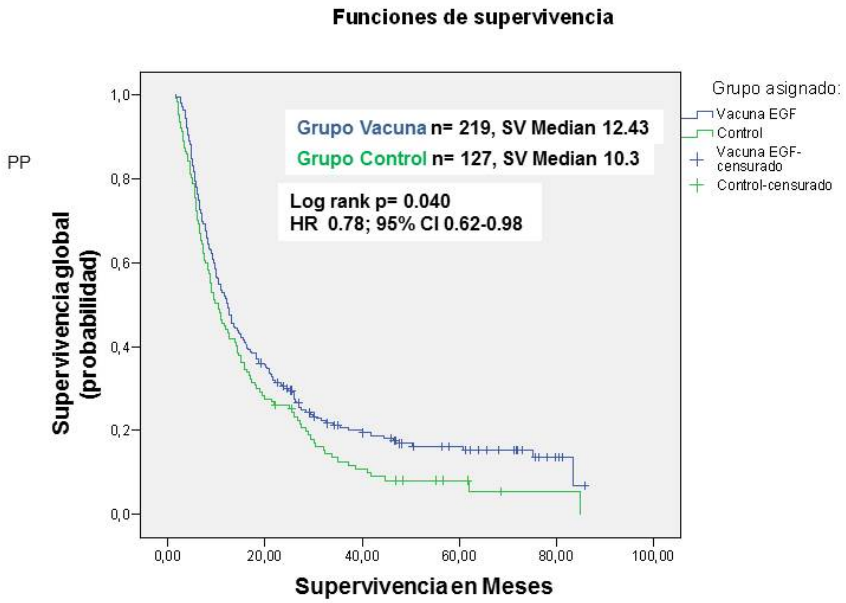
Vivos (n) y tasas de SV (%) por grupos.

4.3.3 Per protocol survival analysis

Graph 4.3-2 shows significant statistical difference (Log Rank p= 0.04) when comparing the survival median by groups, the GV surpassing in 2.13 months the GC (12.43 months vs. 10.3 months respectively).

Starting from the 36 month, the GV sustains survival rates higher than 8% with respect to the control and reaches 7% of living patients at month 84; meanwhile survival is zero in the GC.

Graph 4.3-2: Per protocol survival analysis.



Grupo	0	6	12	24	36	48	60	72	84
Vacuna	219(100)	178(77)	113(52)	65(31)	35(21)	25(17)	22(16)	18(15)	1(7)
Control	127(100)	91(72)	56(44)	33(26)	14(13)	9(8)	9(8)	2(6)	0(0)

Vivos (n) y tasas de SV (%) por grupos.

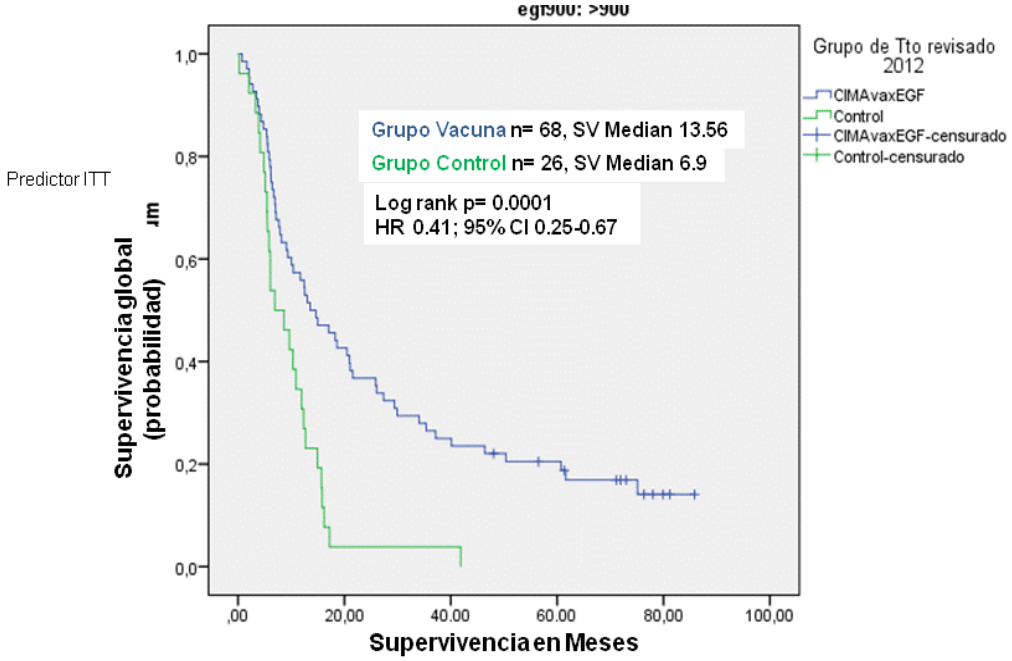
4.3.4 Efficacy results of the EGF basal concentration in serum as predictor biomarker of the response to vaccination.

