Expert Review of Vaccines
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ierv20

CIMAvax EGF (EGF-P64K) vaccine for the treatment of non-small-cell lung cancer

Tania Crombet Ramos[a], Pedro Camilo Rodríguez[a], Elia Neninger Vinageras[b], Beatriz Garcia Verdecia[a] & Agustin Lage Davila[a]
[a] [1]Center of Molecular Immunology, Havana, Cuba
[b] [2]Hermanos Ameijeiras Hospital, Havana, Cuba
Published online: 21 Aug 2015.

EXPERT REVIEWS

CIMAvax EGF (EGF-P64K) vaccine for the treatment of non-small-cell lung cancer

Expert Rev. Vaccines Early online, 1–9 (2015)

Tania Crombet Ramos*[1], Pedro Camilo Rodríguez[1], Elia Neninger Vinageras[2], Beatriz Garcia Verdecia[1] and Agustin Lage Davila[1]

[1]*Center of Molecular Immunology, Havana, Cuba*
[2]*Hermanos Ameijeiras Hospital, Havana, Cuba*
**Author for correspondence: taniac@cim.sld.cu*

Epidermal growth factor receptor (EGFR) is overexpressed in many epithelial tumors and its role in the development of non-small-cell lung cancer (NSCLC) is widely documented. CIMAvax-EGF is a therapeutic cancer vaccine composed by recombinant EGF conjugated to a carrier protein and emulsified in Montanide ISA51. Vaccination induces antibodies against self-EGF that block EGF–EGFR interaction and inhibit EGFR phosphorylation. Five clinical trials were conducted to optimize vaccine formulation and schedule. Then, two randomized studies were completed in advanced NSCLC, where CIMAvax-EGF was administered after chemotherapy, as 'switch maintenance'. The vaccine was very well tolerated and the most frequent adverse events consisted of grade 1/2 injection site reactions, fever, headache, vomiting and chills. CIMAvax was immunogenic and EGF concentration was reduced after vaccination. Subjects receiving a minimum of 4 vaccine doses had a significant survival advantage. NSCLC patients with high EGF concentration at baseline had the largest benefit, comparable with best maintenance therapies.

KEYWORDS: cancer vaccine • clinical trials • epidermal growth factor • epidermal growth factor receptor • lung cancer • non-small-cell lung cancer

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related death in the world [1,2]. Treatments for late stage are largely palliative and platinum-based chemotherapy regimens have reached a benefit plateau [3]. Regrettably, patients diagnosed at an advanced stage have a life expectancy of <1 year [4].

Therapeutic choices and overall disease course for squamous NSCLC have remained relatively unchanged over the past several years. On the other hand, there have been some recent advances in the treatment of non-squamous NSCLC for which several 'actionable' genetic alterations that trigger cancer growth have been identified [5,6]. The anaplastic lymphoma kinase (ALK) gene rearrangement characterizes a population in whom dysregulation of ALK-tyrosine kinase leads to uncontrolled proliferation of cancer cells, providing the basis for the therapeutic use of ALK-TK inhibitors [7]. Crizotinib is recommended as first- or second-line therapy for stage IIIB/IV non-squamous NSCLC patients bearing the ALK mutation, while FDA and EMA recently approved ceritinib for individuals who progress on crizotinib [8,9].

Alternatively, constitutive activation of epidermal growth factor receptor (EGFR) due to mutations in tumor cells leads to deregulated downstream cellular signaling pathways [10]. Therefore, EGFR was identified as another important therapeutic target. Several small-molecule inhibitors of EGFR (gefitinib, erlotinib and afatinib) are effective in patients whose tumors harbor activating mutations of the kinase domain [3,11]. Eight large Phase III trials demonstrated that EGFR-tyrosine kinase inhibitors (TKIs) are the best frontline therapy in EGFR-mutant NSCLC patients [12]. In subjects with ALK or EGFR wild-type lung cancer, platinum-based chemotherapy remains the standard of care [12].

Currently, 'continuation' or 'switch maintenance' for non-progressive disease after frontline therapy is the recommended alternative for patients with good performance status [13–17]. 'Continuation maintenance' implies that a chemotherapy or biological that was part of the initial platinum-based therapy is continued. Alternatively, switch maintenance means that a third agent is initiated prior to

 10.1586/14760584.2015.1079488 ISSN 1476-0584

evidence of disease progression after completion of platinum-based therapy [13–17]. Several drugs used as maintenance only improve the progression-free survival, while agents like pemetrexed or erlotinib also increase the overall survival of the non-squamous NSCLC patients [13–17].

Two antibodies targeting EGFR, cetuximab and necitumumab, were evaluated in Phase III studies in advanced NSCLC patients. Cetuximab improved the survival of the NSCLC patients, but did not gain approval by the FDA [18]. In the 'FLEX' trial, overall survival was significantly prolonged in the chemotherapy plus cetuximab group compared with the chemotherapy-alone group (hazard ratio [HR]: 0.871; p = 0.044). The median overall survival was 11.3 months in the chemotherapy plus cetuximab group and 10.1 months in the chemotherapy-alone group. EGFR expression was retrospectively correlated with survival benefit [19]. For patients with high EGFR expression (31 %), overall survival was longer in the chemotherapy plus cetuximab group than in the chemotherapy-alone group (median 12.0 vs 9.6 months; HR: 0.73; p = 0.011) [18,19].

The relatively modest survival benefit (HR: 0.87) combined with the absence of prospectively validated biomarkers and the high toxicity of the cisplatin/vinorelbine/cetuximab combination have limited the clinical utilization of cetuximab [20].

Necitumumab, a second-generation anti-EGFR monoclonal was evaluated in combination with pemetrexed/cisplatin versus chemotherapy alone in patients with previously untreated, stage IV, non-squamous NSCLC [21]. There was no difference in overall survival between treatment groups, with a median overall survival of 11.3 months in the necitumumab-pemetrexed/cisplatin group versus 11.5 months in the pemetrexed/cisplatin group (HR: 1.01; p = 0.96) [21]. The authors concluded that unless future studies identify potentially useful predictive biomarkers, necitumumab is unlikely to provide benefit in non-squamous NSCLC when combined with pemetrexed and cisplatin [21].

