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Review article

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Development of a new formulation of interferons (HEBERPAG) for BCC treatment

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Abstract

Purpose: This work is aimed to show briefly, the clinical development of a new pharmaceutical formulation of interferons for the treatment of basal cell carcinoma. *Methods:* A rationale design of the combination of IFN-α2b and -γ based in their anti-proliferative synergism on several tumors cell lines identified adequate proportions to be combined to obtain the best clinical results. The potential mechanism of antitumoral effect was studied by qPCR mRNA quantification. HEBERPAG (anti-proliferative synergistic combination of co-formulated recombinant interferons-α2b and –γ) was used in clinical trials in adult patients with non-melanoma skin cancer. Trials were conducted after approval by the ethics review boards of the institutions participating in trials; and the patients gave their written informed consent to be enrolled in the studies and receive HEBERPAG. *Results:* HEBERPAG inhibits the proliferation of several tumor cell lines *in vitro* and *in vivo*. The combination has improved pharmacodinamic properties. Several clinical trials have demonstrated the efficacy of HEBERPAG in BCC, with excellent cosmetic effect and well tolerable, mild side effects. HEBERPAG was approved by State Control Center for Drug, Medical Equipment and Devises in Cuba, for the treatment of basal cell carcinoma of any subtype, size and localization, and adjuvant to other treatments, surgical or not. After 3-year follow-up, a recurrence rate of 0.03% was detected in treated patients. *Conclusions:* HEBERPAG is a novel formulation of IFNs, more potent than separated IFNs for the treatment of basal cell carcinoma, with more rapid and prolonged clinical effect and excellent cosmetic effect and safety profile.

Keywords: BCC; clinical trial; HEBERPAG; interferon; non-melanona skin cancer

Introduction

Basal cell carcinoma (BCC) is the most common form of skin cancer, largely caused by exposure to ultraviolet radiations [1], presumably partly because of resulting immune suppression [2]. P53 mutations have been shown in 30% to 50% of BCCs studied, and more than half of these mutations were UV-specific. BCC has been associated with increase vascularity likely because of increased expression of CXCR-4, a receptor for stromal cell-derived factor 1 alpha (SDF-1) [3] and with inappropriate activation of the Hedgehog (Hh) signaling pathway where Hh stimulus is required for growth of established BCCs [4]. Additionally, BCC has been shown to evade T cell response by secreting IL-10, by shedding ICAM-1 or by down-regulation of IFN-γ receptors and some of its mediated activities and by killing infiltrating cytotoxic T cells [5, 6].

Recurrence of BCC is not uncommon, approximately 12% with most treatment modalities. An estimated 40%–50% of patients with a primary carcinoma will develop at least one or more further BCC within 5 years [7]. The rate of recurrence is positively correlated with tumor size and facial location. Up to 90% of recurrent cases occur on the head and neck. Aggressive histological BCC types are more prone to incomplete excision, recurrence, and metastasis. Some techniques, as cryosurgery, curettage, radiotherapy (RT), photodynamic therapy (PDT), do not allow histological confirmation of tumor clearance [8].

The efficacy of interferons (IFNs) has been demonstrated in the treatment of BCC and squamous cell carcinoma of the

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skin (SCCS) [9-10] with a broad range of response (60%-100%) and lower rate of recurrence (4%) [12]. IFN-α has been employed in the treatment of aggressive epithelioma with objective response (OR) approximately of 60% [27% complete response (CR) + 33% partial response (PR)] [13] and 88.2% of CR in invasive spinocellular carcinoma with tumor diameter lower than 2 cm [10].

However, previous report showed that IFN was inefficacious in the treatment of aggressive BCCs. IFN alone or in combination with retinoid has failed to cure aggressive non-melanoma skin tumors (NMSC) [13]. With high rates of tumor recurrence and with second primary tumors, patients with aggressive BCC and SCCS continue to have an unmet medical need, with devastating mortality, morbidity, and financial consequences [14]. The rising incidence, morbidity, and mortality of advanced BCC and SCCS are a major challenge for clinical oncologists. Promising agents with preclinical and early clinical results relevant to aggressive NMSC deserve a high priority.

The combination of IFNs for the treatment of cancer has been experimented since the firth years of their discovery. However, nor rationally design of combinations aborted many efforts to exploit the truly biological potency of these cytokines.

