

Medical Practice Confirms Clinical Trial Results of the Use of Intralesional Human Recombinant Epidermal Growth Factor in Advanced Diabetic Foot Ulcers

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Abstract

The intralesional injection of recombinant human epidermal growth factor (rhEGF) has been recently approved and introduced in several countries for the treatment of advanced diabetic foot ulcers (DFU), based on the results of five exploratory and one confirmatory, phase III clinical trials in 344 subjects. A significant stimulatory effect of this product on the healing process, given by development of granulation tissue and re-epithelization was shown in these trials, as well as a reduction in lesion recurrences during follow-up, and a tendency to a reduction of the risk of amputations, with an acceptable safety profile. However, products not always perform the same way in current medical practice. The present review summarizes the clinical information available from the intralesional use of rhEGF for advanced DFU and shows that in this case the postmarketing experiences in more than 2000 subjects confirm the results of the clinical trials, with 75% probability of complete granulation response, 61% healing, and a 16% absolute and 71% relative reduction of the risk of amputation. The benefit includes ischemic patients. The safety profile in current practice was satisfactory. Serious adverse events are not attributable to the treatment but to the underlying conditions of the patients. No evidence of neoplasia promotion by the growth factor has been found. The benefit-risk ratio of the procedure is favorable.

Keywords: Epidermal growth factor; Diabetic foot ulcer; Intralesional injections

Abbreviations: DFU: Diabetic Foot Ulcer; EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; rhEGF: Recombinant Human Epidermal Growth Factor; ELISA: Enzyme Linked Immunosorbent Assay; PDGF: Platelet Derived Growth Factor; SAE: Serious Adverse Event

Introduction

Diabetes mellitus is a world-wide health problem with a reported prevalence in more than 170 million in people more than 20 years-old [1]. Foot ulceration is a significant complication of diabetes with an annual incidence of slightly more than 2% of all patients with diabetes, which increases to between 5.0 and 7.5% in those with peripheral neuropathy [2]. It is estimated that 15% of patients with diabetes develop ulcers at some point in their lives. Between 10-30% of diabetic patients with ulcers will progress to amputation. The 5-year mortality rate of patients who have undergone the amputation of a lower extremity is 50-60% [3].

The therapeutic management of a patient with a diabetic foot ulcer (DFU) is currently based on: metabolic control, debridement, moist cures, wound dressing, local pressure off-loading, antimicrobial treatment of infections, and revascularization procedures, when indicated [4]. More recent therapies such as topical growth factors [4-9], engineered skin [10,11], and others have shown efficacy in pure neuropathic, non-complicated ulcers. However, these products would still have to be tested in advanced lesions including those with ischemic etiopathogenesis, which are usually exclusion criteria in DFU clinical trials [12]. Additionally, in some cases their impact on clinical practice

has not fulfilled the expectations raised by clinical trial results [13,14].

Epidermal growth factor (EGF) is a 53-aminoacidpolypeptide, isolated for the first time by Cohen from mice submaxillary glands [15]. It stimulates the proliferation of fibroblasts, keratinocytes and vascular endothelial cells, which contribute to its scar tissue formation properties. Its mechanism of action is based on the interaction with specific receptors (EGFR) with tyrosine kinase activity [16]. The presence of these receptors has been reported in most human tissues (not in hematopoietic linages, although recently it was reported in leukemia cells [17]) with a relative abundance in the skin [18].

The rationale of the use of EGF for the treatment of DFU is based on (i) impairment of healing in diabetic patients, partially due to a relative deficit of growth factors (EGF among them) in the wound area [19]; (ii) the growth stimulating, healing promoting, and cytoprotective actions

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of EGF, including angiogenesis [20]; (iii) the nerve restoration action of EGF as shown in sciatic nerve section experiments, where it prevented distal limb ulceration and the loss of toes. This model resembles the trophic damages in diabetic neuropathy, which is in the basis of the DFU physiopathology [21].

On the other hand the availability of the growth factor on the surface of the wound is limited as it can be degraded by proteases from the biofilm that covers the lesion and/or from its fluid [22-27]. Previous clinical evidences of topically applied EGF had already rendered disappointing results, possibly due to local bioavailability limitations [28,29]. Additionally, EGF-responding granulation tissue develops from the deep layers of the wound as shown by the inverse expression of the phosphorylated EGFR and prohibitin [30], a cell cycle progression inhibitor [31] throughout different layers of the chronic wound.

Based on the concepts issued above, the local (intralesional) instillation of recombinant human EGF (rhEGF) to promote granulation and healing of chronic, advanced DFU has been developed in several clinical trials, which have led to approval in several countries. Additionally, postmarketing information of the product has been gathered. This paper will briefly recall this development and show non-previously published data indicating that the clinical performance of this procedure fits with the clinical trial results, both in terms of safety and efficacy. Not published materials were reviewed from the final reports of the corresponding studies.