On the other hand, 1093 patients with metastatic squamous carcinomas of the lung were randomized to gemcitabine/cisplatin with or without necitumumab in the 'Squire' trial [22]. The study met its primary end point and demonstrated a statistically significant improvement in overall survival with the addition of necitumumab to chemotherapy (11.5 vs 9.9 months; HR: 0.84; p = 0.012). Patients with an EGFR H score >200 (34%) have a larger benefit in survival (HR: 0.75) [22]. Overall, antibodies like cetuximab and necitumumab seem to be active in patients with squamous cell carcinomas that overexpress EGFR.

Very recently, immune checkpoint therapy targeting regulatory pathways in T cells has led to important clinical advances and provided a new weapon against cancer [23–25]. Checkpoint inhibitors release the breaks of the immune system and consequently enhance the 'natural or induced immunity' against tumor cells. Several antibodies against inhibitory receptors in T lymphocytes or its ligands, like cytotoxic T-lymphocyte-associated protein 4, programmed death-1 (PD1) or programmed death ligand-1 (PDL1) are under extensive clinical evaluation in lung cancer patients [26,27]. Ipilimumab is an anti-cytotoxic T-lymphocyte-associated protein 4 mAb. A Phase III study for patients with metastatic squamous cell NSCLC is ongoing to compare the standard therapy of carboplatin/paclitaxel versus concomitant administration of ipilimumab. In a Phase II study in advanced NSCLC, a 'phased' schedule of two chemotherapy cycles followed by ipilimumab and chemotherapy for the next 4 cycles demonstrated the largest efficacy in squamous NSCLC [28,29].

Recent results suggest durable responses after using anti-PD1 or anti-PDL1 antibodies, even in heavily pretreated patients [30,31]. Data from initial clinical trials with the anti-PD1 antibodies (nivolumab, pembrolizumab) and the anti-PDL1 antibodies (MPDL3280A, BMS936559, MEDI4736) showed response rates ranging from 10 to 24% in advanced NSCLC patients [32,33].

In a controlled, Phase II trial, 272 patients with metastatic squamous NSCLC who had disease progression after one prior platinum-based chemotherapy regimen received nivolumab or docetaxel. The trial demonstrated a statistically significant improvement in survival for patients treated with nivolumab as compared with docetaxel [34]. Nivolumab has been approved as second-line treatment for squamous NSCLC in March 2015.

Another avenue of cancer immunotherapy is cancer vaccination, which is anticipated to induce a T-cell or humoral response against tumor specific or associated antigens [35]. Sipuleucel-T, an autologous dendritic cells vaccine, increased the overall survival of patients bearing castration-resistant prostate cancer as compared with placebo and was approved by the US FDA in 2010 [36,37]. Sipuleucel-T approval raised the interest in the field of therapeutic vaccinology for cancer.

However, the history of vaccination for advanced NSCLC has not been fully successful, so far. L-BLP-25 is a mucin 1-based vaccine. Mucin 1 glycoprotein is overexpressed and abnormally glycosylated in NSCLC and other cancers. To evaluate whether the L-BLP-25 vaccine enhances the survival of patients with stage III NSCLC, a multinational, randomized, double-blind Phase III trial, was done. Randomly, 829 patients were assigned to receive L-BLP-25 and 410 were allocated to placebo. The overall survival in patients who received L-BLP-25 after chemo-radiotherapy was not significantly different from those who received placebo (25.6 months with L-BLP-25 vs 22.3 months with placebo; HR: 0.88; p = 0.123). Interestingly, subgroup analysis revealed that patients who received concurrent chemo-radiotherapy before vaccination had a survival improvement [38,39].

Belagenpumatucel-L is an allogeneic whole-cell vaccine composed by four NSCLC cell lines, transfected with a TGF-β2 antisense plasmid. The placebo-controlled, -blind clinical trial enrolled 532 stage IIIA, IIIB and stage IV NSCLC patients who did not progress after first-line platinum-based chemotherapy. Median overall survival was 20.3 versus 17.8 months (HR: 0.94; p = 0.594). Multivariate analysis revealed that patients with pre-treatment radiotherapy had a

median survival of 40.1 months for belagenpumatucel-L versus 10.3 months for placebo (HR: 0.45; p = 0.014) [40,41].

A recent disappointment came from the MAGRIT trial, where the MAGE-A3 vaccine was evaluated in patients with NSCLC who had undergone surgical resection. MAGRIT was a double-blind, randomized, placebo-controlled study where 1515 subjects were assigned to immunotherapy with the MAGE-A3 vaccine and 707, to placebo. At a median follow-up of 38.8 months, the median disease-free survival was 60.5 months for the vaccine group and 57.9 months for the placebo group (HR: 1.02; p = 0.7379). No patient subgroup benefited from vaccination. This was the largest vaccine trial ever conducted in lung cancer [40–42].

Racotumomab is another cancer vaccine under clinical evaluation. Racotumomab consists on a murine mAb with a variable fraction that mimics *N*-GlycolylGM3 ganglioside (NGcGM3). The vaccine is absorbed in Alum and it is envisioned to induce T cell and antibody response against NGcGM3. This ganglioside is a truly tumor-specific antigen, since the enzyme that catalyze the synthesis of the *N*-glycosylated GM3 from the *N*-acetyl ganglioside is absent in humans. A Phase II/III clinical trial showed that stage IIIB/IV patients, with stable disease after first-line chemotherapy that received racotumomab-Alum, had a significant benefit in progression-free and overall survival. Two confirmatory trials are ongoing to definitively assess the efficacy of racotumomab in advanced NSCLC patients, as 'switch maintenance' [43,44].

EGF-based cancer vaccine

CIMAvax-EGF is a therapeutic cancer vaccine composed of human recombinant EGF coupled to a carrier protein, recombinant P64. The vaccine is emulsified in Montanide ISA51, an oily adjuvant from Seppic, France. P64 is one of the most immunogenic proteins of the meningitis B bacteria. The carrier protein and the adjuvant are aimed to break the tolerance against EGF, a self-protein in humans [45,46].