Like many low molecular weight protein drugs, IFNs therapies suffer from a relatively short serum half-life of the products. Consequently, if vascular retention is considered to be desired for enhanced efficacy, strategies that can improve a drug's pharmacokinetic and pharmacodynamic properties might improve its therapeutic benefits.

This can be achieved either by modification of the drug molecule itself (e.g. pegylation) or through a change in formulation (e.g. controlled-release formulations, liposomal preparations). In either case, the primary objective is to improve the therapeutic activity of the drug material [15]. Another approach is to potentiate the pharmacodinamic of the therapeutic drug by combining two active principles that can act synergistically. A promising approach is the use in combination of two biological as IFN-α2b and -γ, with recognized synergistic anti-proliferative effect on several tumor cells [16].

Clinical trials in patients with NMSC with the use of HEBERPAG has demonstrated the right design of this new, first in class, pharmaceutical combinations of recombinant IFN-α2b and -γ with synergistic anti-proliferative effect.

Material and methods

Design of the HEBERPAG formulation

Recombinant IFN-α2b and -γ produced in *E. coli* at the Center for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba, were employed. In vitro anti-proliferative assays were used with several tumor cells lines and xenograph mouse models to evaluate tumor growth inhibitions by the combination of IFNs-α2b and -γ. Isobologram analyses of inhibitory combinations of both IFNs established the optimal synergistic combination.

Molecular characterization mRNA expression of genes potentially involved in the antitumoral effect of HEBERPAG was done using qPCR after incubation of cell lines with HEBERPAG during 72 h, in tissue culture conditions [19].

Clinical investigations

HEBERPAG (anti-proliferative synergistic combination of co-formulated recombinant interferon-α2b and -γ) was produced at CIGB. Vials containing 3.5 MIU of IFNs and sodium hydrogen-phosphates, dextran 40, sodium chloride, and human albumin were employed for the treatment of patients.

Each vial was reconstituted with 1 mL bacteriostatic water for injection and applied perilesional and/or intralesional three times per week for 3 weeks. The applications of IFN combination were practiced by medical doctor specialized in oncology with several years practical experience. For lesions > 4 cm, the tumor area was measure, distributing it as surface of 1.5 cm^2 each. In areas, < 4 cm the product was inject the in equidistant areas. The amount of product to be injected in 1 mL of total doses (10.5 MIU) per each 1.5 cm^2 of lesion surface was calculated. If the total amount to be injected is higher than 2 mL, the product was dissolved in appropriated volume in a sterile flask of approximately 10 mL. Overall, the procedures consumed approximately no more than 5 min. This formulation was selected on the basis of *in vitro* antiproliferative studies using isobologram analysis.

HEBERPAG was evaluated in clinical trials. The trials were designed jointly by the principal investigator and the sponsor (CIGB). Data were collected by the site investigators under a confidentiality agreement and were retained and analyzed by the sponsor.

Patients, both genders, older than 18 years, who gave their written informed consent to participate, were enrolled in the trials. Basal cell carcinoma (BCC) was every subtype, localization and size. The studies excluded pregnant or nursing women, patients with known hypersensitivity to IFN or with history of autoimmune diseases.

The trial protocols were approved by the Ethics Committee and the Scientific Review Board (SRB) of participating health institutions in accordance with the ethical principles stated in the Declaration of Helsinki. All the recluted patients were treated in the medical oncological cabinets suited in the participating health centers.

Before treatment, each patient had a medical history, a physical and skin examination for disease assessment, documentation of concurrent medications, a punch biopsy of not more than 25% of the total lesion size

confirmed the diagnosis before enrolment and laboratory tests (hemoglobin determination and platelets and white blood cell total and differential counts). Patients were asked to avoid any other medication that could interfere with IFN action.

Clinical Evaluation

Clinical response was categorized as complete response (CR): The CR of evaluated cohorts was defined as no residual BCC on sampling tumors biopsy; partial response (PR) as ≥30% reduction in lesion size (sum of the longest diameter from the baseline in target lesions) and stabile diseases (SD): < 30% reduction in the tumor size; and progression (P) defined as any increase in the lesion size. The CR responses were confirmed by histological examinations or computerized axial tomography.