Graph 4.3-3 shows a very significant statistical difference (Log Rank p= 0.0001), when comparing the survival medians by groups, the GV surpassing in 6.66 months the GC (13.56 months vs. 6.9 months respectively).

The survival rates start to separate from the month 12, reaching its maximum separation at month 24, in which 37% of the GV patients are alive, against 4% of living patients for the GC.

As from the month 48 there are no living patients in the GC, meanwhile 23% of the patients of the GV remain alive until month 60 and after 84 months of monitoring still remains alive the 14% of this group.

Graph 4.3-3: Survival analysis of the EGF basal concentration in serum as biomarker predictor of the response to vaccination.



Grupo	0	6	12	24	36	48	60	72	84
Vacuna	68 (100)	54 (80)	38 (55)	25 (37)	18 (27)	16 (23)	16 (23)	9 (17)	5(14)
Control	26 (100)	16 (62)	8 (31)	1 (4)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)

Vivos (n) y tasas de SV (%) por grupos.

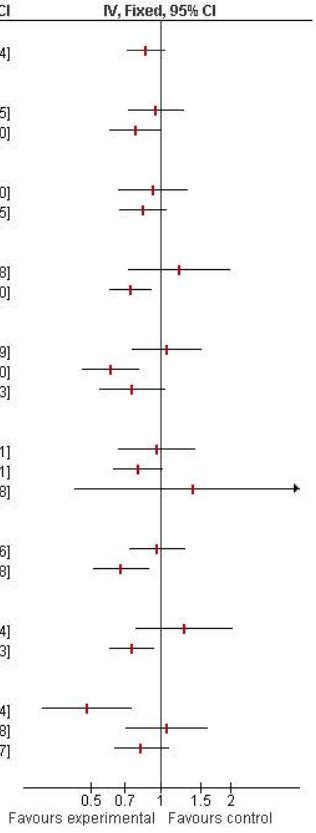
4.3.5 Results of the Cox regression by the study's control variables

Graph 4.3-4 shows a statistically significant influence favoring the following co-variables:

Caucasian race (p= 0.03), non-smokers (p= 0.034), stage IV of the illness (p= 0.055) and the histological type of the non-adenocarcinomas (p= 0.056). No influence is observed in favor of the objective antitumor response obtained by the patients to previous Chemotherapy treatment.