Trials Performed in the Clinical Development Program

A formulation of recombinant human epidermal growth factor

(rhEGF) for intralesional administration in DFU has been developed. The growth factor is purified from a transformed *Saccharomyces cerevisiae* strain and presented as a lyophilized preparation containing 25 or 75 µg of rhEGF per vial under the brand Heberprot P®. In all studies, the intralesional rhEGF was used in-hospital, adjuvant to the standard wound care, which included metabolic control, pressure off-loading, thorough debridement or minor amputation of necrotic and infected tissue, moist dressings, and systemic antibiotics, if necessary, in order to clear signs of infection before the rhEGF injections started. The product was dissolved with 5 ml of water for injection (QUIMEFA, Havana). In every visit this volume was distributed throughout the lesion, in 0.5–1 ml injections. After sharp debridement, lesions were washed with saline (QUIMEFA, Havana) and the rhEGF solution was injected using a standard disposable syringe with 27G × 0.5 needles, first into the dermoepidermal junction at equidistant points all over the lesion contours and then downward into the wound bottom to ensure a uniform distribution. The needle was changed for each puncture. Then, the wounds were dressed with sterile gauze. Infiltrations were performed thrice weekly on alternate days up to the eighth week or less if complete granulation was achieved.

Five exploratory and one confirmatory randomized, double-blind, placebo-controlled studies were performed for the clinical development of this product in advanced DFU (Wagner’s grades 3 and 4, which correspond approximately to the University of Texas grades II or III; stages B, C, or D). Those were studies No. 0102 [32], 0202 [33], 0504 (not published), 0604 (not published), 0705 [34], and 0503 [35]. Their main features, sample size, and the proportion of ischemic

Study	DFU type	Main study features	Subjects (% ischemic)
0102 [32]	Wagner 4	Exploratory. Lineal. 25 µg 3 tpw until healing or maximal dose (500 µg) reached	29 (79%)
0202 [33]	Wagner 3–4	Exploratory. Randomized, double-blind, multicenter (5 sites) Group 1: 75 µg; Group 2: 25 µg 3 tpw, for 8 weeks or until complete granulation	G1: 23 (74%) G2: 18 (56%)
0504 np	Wagner 3-4.	Exploratory. Lineal. 75 µg 3 tpw, for 8 weeks or until complete granulation	12 (67%)
0604 np	Wagner 3-4	Phase IV, multicenter (19 sites). While conditional approval. Lineal. 25 µg 3 tpw for 8 weeks or until complete granulation	93 (50%)
0705 ref [34]	Wagner 3-4	Exploratory. Lineal. 75 µg, 3 times per week, until complete healing	20 (25%)
0503 ref [35]	Wagner 3-4	Confirmatory. Randomized, double-blind, multicenter (20 sites). Group 1: 75 µg; Group 2: 25 µg; Group 3: placebo. 3 tpw, for 8 weeks or until complete granulation. If no minimal response at 2 weeks, patient shifted from group 3 to 2 and from group 2 to 1	G1: 53 (55%) G2: 48 (65%) G3: 48 (46%)
0704 np	All	Postmarketing. Pharmacovigilance. Lineal, multicenter (60 sites). 75 µg or 25 µg, 3 tpw, for 8 weeks or until complete granulation	ulcers 1835 (43%)
1111 np	All	Postmarketing Retrospective review of clinical records from the 2nd semester of 2010 in 8 sites. Group 1: rhEGF treatment assumed as labeled: 75 µg or 25 µg, 3 tpw, for 8 weeks or until complete granulation. Group 2: standard care only	G1: 199 (41%) G2: 439 (51%)
1217 np	All	Postmarketing Retrospective review of clinical records from 2011 in 5 sites. Group 1: rhEGF treatment assumed as labeled: 75 µg or 25 µg, 3 tpw, for 8 weeks or until complete granulation. Group 2: standard care only	G1: 445 (40%) G2: 542 (51%)
IS Cfgos [37]	Wagner 2 and 4	Postmarketing, controlled. rhEGF vs. standard care alone. Treatment assumed as labeled: 75 µg or 25 µg, 3 tpw, for 8 weeks or until complete granulation	rhEGF: 120 Control: 60 (not available)
IS “10/10” [38]	Wagner 3-4	Postmarketing series. Lineal 25 µg, 3 tpw for 8 weeks or until complete granulation	13 (15.4%)
IS Mtzas [39]	All	Postmarketing. randomized comparison of two vehicles 75 µg, 3 tpw, for 8 weeks or until closure	20 (30%)
0707 np	Wagner 1-2	Pharmacokinetics. Randomized, double blind, 25 µg or 75 µg 3 tpw for 12 weeks or until closure. Only taken into account in this review for safety data.	25 µg: 8 (0) 75 µg: 8 (0)
0809 np	Wagner 2	Dose exploratory. Randomized, double blind: placebo, 2.8, 8.3; 25, or 75 µg. 3 tpw for 12 weeks or until closure. Only taken into account in this review for safety data.	35 (0)

tpw: times per week; np: not published; IS: investigator-sponsored study; Cfgos: Cienfuegos province; “10/10”: “10 de octubre” Hospital in Havana; Mtzas: Matanzas province