Subjects were examined as outpatients. At each visit during and after treatment, the investigators assessed the lesions and evaluated tissue conditions. Patients' follow-up examinations were done at weeks 1, 2, 3 and 12 after treatment onset. At week 12, laboratory tests were repeated. Lesion diameter (d) measurements were done using a Folding Magnifier, rulers or calipers, systematically during the treatment time and until week 12 and documented with photographs. Twelve weeks following therapy, the treatment sites were examined for clinical and/or histological evidence of remaining tumor. Patients were fallowed during five years.

Data were collected at the institutions were the patients were treaded. The previously designed and approved by CIGB and Ethics Committee and the SRB of health institutions CRD were filled by principal or responsible investigators or designed specialists. The data were monitoring and then imported by double entry in electronic base data and processed by the statistic group at CIGB.

All investigators had full access to the data and vouch for the accuracy and completeness of the data and analysis and the fidelity of the study to the protocol. The first draft of the manuscript was written by the first author who is an employee of the sponsor. All authors contributed to subsequent drafts and decided to submit the manuscript for publication. Writing assistance was provided by the sponsor.

Safety evaluation

Safety and tolerability were monitored by means of a rigorous adverse events control and their frequency calculated. During the treatment, patients were carefully monitored for side effects. Additionally, blood samples were taken for routine hematological and biochemical determinations.

Statistic

Data were double entered and validated on Microsoft Access and then imported to SPSS version 13.0 for analysis. The frequency distributions for qualitative variables and central tendency and dispersion were estimated: mean, standard deviation, median, interquartile range (OR), maximum and minimum values (range) for quantitative variables.

For each type of adverse event, were estimated frequency distribution (IC95%) with classical and Bayesian statistic. Overall survival and duration of the response were based on te Kaplan-Meier method. The influence of demographic characteristics and appearance of adverse events were evaluated. The laboratory data were analyzed as a paired (initial-final result) result using paired T student and Wilkilson tests, depending from Shapiro-Wilk test results.

Study InCarbacel-II, code: IG/IAI-IGI/NB/9901: The efficacy and safety of HEBERPAG (1.75 MIU, intratumoral, 3 time a week/ 3 weeks) with respect to separated IFNs was evaluated in a three-arm, randomized, double blind, and one-cohort trial, involving patients with BCC at diagnostic, size < 4 cm. Study InCarbacel-III, code: IG/ IAI-IGI/ NB/ 0601: The trial was conducted to establish the most efficacious doses of HEBERPAG. The phase II clinical trial, randomized, controlled, double blind in one center was conducted in 70 patients with BCC at diagnostic, size < 4 cm.

Study InCCNM-I, code: IG/ IAI-IGI/ NNM/0101: An open, exploratory, prospective, non-controlled two-cohort trial in patients with advanced BCC or SCCS was done. Patients with locally advanced NMSC were required to have non-operable lesions, resistant to several previous therapies (surgery, radiotherapy, chemotherapy or combination of two or three of them). The lesions resistant to multiple previous treatments were treated with HEBERPAG (doses up to 21 MIU, peri/intralesional, 3 time a week/4-8 weeks) combined or not with systemic chemotherapy.

Study MYFIC (reference number: 999/16.016.09.B): A pharmacokinetic and pharmacodynamic (PK/PD) study was done as part of an exploratory, prospective, open-label clinical trial with mycosis fungoides.

Twelve patients (females and males) with mycosis fungoides, refractory to previous treatments, were eligible for the study. Other eligibility criteria included measurable disease, a life expectancy of at least 24 weeks, Karnofsky ≥60%, with more than 1 month of previous disease specific treatments or more than 3 months in the cases of the steroids use. Patients also had adequate hematological, hepatic, and renal function. Patients with before treatments of IFNs were not excluded from the trial. Patients were excluded if they had an absolute neutrophil count of <1500 cells /mm^3, a platelet count of <90 000 /mm^3, a haemoglobin concentration of <12 g/ dL (women) or <13 g/ dL (men), or a serum creatinine level of >1.5 times the upper limit of normal.

The study was designed to evaluate the pharmacokinetic and pharmacodinamyc parameters after first single high dose administration of co-administration of IFNs alpha-2b and gamma (HeberPAG). After one week the study continue as a clinical trial to evaluate the efficacy and adverse effects of the product in patients.