Graph 4.3-4 Multivariate analysis for prognosis factors

Study or Subgroup	log[Hazard Ratio]	SE	Experimental Total	Control Total	Hazard Ratio IV, Fixed, 95% CI	P value
1.1.1 Global						
Overall	-0.15015844	0.09639729	270	135	0.86 [0.71, 1.04]	0,180077725
1.1.2 Age						
Age GT 60	-0.05477846	0.14021039	149	75	0.95 [0.72, 1.25]	0,711485365
Age LE 60	-0.25647155	0.13306154	121	60	0.77 [0.60, 1.00]	0,135849001
1.1.3 Sex						
Female	-0.0819028	0.17494819	176	85	0.92 [0.65, 1.30]	0,195074442
Male	-0.18234049	0.11726945	67	50	0.83 [0.66, 1.05]	0,66622145
1.1.4 Ethnic origin						
African-American	0.18073656	0.25722031	77	38	1.20 [0.72, 1.98]	0,399873066
Caucasian	-0.30674827	0.10413362	176	85	0.74 [0.60, 0.90]	0,030191828
1.1.5 Smoking status						
Ex-Smoker	0.05331806	0.17549937	138	61	1.05 [0.75, 1.49]	0,748630461
Non-smoker	-0.50662884	0.14416686	62	29	0.60 [0.45, 0.80]	0,034227802
Smoker	-0.29325401	0.16565786	70	45	0.75 [0.54, 1.03]	0,324054003
1.1.6 Performance Status						
0	-0.03976194	0.19378051	95	44	0.96 [0.66, 1.41]	0,843677372
1	-0.23018715	0.12353516	136	69	0.79 [0.62, 1.01]	0,138818876
2	0.31712884	0.60373843	13	11	1.37 [0.42, 4.48]	0,470726694
1.1.7 Stage						
IIIB	-0.03924494	0.1381324	153	88	0.96 [0.73, 1.26]	0,78471712
IV	-0.40220371	0.14043636	89	36	0.67 [0.51, 0.88]	0,055423431
1.1.8 Histological Type						
Adenocarcinoma	0.22841176	0.24695026	96	46	1.26 [0.77, 2.04]	0,245125896
Non Adenocarcinoma	-0.29325401	0.11451634	134	73	0.75 [0.60, 0.93]	0,05614197
1.1.9 Response Evaluation						
CR	-0.74228988	0.22594998	15	9	0.48 [0.31, 0.74]	0,117858087
PR	0.05427249	0.2056849	103	44	1.06 [0.71, 1.58]	0,780568305
SD	-0.19802451	0.13777218	107	62	0.82 [0.63, 1.07]	0,238352075



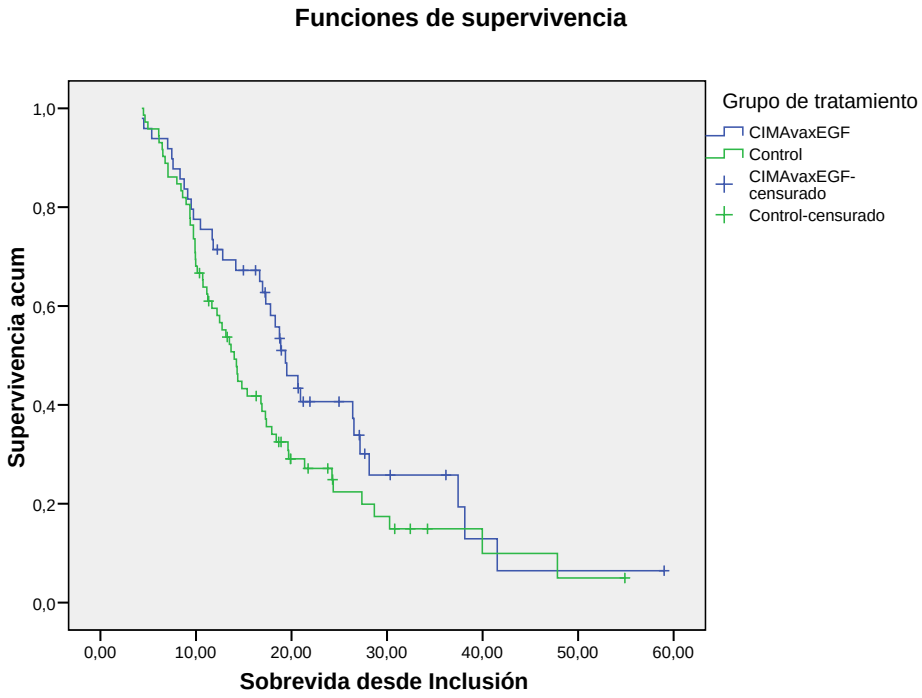
4.4 Phase II clinical assay (IICRDEC077)

Phase II multicenter, randomized, controlled, open and sequential assay, with two groups: the first group treated with the CIMAvax-EGF vaccine and the control group in which was included a total of 199 masculine sex patients, carrying prostate cancer confirmed by cyto-histological techniques and classified as hormone-resistant. The patients included were assigned to one of the two treatment groups: The first one receiving CIMAvax-EGF + chemotherapy in a VCV scheme and the second group receiving chemotherapy alone.