Table 1: Clinical Studies with intralesional rhEGF in DFU considered.

lesions treated (which is the most important baseline characteristic that influences outcomes) are listed in table 1. The trials were all conducted in Cuba according to local Good Clinical Practice guidelines [36], which include the Declaration of Helsinki ethical guidelines, protocol approval by Ethics Committees, and subjects' informed consent to participate. A total of 344 subjects received rhEGF in these studies. Besides, a pharmacokinetics study with the usual doses (25 µg and 75 µg) was finished on 16 subjects with non-complicated DFU (Wagner's 1 and 2, which correspond to University of Texas grades I or II; stage A), and a placebo-controlled dose-exploratory trial, done also on non-complicated ulcers in 35 individuals, were taken into account, only for the safety data.

Table 1 shows the postmarketing studies as well. The nationwide introduction of the use of intralesional rhEGF in DFU in Cuba (study 0704) provided the report of the first 1851 treatments applied on 1835 lesions from 1788 subjects, including pharmacovigilance information. Additionally, two retrospective studies (number 1111 and 1217), based on the review of clinical records of DFU hospital discharges, were done to characterize the management of DFU in a group of sites (3 hospitals coincided in both periods) and to evaluate the difference made by the use of rhEGF on the patients' outcome. Besides, three investigator-sponsored studies were published [37-39] providing reports of the use of the product in routine clinical practice.

Distinctiveness of the Approach Undertaken

This clinical development has dealt with a type of subject not usually included in clinical trials on DFU. The characteristics of the population recruited have been quite homogeneous across the trials, and are essentially diabetes mellitus, mostly type 2 (83%); median age: 65 years, range 21-87; chronic ulcer (more than one month of existence), with exposure of subcutaneous tissue and/or tendons, and/or a joint capsule; advanced ulcers, given by Wagner's grades 3 or 4, large size (median area: 23 cm², range 1-300); 70% with baseline infection, treated before the use of rhEGF; 30% with gangrenous necrotic tissue that had to be removed surgically; 50% of the subjects with deficient circulation of the affected leg (ischemia). The three latter characteristics are often exclusion criteria in clinical trials [12]. However, it is in these patients (who may represent >50% of the whole DFU population) that DFU-related amputations most commonly occur.

Relevant features of the patient populations

The subject populations across the different efficacy exploratory and confirmatory studies have been homogeneous. In comparative trials there has not been any noteworthy difference among concurrent groups. The main exclusion criteria kept in most trials were: (i) uncontrolled chronic diseases including, diabetic coma, cardiac, pulmonary, liver, and renal (creatinine >200 µmol/l) failures; (ii) hemoglobin <100 g/l at inclusion; (iii) history or suspicion of any malignancy; (iv) neurologic or psychiatric diseases; (v) pregnancy or breastfeeding.

The primary outcome studied in most of the clinical trials was the development of granulation tissue covering the lesion area. It can be argued that in serious ulcers this is a valid outcome since (i) granulation is part of the healing process and precedes the final healing (re-epithelialization); (ii) aDFU covered with granulation tissue may allow skin grafting to attain final healing; thus, this type of subjects cannot be necessarily evaluated until complete healing (re-epithelialization) by second intention; (iii) a significant correlation was found in the confirmatory study no. 0503 between granulation tissue development and complete healing. This suggested that granulation can be a surrogate variable for healing in advanced DFU. Previous studies had identified partial wound closure as predictive of complete healing for Wagner's grade 1 or 2 DFU [40], and other ulcers [41,42], but this was the first report of an early surrogate endpoint in Wagner's grade 3 or 4 DFU [35]. This correlation was confirmed when calculated for the other randomized study No. 0202 and for the results of the postmarketing extension program No. 0704 with a larger number of subjects. The further statistical development of these data showed that, for advanced ulcers, granulation tissue development variables are better surrogates than partial closure variables [43].