CIMAvax-EGF induces antibodies against EGF, which is a potent growth factor for EGFR positive neoplastic cells. EGF–EGFR interaction activates a signal transduction cascade that results in cellular proliferation, angiogenesis and survival. Moreover, our group has found that some NSCLC patients have high EGF serum concentration, which anticipates a worse prognosis. The goal of vaccination is to induce neutralizing antibodies against EGF that can 'sequester' soluble EGF and hamper the EGF–EGFR interaction [45,46].

The vaccine is not intended to induce a CD8 response since EGF is not expressed in the tumor cells membrane. The adjuvant (Montanide ISA51) was chosen on account of its capacity to induce a potent humoral response.

It the preclinical setting, mice were able to produce antibodies against EGF, after immunization with either murine EGF conjugated to a carrier protein or with unconjugated human EGF. The anti-EGF antibody response correlated with increased survival when mice were challenged with EGFR-expressing transplantable tumors, but not with tumors lacking EGFR [47].

A toxicology study was done to assess CIMAvax-EGF effect in Sprague-Dawley rats after repeated immunizations during 6 months, by the intramuscular route. Rats were randomly distributed into four groups: control, Montanide ISA51, CIMAvax-EGF (human dose) and CIMAvax-EGF, 15-times the total dose used in humans. Vaccine administration for 26 weeks at doses up to 15-times the human total dose was well tolerated [48].

Clinical summary

Several clinical trials have been done with CIMAvax-EGF since 1996. The first five trials were designed to optimize vaccine formulation, dose and schedule, in terms of immunogenicity and safety. In all trials, anti-EGF antibody titers were measured through an ELISA. Patients were classified as 'good antibody responders' (GAR), if they developed anti-EGF antibody titers equal or higher than 1:4000 sera dilution and at least four-times their pre-immunization values, and 'poor antibody responders' (PAR), otherwise. This good antibody response criterion was established arbitrarily in 1996 and was used repetitively to optimize the vaccine composition and schedule. Antibody response was correlated with survival (Tables 1 & 2) [49].

Pilot clinical trial 1 was done in 10 advanced cancer patients, not amenable to receive further chemotherapy. Two carrier proteins were evaluated: tetanus toxoid and P64 from the meningitis B bacteria. Alum was used as adjuvant. Only two doses of the vaccine (2 weeks apart) were administered by the intradermal route. This study demonstrated that vaccination was safe and immunogenic since 60% of patients developed high antibody titers against EGF. P64 was chosen as the carrier protein. Adverse events consisted of injection site reactions including erythema and itching. Adverse reactions were fully reversible without any medication (Table 1) [49].

After the first pilot study, all clinical trials were performed in advanced (stage IIIB/IV) NSCLC patients. The second and third exploratory studies, evaluated Alum versus Montanide ISA51, as adjuvants as well as the impact of pre-treatment with an immunomodulatory dose of cyclophosphamide. Before enrolment, patients completed frontline platinum-based chemotherapy. The vaccine was administered intramuscularly on days 1, 8, 15, 22 and 52. Re-immunizations were done when antibody titers decreased. Adverse events were mild and moderate and comprised chills, fever, vomiting, nausea, hypertension, headache, dizziness, flushing and pain at the injection site. In both studies, the vaccine using Montanide ISA51 as adjuvant induced higher antibody titers. Patients receiving cyclophosphamide at 200 mg/m^2, 3 days before vaccination were the best responders. Even when re-immunizations provoked an increase in antibody titers, it was only up to the maximal levels previously reached. For maintaining antibody titers, repeated vaccinations were required. The median survival time (MST) of all NSCLC patients was 8.17 months (mean 9.64 months). Individuals were classified into GAR (n = 19) and PAR (n = 21) for survival comparison. The median survival of the GAR group was 9.1 months (mean 12.41 months) versus 4.5 months, for the PAR group (mean 5.47 months) (Table 1) [50].

doi: 10.1586/14760584.2015.1079488

Table 1. Summary of exploratory trials intended to optimize vaccine formulation and schedule.

Clinical trial	Target population (n)	End point	Main result	Ref.
Exploratory trial Pilot Clinical Trial 1	Solid tumor (10 patients)	Carrier selection (TT vs P64)	Selection of P64 as a carrier protein	[49]
Exploratory trial Pilot Clinical Trial 2	NSCLC (20 patients)	Adjuvant selection (Alum vs Montanide ISA51)	Selection of Montanide ISA51 as the best adjuvant	[50]
Exploratory trial Pilot Clinical Trial 3	NSCLC (20 patients)	CPM pre-treatment (CPM or not)	Selection of CPM pre-treatment	[50]
Exploratory trial Pilot Clinical Trial 4	NSCLC (20 patients)	Dose escalation (2 doses)	Selection of the higher dose of EGF	[51]
Exploratory trial Pilot Clinical Trial 5	NSCLC (20 patients)	Schedule dependence: Vaccine-CTP-Vaccine 4 injection sites 14 days interval between induction doses	Selection of: 4 injection sites 14 days interval between induction doses	[52,53]

A fourth exploratory study assessed the impact of increasing the dose of the antigen. Two doses of the recombinant EGF were compared. Stage IIIB/IV NSCLC patients who had received first-line chemotherapy were randomized to receive a single or double dose of CIMAvax-EGF, weekly for 4 weeks and monthly, thereafter. No significant toxicity was observed after vaccination, even after the injection of a high EGF dose. Adverse reactions were primarily fever, chills, nausea, vomiting and flushing. The geometric mean of the antibody titers was higher in the double dose group. EGF concentration in serum was measured with a commercial ELISA (Quantikine; R&D Systems Inc., Minneapolis, MN) as a surrogate mechanism of action of the vaccine. EGF concentration decreased with vaccination and a significant inverse correlation between the anti-EGF antibody titers and serum EGF was seen after immunization. We concluded that double dose of CIMAvax-EGF was safe and more immunogenic. The median survival of patients treated with single or double dose of the EGF vaccine was 6.43 and 8.40 months, respectively. Subjects classified as GAR had a median survival of 11.87 months, while poor responders had a median survival of 7.07 months ($p = 0.0095$). Patients with EGF <168 pg/ml after vaccination had a significantly larger survival (median 11.30 months) as compared with those who did not get EGF reductions below this threshold (median 5 months) ($p = 0.0022$) (Table 1) [51].