4.4.1 Analysis of the per protocol survival

For analyzing per protocol efficacy (SV PP analysis) from the 199 patients included in the study were considered 121 patients' data, 49 from the GV who reached the life expectancy foreseen since their inclusion (4 months or more) and received at least 6 doses of CIMAvax-EGF in any moment of the study. They were compared to 72 patients from the control group who reached at least the 4 months foreseen since their inclusion.

In the PP survival analysis (graph 4.4-1, Tables 4.4-1 y 4.4-2), a numerical advantage is observed of 5.3 months in favor of the vaccine group (n=49 patients), with an average of 23.2 months and a median of 19.3 months of survival, with respect to the control group (n=72 patients), with an average of 19.0 months and median of 14 months of survival. The numerical advantage did not reach a statistical difference (p= 0.08), although a benefit is observed in the survival rates corresponding to the vaccine group at 12, 24 and 36 months, which correspond to 71.4%, 40.7% and 25.8% of living patients in the own vaccine group, in each of the respective periods, compared to 59.5%, 27.1% and 14.6% of living patients form the control group, in each of the corresponding periods.



Graph 4.4-1 Survival curves since the inclusion, by treatment group (PP survival analysis).

Table 4.4-1 Survival since inclusion according to per protocol analysis by group (PP survival analysis)

Treatment group	N	SV since inclusion (months)		Significance (p)
		Media	Median	
CIMAvax-EGF®	49	23.2	19.3	0.08
Control	72	19.0	14.0	

Table 4.4-2 Survival rates by group (PP survival analysis)

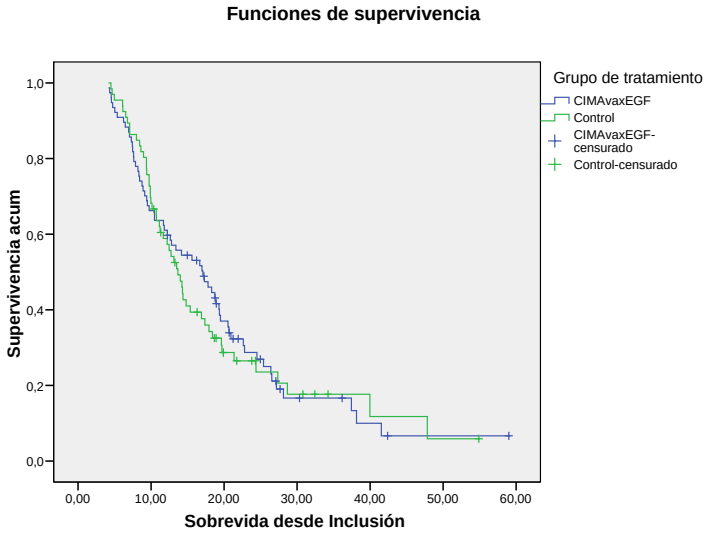
Treatment group	N	SV rates 12 months	SV rates 24 months	SV rates 36 months
CIMAvax-EGF®	49	71.4%	40.7%	25.8%
Control	72	59.5%	27.1%	14.6%

4.4.2 Survival analysis by intention to treat

In the survival analysis by intention to treat (Graph 4.4-2, Table 4.4-3), numerical differences are not observed between the groups, which corresponds to an average of 17.209 months (IC 95%: 13.959; 20.459), and a median of 12.233 months (IC 95%: 6.555; 17.912) for the CIMAvax-EGF group

(n=100 patients) and an average of 18.412 months (IC 95%: 15.041; 21.7784) with median of 13.500 months (IC 95%: 10.671; 16.329), for the Control group (n= 99 patients), obviously, without showing a statistical difference between the groups. (p= 0.539).

For this analysis were considered the 199 patients who received treatment, independently form the number of doses received.