Overview of Efficacy

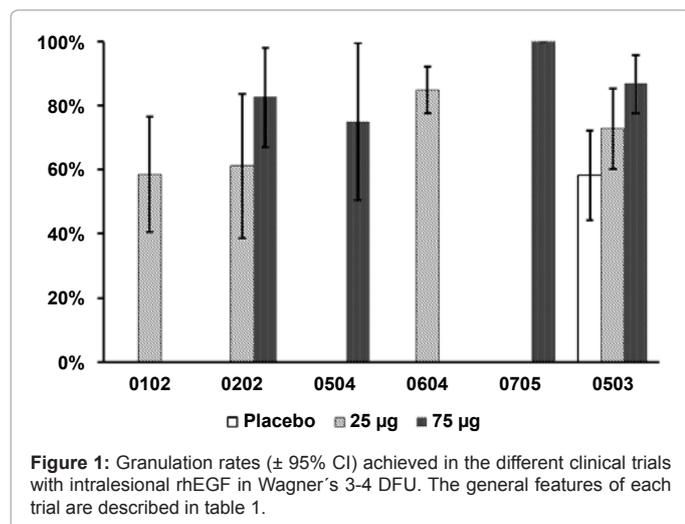
Efficacy evaluation across the different studies

Granulation and re-epithelization: Efficacy measurements across the different clinical trials with rhEGF in advanced, Wagner's grades 3-4 DFU were consistent. Their results were reviewed in details previously [44] and summarized in the supplementary table S1. The complete granulation rates achieved in each study are shown in figure 1 and the pooled results in table 2. More than 75% granulation was obtained globally for both dose levels used, slightly better for the higher dose,

Study	Dose	N	Complete granulation response (%)	Subsequent complete healing (%)	Weeks to complete healing	Amputations
Pooled from clinical trials	Placebo	48	28 (58.3%) (43.4-73.3)	27 (56.3%) (41.2-71.3)	21.4 (8-35)	12 (25.0%) (12.8-37.3)
	25 µg	188	141 (75.0%) (68.8-81.2)	97 (51.6%) (44.5-58.7)	12 (9.7-14.3)	54 (28.7%) (22.3-35.3)
	75 µg	108	94 (87%) (80.7-93.4)	71/96 (74%) (65.2-82.7)	14.0 (11.6-16.3)	18 (16.7%) (9.6-23.7)
IS Mtzas	75	20	15 (75.0%)	14 (70.0%)	NA	NA
0704	25 & 75 µg	1788	1349/1835 (73.5%)	1012/1659 (61.0%)	NA	220(11.9%) Major: 171 (9.2%)
1111	25 & 75 µg	199	147 (73.4%)	NA	NA	Major: 17 (8.6%)
	No rhEGF	439	114 (26.0%)	NA	NA	Major: 98 (22.2%)
1217	25 & 75 µg	445	NA	NA	NA	Major: 22 (4.9%)
	No rhEGF	542	NA	NA	NA	Major: 118 (21.8%)
IS Cfgos	25 & 75 µg	120	74 (61.7%)	87 (72.5%)	NA	Major: 10 (8.3%)
	No rhEGF	60	22 (36.7%)	27 (45%)	NA	Major: 16 (26.7%)
IS "10/10"	25 µg	13	9 (69.2%)	NA	NA	0

NA: Not Available

Table 2: Efficacy results in clinical trials (pooled) and postmarketing experiences of intralesional rhEGF in subjects with DFU.



but the differences were not statistically significant. Overall efficacy results are much better than those observed in the group of subjects that received only standard care (the placebo group of study 0503). The fact that only one study had a control group is a limitation to this comparison, but there is homogeneity among the different studies with respect to the results obtained.

The design of the confirmatory study 0503 was fully randomized and double-blinded just up to the second week of treatment. This constraint was imposed by the ethics committees since it was considered that 2 weeks was enough to detect onset of response and it was unethical to continue non-responders on placebo, particularly when the product had already a conditional approval in Cuba at that moment, and there was risk of an irreversible outcome (amputation). After 2 weeks, if no response was present, the code was opened. Subjects on placebo or 25 µg EGF were offered to continue treatment unblinded with 25 or 75 µg, respectively. Therefore, the main outcome of this trial was the proportion of subjects with at least partial response (50% of the wound area covered with granulation tissue) after 2 weeks of treatment, as this was the time interval when all subjects were blinded and in their originally allocated groups. Subjects whose codes were opened on week 2 (one with 75 µg, 4 with 25 µg, and 5 with placebo) were considered failures for their original groups, on "intention-to-treat" basis, for end-of-treatment response and wound closure.

This design could have some impact on outcome regarding granulation rates at end of treatment visit and closure rates during the one year follow-up. Even if this kind of analysis may introduce bias in the interpretation of the results, the opposite does this too. The "intention-to-treat" evaluation principle has been usually preferred in the analysis of randomized clinical trial data. In this work, the interpretation is further validated by the fact that analyses of secondary variables, after deleting the nine group-shifting patients, yielded similar treatment-dependent outcomes, despite the loss of statistical power.

Results on complete healing (re-epithelization) are also clinically relevant for the 75 µg dose. Treatment-dependency was found for complete closure, despite being reached during follow-up when subjects were treated as outpatients with only general wound care measures (except for study 0705 where treatment was continued until healing). This apparent "EGF-memory" effect can be explained by the granulation tissue stimulation, which was predictive of closure. Granulation can also reduce the probability of infection progression,

since the fresh tissue is better prepared to "fight" against invading micro-organisms and improved circulation irrigation can increase the local bioavailability of systemic antibiotic treatment.