All of them received intramuscularly a single high dose (23×10^6 IU) of HEBERPAG for PK/PD studies. Serum IFN-α2b and IFN-γ concentrations were measured during 96 h by commercial enzyme immunoassays (EIA) specific for each IFN. Other blood IFN-inducible markers and laboratory variables were used as pharmacodynamics and safety criteria.

A retrospective study was conducted in patients with periocular NMSC treated with HEBERPAG. The patients were identified from the data base from department of dermatology at Department of Peripheral Tumors at "National Institute of Oncology and Radiobiology" in Havana (5-year follow-up period, January 2004 to January 2009), Dermatological Department at "Hermanos Ameijeiras" Hospital (2-year follow-up period, July 2010-July 2012), and polyclinics "Noelio Capote", community of Jaruco and "Luis Li Trejent", community of Guines (rural zones), in Mayabeque (1-year follow-up period -July 2011-July 2012).

Patients with periocular (near to the eye), histologically and clinically proven NMSC (BCC or SCCS), treated with HEBERPAG were included. Patients were recorded for age at the time of treatment, periocular location of the skin lesion, complications of the treatment, the final ophthalmic side-effects and the clinical resolution of the lesion following completion of HEBERPAG therapy. The time from the completion of treatment to the last follow-up was recorded. The institutional ethics review board approval was granted for this retrospective study. The employed doses for IFN combination were from 0,875 x 10^6 IU to 27 x 10^6 IU.

Results

Design of the HEBERPAG formulation

During the *in vitro* and *in vivo* preclinical studies, were identified the best combinations that lead to growth inhibition of several tumor cells lines, including basal cell carcinoma primary cultures from biopsies of patients with BCC as well as established cells lines (Hep-2, Cervical carcinoma, ATCC:CCL23), HepG2, Hepatoma, ATCC: HB-8065, and NCI-H125, Non-small cell lung cancer). The rationale design of the combination of both IFNs based in their anti-proliferative synergism on several tumor cell lines, identified which proportions of both IFNs are more adequate to be combined to obtain the best clinical results [17]. With the defined proportions, first in class, pharmaceutical formulation of co-formulated in the same vial IFNs-α2b and -γ in antiproliferative synergistic proportion was developed.

Molecular drug properties

HEBERPAG inhibits the proliferation of several tumor cell lines *in vitro*, representative of BCC, cervical carcinoma (Hep-2), non-small cell lung carcinoma (H-125), and malignant glioma (U87MG). In xenograph mouse model it inhibits the tumor growth of Hep-2 cell line. Additionally, HEBERPAG down regulates expression of anti-apoptotic Bcl-2 and up-regulates apoptotic Bax, p53, and caspases mRNAs; and establishes a favorable antitumoral relationship between gene suppressor activity (STAT-1) and pro-oncogenic activity (STAT-3) [18-20].

Clinical investigations

During the process of clinical development of the HEBERPAG formulation, several clinical trials were conducted.

In the PK/PD MYFIC study, twelve patients with mycosis fungoides were enrolled. Patients, 7 females and 5 males with mycosis fungoides (stage IIa and III), mean age of 53.3 years (33 to 74 years), a body weight mean of 72.7 kg (55 to 97.5 kg), a body mass index between 1,79 m^2 and body surface (1,46 to 2,28 kg/m^2) that represented 3 clinical variants of mycosis fungoides were treated.

The PK evaluation by EIA yielded a similar pattern for IFNs alpha-2b and gamma components of HEBERPAG formulation and the pharmacokinetic described for both IFNs when administered separated. For IFN alpha-2b the parameters' mean were Cmax: 270 ± 147 pg/mL; Tmax: 12.0 ± 6.0 h; half-life (t½): 5.2 ± 0.7 h; and for IFN gamma Cmax: 6.1 ± 11.8 pg/mL; Tmax: 6.0 ± 0.8 h; half-life (t½): 13.4 ± 27.1 h. The pharmacodynamic variables either: serum neopterin and, beta 2-microglobulin (beta-2M) levels, or 2'-5' oligoadenylate synthetase (2'-5' OAS) mRNA expression levels were strongly stimulated by simultaneous combination of both IFNs. The most frequent adverse reactions were fever, flulike symptoms.