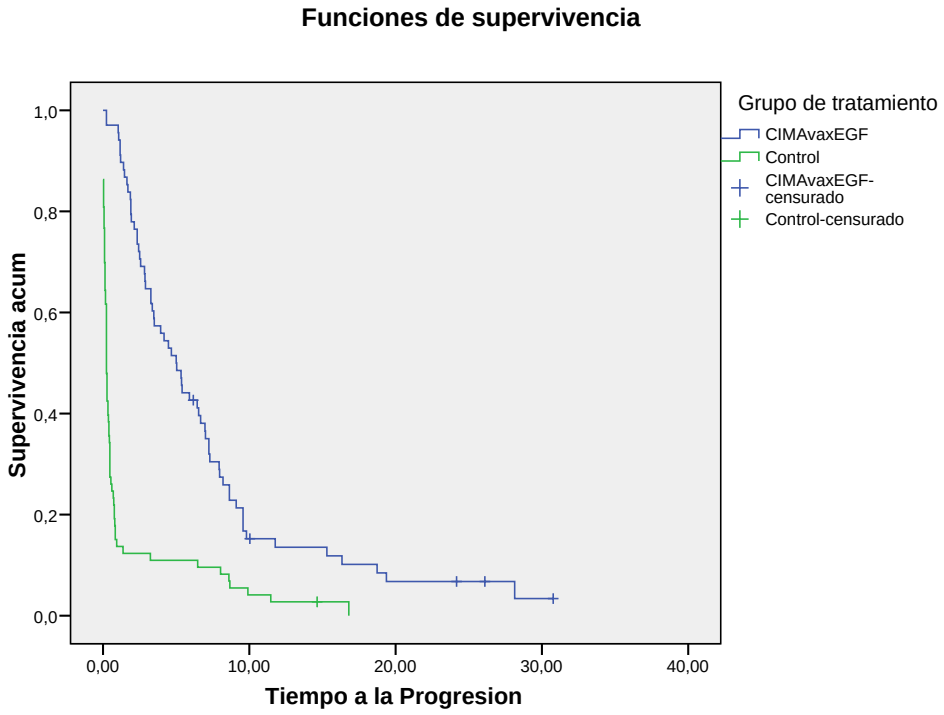
Graph 4.4-2 Survival curves since inclusion, by treatment group (SV by ITT analysis).

Table 4.4-3 Survival curves since inclusion, by treatment group (SV by ITT analysis).

Treatment group	N	SV since inclusion (months)		Significance (p)
		Average	Median	
CIMAvax® EGF	100	17.209	12.233	0.539
Control	99	18.412	13.500	

4.4.3 Time to progression analysis

Time to progression analysis (Graph 4.4-3, Table 4.4-4), shows an average of 7.1 months (IC 95%: 3.124; 10.125) and a median of 5.0 months (IC 95%: 1.00; 9.12) for the CIMAvax-EGF group (68 patients). Meanwhile, for the control group (73 patients) the average was of 1.7 months (IC 95%: 0; 4.021) and the median of 0.23 months. The numerical differences were statistically confirmed (p= 0.0001).



Graph 4.4-3 Time to progression (in months) by treatment group

Table 4.4-4 Time to progression by treatment group

Treatment group	N	TTP(months)		Significance (p)
		Average	Median	
CIMAvax® EGF	68	7.1	5.0	0.0001
Control	73	1.7	0.23	

Note: Information for 58 patients was not reported.

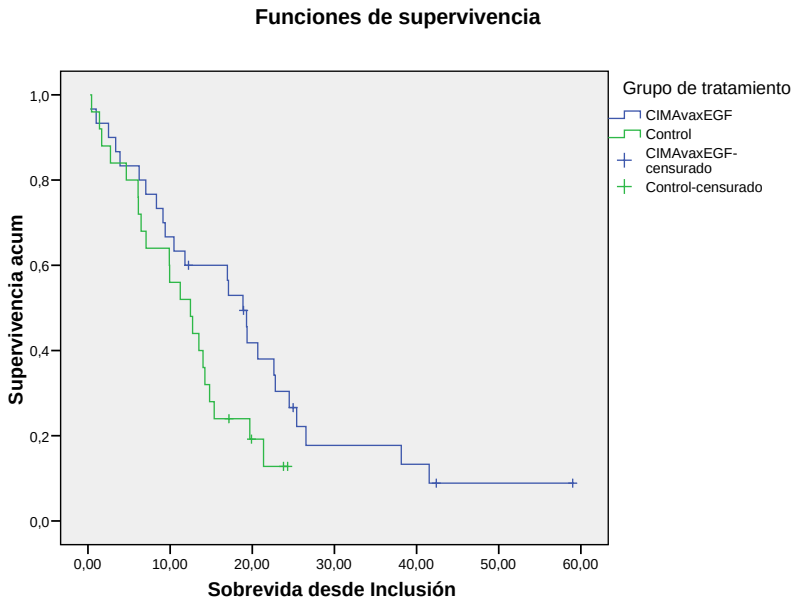
4.4.4 Survival analysis considering histological pattern