Time-to-closure was also shortened in approximately 5 weeks for the 75 µg and 25 µg treatments in the confirmatory 0503 trial. This difference is clinically significant. The healing "acceleration" was more relevant for neuroinfectious subjects. In the confirmatory 0503 trial, this category included those subjects with ankle/brachial pressure index >0.75. Therefore the "mildly ischemic" subjects (0.90 >ABI >0.75) were considered in the "neuropathic" category as well. This is important for further trial design as well as for analyses of the external validity of the results.

Postmarketing information in Cuba fits with that obtained from clinical trials (Table 2) and confirms the results obtained there, despite coming from routine medical practice. Even the retrospective study No. 1111, done with the information taken from archive medical records of 8 hospitals shows a complete granulation rate at discharge, which is consistent with that of the clinical trials.

Relapses: Ulcer recurrence was evaluated during followup upto one year in trials 0102, 0202, and 0503. There were very few recurrences in rhEGF-treated subjects where complete ulcer healing had occurred: 1/7 in study 0102, 1/22 in study 0202, and none (0/66) in study 0503. These subjects were further visited. The mean follow-up time was 2.9 years (maximum 8 years). The frequency of relapses at any moment was significantly lower ($p < 0.001$) in patients that received rhEGF (2.0% and 1.3% person-years of follow-up for the 75 µg and 25 µg doses, respectively) as compared to the control group of the confirmatory, No. 0503, trial (7.9% person-years). This effect was obtained for both neuropathic and ischemic patients. On the contrary, no effect was seen on the appearance of new DFU on other locations (mainly on the contralateral limb). The rates were 8.8%, 8.2%, and 11.6% for patients treated with 75 µg rhEGF, 25 µg, and placebo, respectively. It seems as if the tissue keeps a sort of "memory" of the treatment received, which is not transferable to non-treated zones. These results were confirmed in the postmarketing 0704 study where the relapse and new lesions rates were 5% and 9.5% person-years, respectively.

Amputations: With respect to amputations, the number of events was too small in the clinical trials in order to make a proper statistical analysis; this outcome was secondary in all studies. The pooled analysis of clinical trials is interesting for the 75 µg dose (14% vs. 25% in the control group of study 0503).

The postmarketing studies provide more useful information for this variable (Table 2). Groups of patients treated with rhEGF have similar rates of major amputations: study No. 0704 (National program): 9.2%; retrospective study no. 1111: 8.6%; retrospective study no. 1217: 4.9%; investigator-sponsored study in Cienfuegos: 8.3%. On the contrary, groups not treated with rhEGF, but only with the general standard care measures have higher rates of amputations: retrospective study no. 1111: 22.2%; retrospective study no. 1217: 21.8%; investigator-sponsored study in Cienfuegos: 26.7%. Even if the comparison is limited by the fact that those were not randomized studies and all the baseline characteristics of the patients are not known, the consistency of the results suggests their reliability. The difference is noteworthy. The impact of the use of rhEGF for treating DFU is similar in the three independent series, as shown in table 3. The result is highly significant: 16% absolute difference, 71% relative advantage, and only 6 subjects needed to treat to prevent one amputation.

Survival since the beginning of treatment: The median follow-

Study	ARR (95% CI)	RRR (95% CI)	NNT (95% CI)
Investigator-sponsored study-Cienfuegos [37]			
All patients (ulcer etiology information not available)	18.4% (6.2-30.6%)	69% (23-100%)	5 (3-16)
Retrospective investigation No. 1111			
All patients	13.8% (8.3-19.3)	62% (37-87)	7 (5-12)
Ischemic	16.8% (6.4-27.2)	48% (18-78)	6 (4 -16)
Neuropathic	7.2% (2.7-11.7)	81% (31-100)	14 (9-37)
Retrospective investigation No. 1217			
All patients	16.9% (12.9; 20.9)	78% (59; 96)	6 (5; 8)
Ischemic	25.5% (18.4; 32.6)	73% (52; 93)	4 (3; 5)
Neuropathic	5.1% (1.6; 8.6)	73% (22; 100)	20 (12; 64)
Pooled			
All patients	15.9% (12.8-19.1)	71% (58-85)	6 (5 8)
Ischemic (only 1111 & 1217)	22.3% (16.5-28.1)	64% (48-81)	4 (4-6)
Neuropathic (only 1111 & 1217)	6.0% (3.2-8.8)	76% (40-100)	17 (11-31)

ARR: Absolute risk reduction; RRR: Relative risk of amputation reduction; NNT: Number needed to treat to prevent an amputation

Table 3: Impact of rhEGF on amputation rates in series with control groups.

up period has been 2.9 years (maximum, 8 years) for clinical trials and median 1.2 years; maximum 4 years for the postmarketing study 0704. In both series median survival was longer in patients that had attained ulcer-healing; in clinical trials 5.7 years vs. 4.2 years in those who did not heal (1.5 years difference). The postmarketing study, with shorter follow-up, reproduced this result: mean survival 3.5 years for healers and 2.2 for non-healers (1.3 years difference; median has not been reached yet for healers). Besides, in study 0704, due to its larger population, significant unfavorable effects on survival were detected for the ischemic etiology of the lesion, amputation after treatment, older age, and history of ischemic cardiopathy.