The MYFIC study demonstrated that HEBERPAG has improved pharmacodinamic properties [21] which can sustain more potent biological activities and more rapid and prolonged anti-tumoral effects, with reduced intensity of characteristic for IFNs side effects.

During the InCarbacel-II study were enrolled 19 patients with BCC, average 67.0 years–old with predominance of females (57.9%) in the group of HEBERPAG, where 89.5% of patients were white (Table 1). The HEBERPAG OR of 95% vs IFNα-2b with 90% (n = 21) was detected. CR of 42.1% and 33.3% were observed in the HEBERPAG and IFNα-2b groups, respectively. The CRs in the group of HEBERPAG were obtained one month before than those in IFN α2b group (Figure 1). No tumor progressions were observed during the study. Duration of CR was at least 1 year in the 100% of patients treated with HEBERPAG (Table 2).

In the InCCNM-I study (Tables 1 and 2), 16 elders (average 64 years–old) patients were included. They beard 12

Table 1 Demographic and clinical characteristic of patients at baseline.

Trial	*Characteristic (%)*		*BCC at diagnostic*	*Advanced*	
				BCC	*SCCS*
InCarbacel-II Collazo-Caballero et al. (2005)	Sample size–no.		19		
	Age–yr. (range)		67.0		
	White–no. (%)		17 (89.5%)		
	Sex–no. (%)	Male	8 (42.1%)		
		Female	11 (57.9%)		
InCarbacel-III Rodríguez-García MA et al. (2012)	Sample size–no.		75		
	Age–yr.		61.5 (29-82)		
	Sex–no. (%)	Male	40 (53.3)		
		Female	35 (46.6)		
	White		66 (89.2)		
InCCNM-I [21] Anasagasti-Angulo L et al. (2008)	Sample size–no. (%)			12 (75)	4 (15)
	Age–yr. (range)			66.4 (31-81)	72 (54-89)
	Sex–no. (%)	Male		7 (70)	3 (30)
		Female		5 (83.3)	1 (16.6)
	White–no. (%)			16 (100)	
Retrospective [22] Garcia-Vega Y et al. (2013)	Sample size–no. (%)			18 (85.7)	3 (14.3)
	Age–yr. (range)			66 (58-60)	
	Sex–no. (%)	Male		9 (42.9)	
		Female		12 (57.1)	
	White–no. (%)			19 (90.5)	

Table 2 Primary and secondary efficacy end points of patients with BCC at diagnostic.

Trial	*Outcome*	*BCC at diagnostic*
*InCarbacel-II (doses 3.5 MIU) Collazo-Caballero et al. (2005)	OR–no. (%)	18 (95%)
	SD–no. (%)	1 (5%)
	P–no. (%)	0
	Data missing	0
	Median duration of response–yr. (%)	1 (100%)
	Duration of treatment–day	9
**InCarbacel-III Doses: 1.75 –10.5 MIU Rodríguez-García MA et al. (2012)	OR–no.	60 (83.3)
	SD–no.	12 (16.7)
	P–no.	0
	Median duration of response–yr. (%)	†3 (100%)
	Duration of treatment–day	18.4

*Clinical response at week 16, and **week 12.
† doses 10.5 MIU

BCC and 4 SCSC ranging from 2 to 21 cm in the longest dimension. The clinical forms were terebrant (9), nodular (3), mixed (2), and ulcerated (2). Sites of tumors included face, neck and trunk. The median of initial tumor size was 9.0 cm. The main localization of tumor was in patient face (68.8%) and the terebrant clinical form was the most frequent (56.3%). The 81.2% of patients completed the treatment schedule.

At the end of treatment 47% CR, 40% PR and 13% stable disease were obtained. None of the patients relapsed during the treatment period. The median duration of the response was 38 months. Only one patient with CR had relapsed after 5 years follow-up. Five patients (3 SCSC and 2 BCC) dyed in the study (31.3%). The causes of the death were hepatic metastasis, pneumonia, and tumor progression. The mean survival was 42.3 months (95%, 29.4 – 55.2). Most of the deaths were before 2 year [22].

A thirst trial, InCarbacel-III study, was conducted to establish the most efficacious doses of HEBERPAG. Table 1 shows the demographic characteristics of 75 included patients, distributed in 5 groups (0.875, 1.75, 3.5, 7.0, 10.5 MIU). Patients were predominantly men (53.3%), between 29 and 82 years-old, of them, 89.2% were white.