For this analysis were considered the 79 patients for which were available the results of their histological pattern evaluation at their inclusion in the study. In the vaccine group, 8 patients showed a Well differentiated pattern (Gleasson 2-4), 12 patients Moderately Differentiated (Geasson 5-6) and 21 patients a Poorly differentiated or undifferentiated pattern (Geasson 7-10). Meanwhile, in the Control Group 7 patients showed a Well Differentiated pattern (Gleasson 2-4), 10 patients a Moderately Differentiated pattern (Geasson 5-6) and 20 patients a Poorly Differentiated or Undifferentiated pattern

In the survival analysis considering the histological type (Graph 4.4-4, Tables 4.4-5 and 4.4-6), a numerical advantage is observed in favor of the Vaccine group (n= 21 patients), with an average of 20.2 months and a median of 18.8 months of SV, with respect to the Control group (n= 20 patients), with an average of 12.0 months and a median of 12.4 months of SV.

In the survival analysis considering the histological pattern (Highly Differentiated Graph, Gleasson 7-10), a numerical advantage of 6.4 months is observed in favor of the Vaccine group. The numerical advantage did not reach a statistical difference (p= 0.071), although a benefit is observed in the survival rates corresponding to the Vaccine group at 12, 24 and 36 months, corresponding to the 52%, 30.4% and 17% of living patients of the Vaccine group in each respective period, compared to 42%, 12.8% and 0 living patients in the Control group in the corresponding periods.

When performing the survival analysis according to the histological pattern in each group, there were not differences observed between the groups, considering the Well Differentiated and the Moderately Differentiated histological patterns.



Graph 4.4-4 Survival curves since the inclusion by treatment group and according to the Highly Undifferentiated histological pattern. (Gleasson 7-10).

Table 4.4-5. Survival since the inclusion by treatment group and according to the Highly Undifferentiated histological pattern (Gleasson 7-10)

Treatment Group	N	SV since the inclusion (months)		Significance (p)
		Average	Median	
CIMAvax-EGF®	21	20.2	18.8	0.071
Control	20	12.0	12.4	

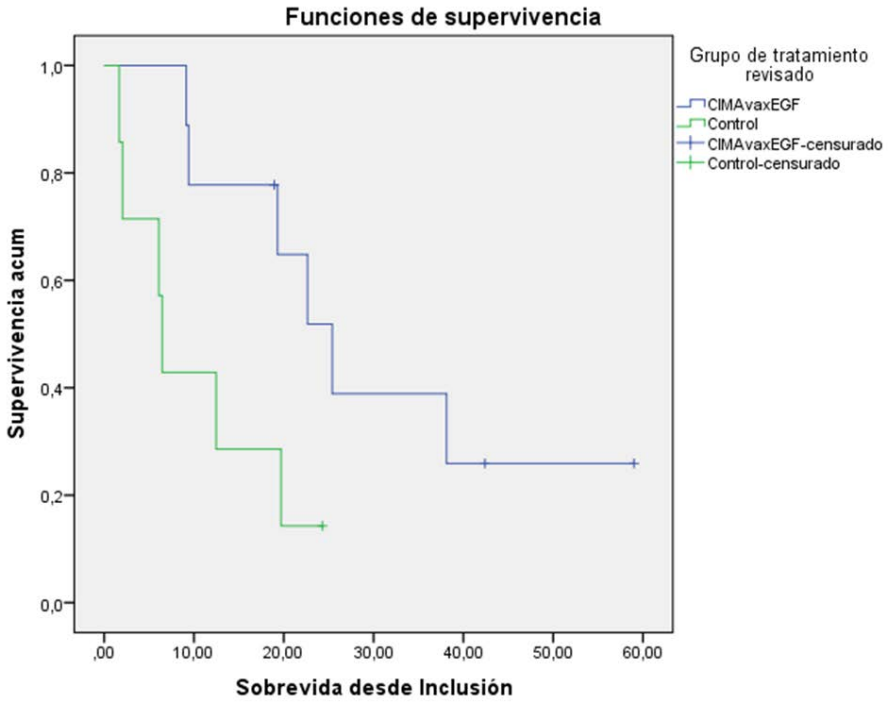
Table 4.4-6. Survival rates by treatment group and according to the Highly Undifferentiated histological pattern (Gleasson 7-10).