Overview of Safety

The people from whom there is exposure data of rhEGF injections and safety information are: 51 subjects that received placebo in clinical trials; 364 that received rhEGF in clinical trials, and 1788 that received 1851 treatment cycles of rhEGF in the national pharmacovigilance program in Cuba. The placebo individuals are included in the analyses as reference, but also to evaluate the safety of the intralesional injection procedure itself. Most of the subjects received the product 3 times per week, but there have been daily and once or twice per week applications as well. The median number of applications ranged in the different studies from 8 to 16. The maximum number of injections received by anybody was 51 in a period of 10 months with the 75 µg dose in a patient that came twice to be treated on different ulcers. The amount of rhEGF received by this subject was 3.8 mg. He has been followed-up for 637 more days without any relevant clinical issue.

Common adverse events

Recombinant EGF administered intralesionally was well tolerated. Around one half of the subjects (63.1% in the clinical trials and 46.2% in the postmarketing pharmacovigilance) reported some kind of adverse event. Adverse events reported have been more frequent with the 75 µg dose than with 25 µg. The proportion of patients with adverse events are 69.7% vs. 54.4% in the clinical trials and 51.9% vs. 40.0% during the pharmacovigilance in Cuba for 75 µg and 25 µg, respectively. Seriousness of the events seems higher for the 75 µg too: in the clinical trials 18.3% vs. 11.8% of the subjects had serious adverse events; in the

pharmacovigilance, 80% of the serious adverse events occurred with the 75 µg dose.

The more common adverse events (occurring in more than 1% of the subjects) are summarized in table 4. Pain and burning sensation at the administration site were the main adverse events described in all groups, with similar frequencies among them. They are most likely associated to the injection procedure itself. Chills and shivering are the other most frequent adverse events in all trials. They are clearly dose-dependent. These events were never severe or caused treatment interruptions. Their frequency decreases in subsequent applications. In most cases their relation with treatment was considered definite or probable.

Local infection was reported in 53 individuals in the clinical trials and 69 during the pharmacovigilance, mostly moderate or severe. It was the most frequent serious adverse event (SAE): 29/58 SAE in clinical trials; 10/31 SAE in the pharmacovigilance, leading to hospitalization and/or amputation in all dose groups, including the placebo. Its relation to treatment is not likely. Ulcers, even without signs of infection, are not sterile and infection progression is one of the most common complications of DFU. It occurred in the placebo group as well, at a similar rate. In those patients with Wagner's grade 3 and 4 ulcers where healing did not occur, local infection was deemed the reason for the non-healing of the ulcers. Indeed, it has been reported that the infection of lower extremities is the most frequent reason for the hospitalization of subjects with diabetes [45]. Some of the factors that leave subjects predisposed to the development of infection include the presence of an entryway for bacteria and the fact that the immune response of diabetic patients is often compromised.

The safety profiles in clinical trials and postmarketing pharmacovigilance were similar, except for the frequency of local infections, which is lower in the postmarketing. This could be due to (i) confusion of this event with "lesion worsening", which was not always reported as adverse events by the treating personnel; (ii) improvement of wound management in the main sites with more experience on the issue; (iii) sub-reporting during usual medical practice of an event that is seen as a common complication of the ulcer.

Serious adverse events

Serious adverse events (SAE) were reported in 58 (14%) of the

Adverse event	In clinical trials				Pharmacovigilance (study 0704)	
	<25 µg (n=13)	25 µg (n=204)	75 µg (n=142)	Placebo n=48	25 µg (n=734)	75 µg (n=1103)
Burning sensation on the injection site	9 (69.2%)	31 (15.2%)	30 (21.1%)	19 (33.9%)	102 (13.90%)	194 (17.59%)
Pain on the injection site	9 (69.2%)	32 (15.7%)	23 (16.2%)	23 (41.1%)	135 (18.39%)	266 (24.12%)
Chills	1 (7.7%)	18 (8.8%)	43 (30.3%)	3 (5.4%)	40 (5.45%)	131 (11.88%)
Shivering	0 (0.0%)	14 (6.9%)	32 (22.5%)	1 (1.8%)	110 (14.99%)	256 (23.21%)
Local infection	2 (15.4%)	19 (9.3%)	22 (15.5%)	10 (17.9%)	25 (3.41%)	44 (3.99%)
Fever	0 (0.0%)	19 (9.3%)	10 (7.0%)	7 (12.5%)	9 (1.23%)	33 (2.99%)
Vomiting	0 (0.0%)	6 (2.9%)	4 (2.8%)	1 (1.8%)	6 (0.82%)	19 (1.72%)