The last exploratory trial evaluated the impact of starting vaccinating before chemotherapy in advanced NSCLC patients. CIMAvax-EGF was administered before and after chemotherapy. The rationale of this particular schedule was based on studies of homeostatic lymphocyte repopulation after chemotherapy, according to which the lymphocyte pool is recovered from the expansion of the peripheral pool (including memory cells) rather than from *de novo* export of naïve cells from the bone marrow. The goal was to achieve a preferential recovery of the CIMAvax-EGF pre-expanded lymphocytes before the platinum-doublet. The vaccine was administered by the intramuscular route, in four injection sites, at 14 days interval for the four induction doses. After the fourth dose, vaccination continued monthly. As a result, anti-EGF antibody titers were 20-times higher than those previously obtained. To assess immunogenicity, a more stringent criterion (anti-EGF titers >1:64000) was established, according to which 16 of 20 vaccinated subjects were considered 'super good antibody responders'. Serum EGF concentration decreased to undetectable levels in all patients. Overall, dose-dense platinum chemotherapy did not affect CIMAvax-EGF immunogenicity.

Table 2. Summary of randomized clinical trials designed to assess vaccine efficacy.

Clinical trial	Target population (n)	End points	Main result	Ref.
Preliminary Efficacy Trial (Randomized Phase II) Vaccine versus BSC as switch maintenance or second line	NSCLC with disease control or PD after CTP (80 patients)	Preliminary survival benefit Immunogenicity Safety	Vaccine was safe and immunogenic Trend toward survival benefit for all vaccinated patients	[54,55]
Efficacy Trial (Randomized Phase III) Vaccine versus BSC as switch maintenance	NSCLC with disease control after CTP (405 patients)	Survival benefit Immunogenicity Safety	Vaccine was safe and immunogenic. Significant survival benefit in patients receiving at least 4 vaccine doses and in patients with (EGF) >870 pg/ml	

Toxicity of chemotherapy or CIMAvax was not increased and the commonest related adverse events were chills, fatigue, nausea, vomiting, arthralgia and pain at the site of injection. Median survival for all 20 patients was 12.8 months (mean 18.74 months). Super good antibody responders patients survived significantly more (median 19.3 months; mean 25.6 months) as compared with GAR (median 8.4 months, mean 10.5 months). A radio-immunoassay was used to evaluate the capacity of the anti-EGF antibodies to inhibit EGF-EGFR binding. All post-immune serum samples inhibited the EGF-EGFR binding and the mean post-immunization inhibition percentage was very high (67.8%) (Table 1) [52].

Then, to evaluate the preliminary efficacy of CIMAvax-EGF, a controlled, randomized study was done in 80 patients bearing stage IIIB/IV NSCLC, after finishing first-line chemotherapy. Once more, vaccine was very safe and immunogenic. Vaccinated patients achieved a median survival of 6.47 months (mean 12.73 months), whereas the control arm survival was 5.33 months (median, 8.52 months). There was a trend toward a survival advantage for the vaccine group, which was not significant at this sample size. However, vaccinated patients younger than 60 years survived significantly longer (median, 11.57 months; mean, 18.53 months) than controls (median, 5.33 months; mean, 7.55 months). Immune response strongly correlated with survival and GAR patients survived significantly more (median, 11.7 months; mean, 19.47 months) than PAR patients (median, 3.6 months; mean, 4.49 months). GAR patients also had a significantly better survival than controls. There was a strong correlation between the decrease in EGF concentration and survival. Vaccinated patients with EGF concentration <168 pg/ml survived significantly longer (median, 13.0 months; mean, 20.44 months) than those who did not reach such reduction (median, 5.6 months; mean, 6.01 months). Besides evaluating the antibody response, the functional capacity of the antibodies was measured. A radio-immunoassay was used to evaluate the binding inhibition capacity and an immunoblotting assay, which detects phosphorylated EGFR, was used to evaluate the ability of the post-vaccinated samples to inhibit the EGFR activation in the presence of EGF. After vaccination, 76.9% of the patients were able to inhibit EGF/EGFR binding (range 22.4–58%). Sera from control patients did not prevent the union between EGF and EGFR. On the other hand, more than 60% of the post-immunization samples inhibited EGFR phosphorylation (range 13.1–62.5% inhibition). Neither sera before immunization nor sera from controls were able to impede the EGF-induced phosphorylation. There was a direct correlation between antibody titers and binding or phosphorylation inhibition. In summary, these experiments demonstrated the functional capacity of the anti-EGF antibodies induced by vaccination, to hamper EGF union and activation of the EGFR (Table 2) [53,54].