Treatment Group	N	SV rate 12 months	SV rate 24 months	SV rates 36 months
CIMAvax-EGF®	21	52%	30.4%	17%
Control	20	42%	12.8%	0

4.4.5 Efficacy results of the EGF basal concentration in serum as predictor biomarker of the response to vaccination

The predictive values of the response to treatment with CIMAvax-EGF®, was evaluated using as a possible biomarker the EGF basal concentration in serum from 83 patients, in which this determination was available. Using 874 pg./mL of basal EGF in serum as cutting value (34 patients with this basal concentration) it was observed that the immunized patients reach an advantage of 6.9 months survival with respect to the controls (p=0.86). As well it was observed that in those patients with basal EGF in serum higher than the cutting value and with Gleasson higher than 5, the vaccinated patients reached an advantage of 19 months survival with respect to the controls. (p=0.38) (Graph 4.4-5)

These results suggest the possibility of predicting the benefit in survival that patients could receive when immunized with the CIMAvax EGF vaccine, and sustains the possibility of performing a clinical assay to prospectively assess the value of basal EGF and Gleasson as potential response predictor biomarkers.



Graph 4.4-5. Survival curves since the inclusion by treatment group and according to basal EGF and histological pattern (Gleasson 5-10).

4.5 Summary and analysis of the adverse events emerged during the clinical assays

4.5.1 Analysis of the adverse events emerged during clinical assays with NSCLC indication

Table 4.5-1 shows the common adverse events (≥ 1% - ≥ 10%) for each clinical assay with NSCLC indication. It includes only the clinical assays in which the current CIMAvax-EGF® formulation was employed (rhEGF-rP64K/Montanide ISA 51), Protocols IIC RD EC025. IIC RD EC033, IIC RD EC062, IIC RD EC056, IIC RD EC081, IIC RD EC111, IIC RD EC120). In the case of assays with control groups, only the adverse events for the group treated with the vaccine were considered.

Table 4.5-1 Integrated summary of the adverse events independently from their relation with CIMAvax-EGF®.

ADVERSE EVENT (EA)	No. EA	%
Hypotension	60	1.00
Induration	67	1.12
Injection site reaction (pain)	467	7.83
Anorexia	134	2.25
Diarrhea	70	1.17
Nauseas	222	3.72
Vomits	241	4.04
Alkaline anorexia (increase)	79	1.32
Arthralgia	63	1.05
Articular pain	60	1.00
Thorax pain	150	2.51
Headache	371	6.22
Tremor	176	2.95
Dyspnea	268	4.49
Cough	258	4.32
Expectoration	66	1.11
Hemoglobin (anemia)	147	2.46
Asthenia	142	2.38
Chills	193	3.23
Fatigue	79	1.32
Fever	425	7.12
Dizziness	91	1.52
General discomfort	118	1.98

The majority of the adverse events notified in patients treated with CIMAvax-EGF® classify as light or moderate, being the most frequent: injection site reaction (pain), headache, fever, vomits, nausea, chills and tremors.

4.5.2 Analysis of the adverse events in the clinical assay with prostate indication

Of the total Adverse Events (EAs) reported, (1274) associated both to the CIMAvax-EGF® group and to the Control group, bones pain was the most frequently described with a total of 215 events (16.8 %), followed by fever with 75 events (5.9%), anemia with 72 events (5.7%) and nausea with 48

events (3.8%). Moreover, with equal frequency were reported asthenia and edemas, 44 events (3.5%) each.

In relation with the CIMAvax-EGF® vaccine's administration, the largest percentage was in the category of light events 488 (63.29%), followed by the category of moderate events 196 (25.42 %).

Of the 802 EAs reported for the CIMAvax-EGF® group, 488 were described with possible causality (60.84%), 107 with probable causal relation (13.34%) and in the improbable category were reported 56 events (6.98%).

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