Table 4: More frequent adverse events in DFU patients treated with intralesional rhEGF.

subjects from the clinical studies: 7 (12.5%) of those that received placebo, 1 (7.7%) with <25 µg, 24 (11.8%) with 25 µg, and 26 (18.3%) with 75 µg. The pharmacovigilance reported SAE in 8 treatment cycles with the 25 µg dose (1.1%) and 23 (2.1%) with 75 µg. The difference is probably given by the less rigorous evaluation in routine clinical practice as compared to the clinical trials setting.

Another important group (apart from local infection, discussed above) of SAE is related to the cardiovascular system. Acute pulmonary edema (7), acute myocardial infarct (6), chest pain (1), sudden cardiac death (2), stroke (1), ventricular fibrillation (1), and uncompensated heart insufficiency (1) account for 19/89 of the SAE reported, but 10/16 of the lethal ones. It is unlikely to associate these events with rhEGF treatment since diabetes is a known risk factor for cardiovascular diseases and mortality rates due to cardiovascular disease is increased among diabetic patients [46,47], moreover if they bear a DFU [48,49]. In data taken from the Cuban death certificate database and the Cuba Annual Health Statistics Report [50], for the period 2007 – 2010, association between diabetes and cardiovascular diseases, particularly acute myocardial infarct was very high. The rates of deaths due to these causes in people with diabetes among the three main causes of death in the certificate almost double that of the general population.

Immunogenicity

The presence of anti-EGF autoantibodies has not been considered detrimental for adult animals physiology [51,52], particularly for the healing process [53]. Nevertheless, immunogenicity of rhEGF was evaluated in five studies (No. 0202, 0504, 0503, 0707, and 0809) by a “sandwich”-type ELISA system since, as a recombinant protein, rhEGF could exert antibody production.

Some subjects (15/174; 8.6%) had anti-EGF antibodies before treatment. The presence of natural anti-EGF autoantibodies was already reported [54]. A total of 16 subjects out of 131 previously negative, evaluated after rhEGF treatment (any dose) developed anti-EGF binding antibodies. The amounts of antibodies found were very low since the value of absorbance was close to the positivity cutoff in the confirmatory test, as compared to the negative controls. There was no clear relationship between the presence of these antibodies and any efficacy or safety outcome.

Can parentally administered rhEGF stimulate tumor growth?

Being a growth factor, one of the main safety concerns with the use of parenteral rhEGF is the possibility of development of cancer. Contrary to PDGF, EGF cannot initiate malignant transformation [55]. However, the promotion of a pre-existing tumor, clinically evident or not, is potentially possible. Results with EGF have been contradictory in this sense. Experiments of tumor promotion as well as tumor inhibition have been reported [56]. A study in Min mice suggested that the tumor promoting activity of EGF is minimal, despite a trophic effect on intestinal tissue [57]. EGF inhibited tumor growth in several models in the literature [58,59]. Additionally, EGF overexpression in transgenic animals not always leads to increase in tumor development [60].

At the same time there is no evidence in humans of any tumor promoting action of exogenously administered rhEGF for healing purposes. The negative arguments include: (i) no increase in tumors in subjects treated with topical rhEGF for burns, in a controlled trial done in Cuba in 1993-1994. These subjects were visited in 2008-2009 and their incidence of cancer was not above that expected for the Cuban population in the same age group (non-published result, reported in ref [56]); (ii) little absorption and short residence in blood of rhEGF

administered intralesionally; at 2 hours all rhEGF has been cleared (study 0707); (iii) treatments with rhEGF are short-term: not more than 8-12 weeks, contrary to a tumor promoting action that would require a longer exposure.

Additionally, no evidence was gathered, either in the clinical trials or in the postmarketing study 0704, indicating that intralesional rhEGF administration could stimulate cancer growth. Only one treated patient from the clinical trials, who had received 25 µg rhEGF, was found to develop a malignancy. In the follow-up of the postmarketing series 51 subjects with cancer were identified, nine of them diagnosed before the treatment. The age-adjusted incidence rate per person-year of follow-up (95% CI) for all rhEGF-treated subjects was 0.59% (0.53-0.65%), compared with 0.34% for the whole Cuban population. The larger rates in the DFU patients receiving rhEGF can be explained by the effect of diabetes itself, which is a known cancer-risk factor [61-64]. This has been discussed from a mechanistic point of view [65,66]. Particularly breast cancer is one of the most frequently referred as increased in diabetic populations. Coincidentally, this was the most frequent location in the rhEGF- treated diabetic patients. On the other hand, the fact that cancer was actively screened in the pharmacovigilance database could represent certain higher incidence than in the general population, where it is based on the compulsory, but passive, report to the National Cancer Registry. There are no available Cuban statistics for cancer incidence in diabetic individuals.