To confirm vaccine efficacy, a randomized, controlled Phase III clinical trial was recently concluded in stage IIIB/IV NSCLC patients, with at least stable disease after chemotherapy. CIMAvax-EGF was evaluated as 'switch-maintenance'. The study recruited 405 patients with a 1:2 randomization (1 control for each 2 vaccinated patients). In the intent-to-treat analysis, vaccinated patients had a larger overall survival as compared with controls (MST 10.37 vs 8.93 months), which was significant according to the Harrington-Fleming estimate, a sensitive test when there is a delayed separation of the time to event curves. The Harrington-Fleming test is a weighted log-rank test that can be applied once the non-proportionality of the hazard ratio is verified. Furthermore, vaccinated patients who completed at least four doses of CIMAvax-EGF (induction period) had a significant survival benefit as compared with controls (MST 12.43 vs 10.30 months, log-rank $p = 0.04$). Vaccination continued beyond progressive disease (PD) since tumor progression in the absence of performance status worsening was not an interruption criterion. Most patients did not receive second-line therapy. CIMAvax-EGF was safe and the most common toxicity included grade 1 or 2 injection site pain, fever, headache, chills and vomits. Vaccinated patients developed high anti-EGF antibody titers, mainly IgG3 and IgG4. EGF was measured in serum at baseline and retrospectively correlated with overall survival. Controls with high serum EGF concentration (EGF >870 pg/ml) had a significantly worse prognosis compared with controls with low levels (EGF <870 pg/ml) (MST 8.63 vs 15.06 months, log-rank $p = 0.002$). In contrast, vaccinated patients with high serum levels of EGF at day 0 had a significantly better survival, as compared with control patients with EGF >870 pg/ml (MST 14.66 vs 8.63 months, log-rank $p = 0.0001$) [55,56]. Strikingly, 23% of the vaccinated subjects with high EGF concentration at baseline survived for 5 or more years while none of the controls did (submitted manuscript). Overall survival of patients with high EGF concentration treated with CIMAvax-EGF was comparable with survival gain after the use of pemetrexed, docetaxel or erlotinib as 'switch maintenance'. Other markers of immunosenescence like the proportion of $CD8^+CD28^-$ T cells (terminal differentiation phenotype), the proportion of $CD4^+$ T cells, $CD19^+$ cells and the CD4/CD8 ratio were also associated with improved survival (submitted manuscript). A subgroup exploration including the most important demographic and tumor variables was done in the ITT population. The largest benefit was seen in males with non-adenocarcinoma, smokers, at stage IV. No differences in the mean serum EGF were found in squamous versus adenocarcinoma patients, in smokers versus non-smokers or in stage IIIB versus stage IV NSCLC patients (Table 2).

The vaccine was approved in Cuba and a Phase IV enrolled 1084 advanced NSCLC patients, not amenable to receive any additional chemotherapy. Apart from the conventional cancer trials, the study was conducted in primary care units and not in secondary or tertiary level hospitals. Patients were referred to the family doctors by the oncologists, once inclusion criteria were confirmed. The oncologists evaluated antitumor response together with the family doctors, every 3 months. As in the Phase III study, patients received four bi-weekly doses of CIMAvax-EGF followed by monthly re-immunizations.

doi: 10.1586/14760584.2015.1079488

Vaccine was interrupted only in case of severe deterioration of the performance status or unmanageable toxicity. The research teams from the 45 primary care units participating in the study received special training in oncology, immunotherapy and good clinical practices before study initiation. Feasibility of vaccination in primary care units was confirmed. Administering CIMAvax-EGF by the family doctors granted better access and compliance of the vaccination schedule (chronic vaccination). Overall safety and survival benefit of CIMAvax-EGF was confirmed.

Conclusion

EGFR activation has been associated with high proliferation, invasiveness, metastization and anti-apoptosis of tumor cells. EGF is one of the most important growth factors for certain epithelial tumors including NSCLC. Particularly, our group has found that serum EGF concentration is high in lung cancer patients and seems to be a negative prognostic factor. CIMAvax-EGF is a therapeutic vaccine aimed to generate neutralizing antibodies against autologous EGF that block the union between EGFR and its ligand. Conjugation of recombinant EGF to a carrier protein and emulsification in Montanide ISA51 granted breaking the tolerance against the self-protein. EGF concentration in sera significantly decreased after vaccination and there was an inverse correlation between anti-EGF antibody titers and serum EGF. The anti-EGF antibodies induced by vaccination were capable to hamper EGFR activation.

Regarding safety, CIMAvax-EGF is very well tolerated and most frequent adverse events are grade 1 or 2 injection site reactions, chills, fever, nausea and vomiting.

Concerning efficacy, stage IIIB/IV NSCLC patients receiving at least 4 doses of the vaccine (induction scheme) had a significant advantage in overall survival. Patients with high EGF concentration in sera had the largest benefit and MST time after vaccination was 14.66 months, which is equivalent to the survival gain obtained after pemetrexed, docetaxel or erlotinib, the recommended drugs as 'continuation' or 'switch maintenance'. In the Phase II or Phase III studies, patients did not receive further therapy upon progression. Whether survival time can be increased with the use of second-line therapy at the moment of progressive disease should be explored in new trials.

Expert commentary

Active vaccination with specific antigens or whole cells needs further efficacy validation. The challenge with traditional vaccines intended to induce cytotoxic T cells is to trigger a potent response that ends up in tumor lysis, cross-presentation and a diversified response against many tumor antigens. Antigen spreading can bypass tumor escape mechanisms. 'T-cell vaccines' like L-BLP25, belangepumatucel and MAGE-A3 are anticipated to be more efficacious when combined with immune-modulatory drugs (agonists of activating receptors or antagonists of inhibitory receptors) that would augment T-cell response and circumvent tumor-induced immunosuppression.

Apart from 'cytotoxic T-cell' vaccines, CIMAvax-EGF acts by inducing an antibody-mediated removal of EGF, a soluble key activator of the EGFR. In that sense, its mechanism of action stands alike other molecules intended to inhibit EGFR, a well-validated driver of proliferation, angiogenesis and neoplastic cells' survival.

In other words, CIMAvax-EGF replicates the hormone deprivation concept that is successful for estrogen- or testosterone-dependent tumors. NSCLC seems to be addicted to EGF that behaves as a 'growth hormone' for EGFR overexpressing tumors.

Erlotinib, gefitinib and afatinib are active in patients bearing activating mutations of the EGFR at the kinase domain. These STKI are able to effectively inhibit EGFR mutants while sparing the wild-type receptor. The association between EGFR mutations at exons 19 or 21 and CIMAvax-EGF efficacy has not been evaluated. However, STKI sensitizing mutations are not likely to predict efficacy of CIMAvax-EGF since neoplastic cells with constitutive activation of the EGFR do not rely on ligand binding to transduce the oncogenic signal [57]. CIMAvax-EGF must be efficacious in patients with wild-type EGFR, reliant on the EGF paracrine stimulation. EGFR-activating mutations in the kinase domain are more frequent in individuals with adenocarcinoma histology, non-smokers and female sex [58]. On the contrary, CIMAvax-EGF is most effective in patients with non-adenocarcinoma histology, in smokers and in male sex, highlighting the vaccine distinctive activity in tumors with non-mutated EGFR. Actual correlative studies between EGFR overexpression, amplification or mutations in patients' samples and CIMAvax-EGF efficacy are planned.

CIMAvax-EGF resistance mechanisms are under investigation. Chronic vaccination so far has not resulted in clonal exhaustion and antibody titers were kept at high levels with monthly boosters. Serum EGF remained undetectable in the majority of patients but according to our preliminary results, TGF-α serum levels increased in some patients, 6 months after vaccination. We speculate that one resistance mechanism could be associated with a change in the growth factor addiction. TGF-α increase has been described after blocking EGFR with other antibodies or TKIs [59]. A strategy using a TGF-α vaccine or EGFR-blocking antibodies would be useful in this scenario.

The mean caveats of CIMAvax-EGF development so far are related with adequate patient selection and the use of the vaccine in combination with other drugs administered as second or third line in advanced NSCLC patients. Concerning patient selection, it is critical to validate which are the optimal serum separation protocol and the EGF cut-point that translates into a survival benefit after vaccination. In addition, evaluating the immune-competence biomarkers that allow a 'prompt' seroconversion is crucial.

Overall, immunotherapy marks an entirely different way of treating cancer by targeting the immune system, not the tumor itself. Accordingly, vaccine effects are not anticipated to be immediate because patients need several vaccine doses and time to elicit a protective response. As a consequence, patient selection for immunotherapy is a key issue. For CIMAvax-EGF,

maximum antibody titers are obtained 2–3 months after immunization. Patients without an adequate performance status and a life expectancy of at least 3 months are not good candidates for active vaccination. In addition to this rather 'subjective' assessment, other objective measurements like EGF concentration and immunosenescence markers ($CD4^+$, $CD19^+$ and $CD8^+$ $CD28^-$ counts) could be very relevant to recommend an 'EGF deprivation strategy' by means of vaccination.

Targeting EGFR might not be enough to overcome tumor heterogeneity. CIMAvax-EGF has been used so far, next to platinum-based chemotherapy. After progressive disease, patients may require second-line therapy. However, CIMAvax-EGF administration would better be continued during or after second-line therapy. Generally, it is recommended not to stop immunotherapy in spite of PD, given that slower progression rates, disease stabilizations or even responses can be obtained after an initial tumor progression. Essentially, uninterrupted vaccination might keep 'EGF-addicted' clones under control or allow a late response to occur. Maintenance in spite of PD would only be appropriate for very safe drugs like CIMAvax-EGF, which will not add major toxicity and can be combined with chemotherapy.

Five-year view

CIMAvax-EGF is still under clinical evaluation. A Phase III trial is ongoing to enroll only those NSCLC patients responsive or stabilized after chemotherapy with high EGF concentration. This trial will prospectively validate serum EGF as a predictive biomarker of CIMAvax-EGF efficacy. CIMAvax-EGF was also combined with a second cancer vaccine (racotumomab). Racotumomab targets NGcGM3 ganglioside, a very potent immunosuppressive molecule, which is overexpressed in NSCLC. Preliminary data showed increased immunogenicity of both vaccines when used in an alternating scheme. A Phase III clinical trial comparing CIMAvax-EGF, racotumomab or both vaccines was recently launched in advanced NSCLC patients unfit for chemotherapy.

As well, CIMAvax-EGF was added to frontline platinum chemotherapy or to chemotherapy/radiation in unresectable stage III NSCLC (unpublished results). CIMAvax-EGF immunogenicity was not affected by chemo-radiation. A Phase III trial to evaluate efficacy of CIMAvax-EGF in combination with chemotherapy or chemoradiation will start patient accrual.

There are many questions still pending in the clinical development of this vaccine that will be addressed in the forthcoming years: if CIMAvax-EGF should be better used as continuation or switch maintenance for advanced NSCLC (meaning if starting vaccination together with platinum doublet is better than after chemotherapy), if CIMAvax-EGF can be used as frontline monotherapy for patients with high serum EGF, which other tumor localizations would be sensitive to EGF deprivation, if CIMAvax-EGF immunogenicity and efficacy can be improved after combining with checkpoint inhibitors? Particularly, it was recently established that the PD1-PDL1 pathway contributes to immune escape in EGFR driven tumors [60].

Positioning CIMAvax-EGF in the current algorithm of NSCLC is one of the biggest challenges. According to our current data, CIMAvax-EGF should be best used as 'switch maintenance' in squamous NSCLC patients with high EGF concentration. Adenocarcinoma (ADC) patients without EGFR or ALK mutations might also be good vaccine candidates, provided they had high serum EGF levels. Alternatively, CIMAvax-EGF can be administered together with platinum doublets in squamous or ADC patients lacking EGFR mutations. At the moment of progressive disease, CIMAvax-EGF should be maintained in combination with any second-line therapy including docetaxel, erlotinib, ramucirumab or nivolumab.

In summary, active vaccination with CIMAvax-EGF would be useful for long-lasting control of those tumors addicted to EGF, without adding significant toxicity or lessening the quality of life. Today's priorities include finding the right place of CIMAvax-EGF in the current practices of NSCLC together with confirming the best predictors of its efficacy.

Financial & competing interests disclosure

A Lage, C Rodríguez, B Garcia and T Crombet work for the Center of Molecular Immunology, which sponsored all the clinical studies and produces CIMAvax-EGF. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Key issues

- Epidermal growth factor receptor (EGFR) activation is associated with high proliferation, invasiveness, metastization and anti-apoptosis of tumor cells.
- EGF is one of the most important ligands of the EGFR.
- High EGF concentration is a worse prognostic factor for advanced lung cancer patients after frontline chemotherapy.
- CIMAvax-EGF is a therapeutic cancer vaccine aimed to generate neutralizing antibodies against EGF, which would block the union between EGF and EGFR.
- CIMAvax-EGF is very safe. Most frequent adverse events are grade 1 or 2 injection site reactions, chills, fever, nausea and vomiting.
- Patients receiving at least 4 doses of the vaccine had a significant advantage in overall survival.
- Patients with high EGF concentration in sera had the largest benefit, comparable with best 'switch maintenance' therapies.