Benefits and Risks Conclusions

Benefit

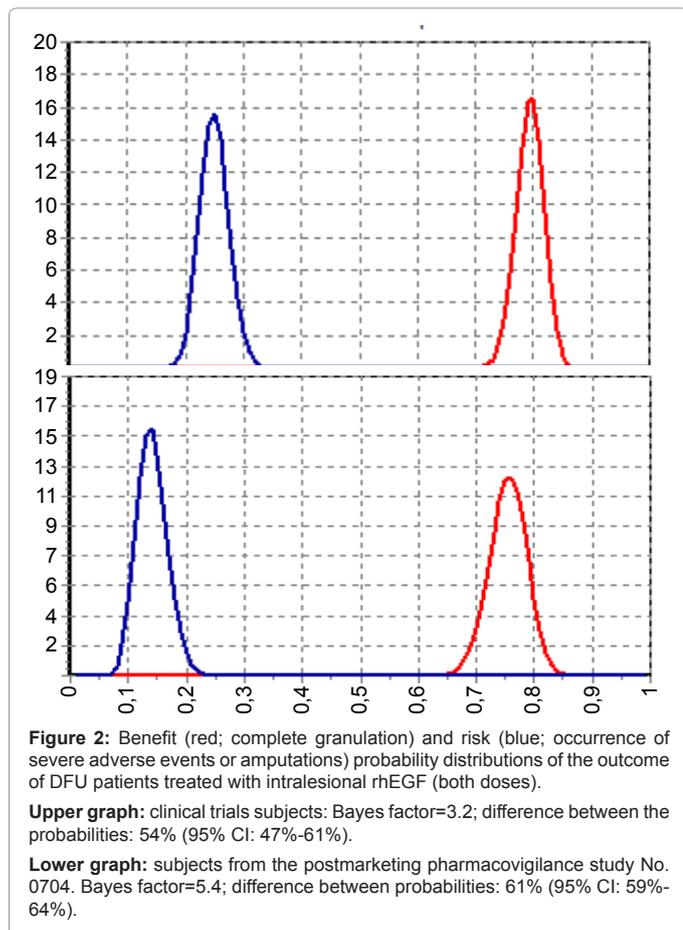
The clinical studies of rhEGF in 344 subjects with advanced diabetic foot ulcers (Wagner's grade 3 or 4, median size >20 cm², ischemic ulcers not excluded) have shown that injected recombinant EGF has the potential to promote complete granulation in more than 80%, with complete wound healing (re-epithelialization) in more than 50% of subjects usually unresponsive to other treatments, one month faster than a group treated only with standard wound care. Injected recombinant EGF has the potential to reduce amputation rates, with a considerable personal and public health improvement, including longer survival. Postmarketing experiences in Cuba in 2702 subjects have confirmed those results and strongly suggest an impact of 16% absolute and 71% reduction of the risk of amputation, both in neuropathic and ischemic subjects. Although the presence of ischemia in the affected limb represents a significant handicap for the healing process and the effect of rhEGF, beneficial impact on this subgroup was obtained as well. All the differences obtained are clinically significant.

Potential risk

Risks arise from the short and long-term adverse event profile. More than 90% of the adverse events were mild or moderate, easily manageable. The occurrence or serious adverse events in 14% of the subjects treated (taking the worst scenario of the clinical trials) constitutes the maximal risk in this sense. The apparent higher cancer incidence rate could also be considered among the risks. However none of them is attributable to rhEGF treatment, but mostly to the underlying conditions. Further investigations should nevertheless continue evaluating these aspects.

Conclusions

The benefit-risk balance seems thus quite favorable. This was also suggested by the analysis done from a Bayesian approach [67] comparing the probabilities of risk (given by the occurrence of serious



adverse events, including amputation) with that of benefit (given by complete granulation or complete re-epithelization) from data from all clinical trials [44]. The comparison of the benefit-risk ratios of the results of the post marketing pharmacovigilance and the clinical trials is illustrated in figure 2. The benefit distributions of both periods, given by the probabilities of complete granulation, overlap. The risk distribution in the post marketing experience was smaller, probably due to more rigorous report of adverse events during the clinical trials. In both cases the differences between benefit and risk probabilities clearly favor the former. The postmarketing information ratifies the results of the clinical trials in terms of efficacy, safety and impact. Information from additional clinical trials and post marketing surveillance in other populations should further validate this conclusion.

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Disclosure of conflict of interests: author JIFM is co-author of the patent covering the product described; the rest of the authors are employees of the Center for Genetic Engineering and Biotechnology, Havana, owner of the product's patent and sanitary registrations, and where product Heberprot P® is produced.

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