doi: 10.1586/14760584.2015.1079488

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Shen H, Shen J, Wang L, et al. Low miR-145 expression level is associated with poor pathological differentiation and poor prognosis in non-small cell lung cancer. Biomed Pharmacother 2015;69:301-5
2. Mah TL, Yap XN, Limviphuvadh V, et al. Novel SNP improves differential survivability and mortality in non-small cell lung cancer patients. BMC Genomics 2014; 15(Suppl 9):S20
3. Tan F, Shi Y, Wang Y, et al. Icotinib, a selective EGF receptor tyrosine kinase inhibitor, for the treatment of non-small-cell lung cancer. Future Oncol 2015;11(3): 385-97
4. O'Mahony S, Nathan S, Mohajer R, et al. Survival Prediction in Ambulatory Patients With Stage III/IV Non-Small Cell Lung Cancer Using the Palliative Performance Scale, ECOG, and Lung Cancer Symptom Scale. Am J Hosp Palliat Care 2015. [Epub ahead of print]
5. Davis KL, Goyal RK, Able SL, et al. Real-world treatment patterns and costs in a US Medicare population with metastatic squamous non-small cell lung cancer. Lung Cancer 2015;87(2):176-85
6. Jiang T, Zhou C. Treatment of patients with ALK Gene rearranged non-small cell lung cancer after resistance to crizotinib. Zhongguo Fei Ai Za Zhi 2015;18(2):69-74
7. Iacono D, Chiari R, Metro G, et al. Future options for ALK-positive non-small cell lung cancer. Lung Cancer 2015;87(3): 211-19
8. Cappuzzo F, Moro-Sibilot D, Gautschi O, et al. Management of crizotinib therapy for ALK-rearranged non-small cell lung carcinoma: An expert consensus. Lung Cancer 2015;87(2):89-95
9. Massarelli E, Papadimitrakopoulou V. Ceritinib for the treatment of late-stage (metastatic) non-small cell lung cancer. Clin Cancer Res 2015;21(4):670-4
10. Jorge SE, Kobayashi SS, Costa DB. Epidermal growth factor receptor (EGFR) mutations in lung cancer: preclinical and clinical data. Braz J Med Biol Res 2014; 47(11):929-39
11. Liao BC, Lin CC, Yang JC. Second and third-generation epidermal growth factor receptor tyrosine kinase inhibitors in advanced nonsmall cell lung cancer. Curr Opin Oncol 2015;27(2):94-101
12. Landi L, Cappuzzo F. Pharmacotherapy targeting the EGFR oncogene in NSCLC. Expert Opin Pharmacother 2014;15(16): 2293-305
13. Paz-Ares LG, de Marinis F, Dediu M, et al. PARAMOUNT: Final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. J Clin Oncol 2013;31(23):2895-902
14. Greenhalgh J, McLeod C, Bagust A, et al. Pemetrexed for the maintenance treatment of locally advanced or metastatic non-small cell lung cancer. Health Technol Assess 2010;14(Suppl 2):33-9
15. Fidias PM, Dakhil SR, Lyss AP, et al. Phase III study of immediate compared with delayed docetaxel after front-line therapy with gemcitabine plus carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 2009;27(4):591-8
16. Wu YL, Kim JH, Park K, et al. Efficacy and safety of maintenance erlotinib in Asian patients with advanced non-small-cell lung cancer: a subanalysis of the phase III, randomized SATURN study. Lung Cancer 2012;77(2):339-45
17. Al-Farsi A, Ellis PM. Treatment paradigms for patients with metastatic non-small cell lung cancer, squamous lung cancer: first, second, and third-line. Front Oncol 2014;4:157
18. Pirker R, Pereira JR, Szczesna A, et al. FLEX Study TeamCetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet 2009;373(9674):1525-31
19. Pirker R, Pereira JR, von Pawel J, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol 2012; 13(1):33-42
20. Pillai RN, Ramalingam SS. Advances in the diagnosis and treatment of non-small cell lung cancer. Mol Cancer Ther 2014;13(3): 557-64
21. Paz-Ares L, Mezger J, Ciuleanu TE, et al. Necitumumab plus pemetrexed and cisplatin as first-line therapy in patients with stage IV non-squamous non-small-cell lung cancer (INSPIRE): an open-label, randomised, controlled phase 3 study. Lancet Oncol 2015;16(3):328-37
22. Sacco PC, Maione P, Rossi A, et al. Necitumumab for the treatment of stage IV metastatic squamous non-small-cell lung cancer. Expert Rev Respir Med 2015;1-10
23. Sivendran S, Glodny B, Pan M, et al. Melanoma immunotherapy. Mt Sinai J Med 2010;77(6):620-42
24. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 2015;33C:23-35
25. Momtaz P, Postow MA. Immunologic checkpoints in cancer therapy: focus on the programmed death-1 (PD-1) receptor pathway. Pharmgenomics Pers Med 2014;7: 357-65
26. Weber JS. Current perspectives on immunotherapy. Semin Oncol. 2014; 41(Suppl 5):S14-29
27. Rini B. Future approaches in immunotherapy. Semin Oncol. 2014; 41(Suppl 5):S30-40
28. Rijavec E, Genova C, Barletta G, et al. Ipilimumab in non-small cell lung cancer and small-cell lung cancer: new knowledge on a new therapeutic strategy. Expert Opin Biol Ther 2014;14(7):1007-17
29. Yoon SH. Immunotherapy for non-small cell lung cancer. Tuberc Respir Dis (Seoul) 2014;77(3):111-15
30. Domingues D, Turner A, Silva MD, et al. Immunotherapy and lung cancer: current developments and novel targeted therapies. Immunotherapy 2014;6(11):1221-35
31. Reiss KA, Forde PM, Brahmer JR. Harnessing the power of the immune system via blockade of PD-1 and PD-L1: a promising new anticancer strategy. Immunotherapy 2014;6(4):459-75
32. Johnson DB, Rioth MJ, Horn L. Immune checkpoint inhibitors in NSCLC. Curr Treat Options Oncol 2014;15(4):658-69
33. Harshman LC, Drake CG, Wargo JA, et al. Cancer Immunotherapy Highlights from the 2014 ASCO Meeting Cancer Immunol Res 2014;2(8):714-19
34. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015;373(2):123-35
35. Liu JK. Anti-cancer vaccines - a one-hit wonder? Yale J Biol Med 2014;87(4):481-9
36. Singh BH, Gulley JL. Therapeutic vaccines as a promising treatment modality against prostate cancer: rationale and recent advances. Ther Adv Vaccines 2014;2(5): 137-48
37. Weber JS. Current perspectives on immunotherapy. Semin Oncol. 2014; 41(Suppl 5):S14-29

38. Bogart JA, Gajra A. Immunotherapy in non-small-cell lung cancer: a good start? Lancet Oncol 2014;15(1):5-6

39. Butts C, Socinski MA, Mitchell PL, et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15(1):59-68

40. Ruiz R, Hunis B, Raez LE. Immunotherapeutic agents in non-small-cell lung cancer finally coming to the front lines. Curr Oncol Rep 2014;16(9):400

41. Cuppens K, Vansteenkiste J. Vaccination therapy for non-small-cell lung cancer. Curr Opin Oncol 2014;26(2):165-70

42. Domingues D, Turner A, Silva MD, et al. Immunotherapy and lung cancer: current developments and novel targeted therapies. Immunotherapy 2014;6(11):1221-35

43. Alfonso S, Valdés-Zayas A, Santiesteban E, et al. A randomized, multicenter, placebo-controlled clinical trial of racotumomab-alum vaccine as switch maintenance therapy in advanced non-small cell lung cancer patients. Clin Cancer Res 2014;20(14):3660-71

44. Hernández AM, Vázquez AM. Racotumomab-alum vaccine for the treatment of non-small-cell lung cancer. Expert Rev Vaccines 2015;14(1):9-20

45. Gonzalez G, Montero E, Leon K, et al. Autoimmunization to epidermal growth factor, a component of the immunological homunculus. Autoimmun Rev 2002;1(1-2): 89-95

•• **The authors highlight the relevance of the EGF as part of the immunological homunculus.**

46. González G, Lage A. Cancer vaccines for hormone/growth factor immune deprivation: a feasible approach for cancer treatment. Curr Cancer Drug Targets 2007; 7(3):229-41

• **The paper summarizes the rationale behind CIMAvax-EGF.**

47. Lage A, Crombet T, González G. Targeting epidermal growth factor receptor signaling: early results and future trends in oncology. Ann Med 2003;35(5):327-36

48. Mancebo A, Casacó A, González B, et al. Repeated dose intramuscular injection of the CIMAvax-EGF vaccine in Sprague Dawley rats induces local and systemic toxicity. Vaccine 2012;30(22):3329-38

• **The authors describe the results of the repeated toxicology.**

49. González G, Crombet T, Catalá M, et al. A novel cancer vaccine composed of human-recombinant epidermal growth factor linked to a carrier protein: report of a pilot clinical trial. Ann Oncol 1998;9(4): 431-5

•• **The authors compare 2 carrier proteins in terms of immunogenicity and safety.**

50. Gonzalez G, Crombet T, Torres F, et al. Epidermal growth factor-based cancer vaccine for non-small-cell lung cancer therapy. Ann Oncol 2003;14(3):461-6

•• **The authors compare two adjuvants in terms of immunogenicity and safety.**

51. Ramos TC, Vinageras EN, Ferrer MC, et al. Treatment of NSCLC patients with an EGF-based cancer vaccine: report of a Phase I trial. Cancer Biol Ther 2006;5(2): 145-9

•• **The authors compare two vaccine doses in terms of immunogenicity and safety.**

52. Neninger E, Verdecia BG, Crombet T, et al. Combining an EGF-based cancer vaccine with chemotherapy in advanced nonsmall cell lung cancer. J Immunother 2009;32(1):92-8

•• **The authors describe the immunogenicity of CIMAvax-EGF in combination with chemotherapy.**

53. García B, Neninger E, de la Torre A, et al. Effective inhibition of the epidermal growth factor/epidermal growth factor receptor binding by anti-epidermal growth factor antibodies is related to better survival in advanced non-small-cell lung cancer patients treated with the epidermal growth factor cancer vaccine. Clin Cancer Res 2008;14(3): 840-6

•• **The authors describe the biological effect of the anti-EGF antibodies induced by vaccination.**

54. Neninger E, de la Torre A, Osorio M, et al. Phase II randomized controlled trial of an epidermal growth factor vaccine in advanced non-small-cell lung cancer. J Clin Oncol 2008;26(9):1452-9

•• **The authors present the results of the first randomized study.**

55. Crombet T, Neninger E, Acosta S, et al. EGF-based cancer vaccine for advanced NSCLC: Results from a phase III trial. J Clin Oncol 2012;30; Suppl; abstr 2527

•• **The authors summarize the preliminary results of the Phase III study.**

56. Crombet T, Neninger E, Gonzalez J, et al. EGF-based cancer vaccine: Optimizing predictive and surrogate biomarkers. J Clin Oncol 2013;31; Suppl; abstr 3013

•• **The authors propose EGF concentration as a biomarker of efficacy.**

57. Jorge SE, Kobayashi SS, Costa DB. Epidermal growth factor receptor (EGFR) mutations in lung cancer: preclinical and clinical data. Braz J Med Biol Res 2014; 47(11):929-39

58. Cress WD, Chiappori A, Santiago P, et al. Lung cancer mutations and use of targeted agents in Hispanics. Rev Recent Clin Trials 2014;9(4):225-32

59. Troiani T, Martinelli E, Napolitano S, et al. Increased TGF-α as a mechanism of acquired resistance to the anti-EGFR inhibitor cetuximab through EGFR-MET interaction and activation of MET signaling in colon cancer cells. Clin Cancer Res 2013; 19(24):6751-65

60. Lin C, Chen X, Li M, et al. Programmed Death-Ligand 1 Expression Predicts Tyrosine Kinase Inhibitor Response and Better Prognosis in a Cohort of Patients With Epidermal Growth Factor Receptor Mutation-Positive Lung Adenocarcinoma. Clin Lung Cancer 2015. [Epub ahead of print]

doi: 10.1586/14760584.2015.1079488