Sixth–eight percent of patients referred concurrent disease, predominantly arterial hypertension and cardiac insufficiency. By confirmatory histology, the OR was 93% (60% CR) and 85% (64% CR) in the groups of higher doses (7.0 MIU and 10.5 MIU, 3 time a week/3 weeks), respectively (manuscript in preparation). See table 2 for efficacy data of the group of higher dose.

The use of HEBERPAG by medical indication for NMSC in the periocular regions was retrospectively recorded (Tables 1 and 2). The series included predominantly females (57.1%); and 90.5% of patients were white. Histological subtypes included 18 BCC and 3 SCCS with predominant clinical forms, nodular (38.1%) and mixed (33.3%) and 3 cases were terebrant, 2 ulcerated and 1 pigmented. The median time of tumor evolution was 16.5 months with an initial diameter of 8.25 cm. At week 12 after the end of treatment, a 47.6% CR rate was obtained. A PR was achieved in 5 patients (23.8%). A high response rate was obtained with OR in 71.4% [23].

Safety

Safety global analysis of HEBERPAG was done. The analysis include patients from InCarbacel-II, InCarbacel-III, and InCCNM-I. Analyzed data from 155 treated patients with BCC or SCCS (52.0% females), median age of 65.04 ± 17.0 (29 to 89 years old), with predominant skin phenotype II (40.7%) and III (40.7%) and lesions localized mainly in the face (68.0%) and trunk (16.7%), indicated that side effects associated with the use of HEBERPAG are considered to be minor (> 95% of patients).

At least one adverse event was observed in the 86% of patients that included 58 different events. The most frequent events (>10.0%) were fever (64.4%), chills (49.7%), arthralgia (35.6%), headache (32.2%), asthenia (29.5%), general malaises (24.8%), anorexia (21.5%), perilesional ertithema and edema (17.4%), myalgias (14.1%), and diarrhea (10.7%). All were well tolerated and preventable with the use of antipyretics. 90% of events were transient and 98.9% of patients with side effects continued without doses adjustments. The 97.0% were events categorized as very probably related to the HEBERPAG.

Discussion

Potential mechanisms of action of HEBERPAG

The clinical trial InCarbacel-II evidenced more rapid CR responses of HEBERPAG when comparing to IFN-α-2b (Figure 1). This effect could be due to the occurrence of several IFN mediated actions that include: apoptosis of BCC cells via the Fas-Ligand-receptor interaction, a mechanism that could be reinforced by IFN-γ through the augmentation of Fas-R; stimulation of IFN-γ receptor expression (IFNGR1, and IFNGR2) by IFN-α that may reverse the observed low levels of this membrane receptor in BCC cells; and stronger signaling of IFN-γ in the presence of IFN-α [21]. The precisely regulation of early mediators of IFN-α and -γ signaling (STAT-1/STAT-3) and of apoptosis as p53, Bax and caspases could also collaborate, in the more potent antitumoral of the HEBERPAG mediating an inhibition of anti-apoptotic and stimulating the pro-apoptotic pathways [18].

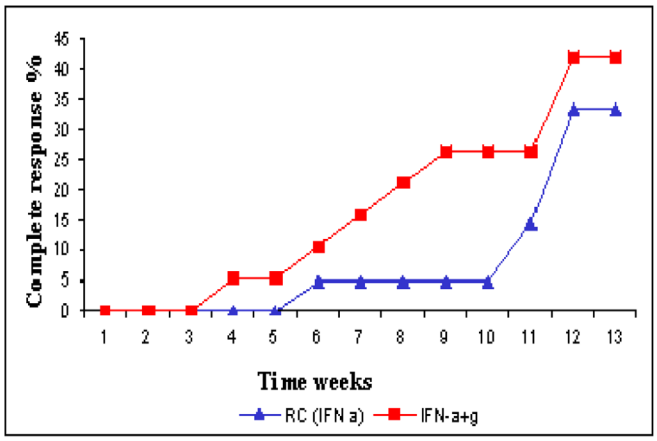

Figure 1 Percentage of patient with complete response (CR) during the clinical trial InCarbacel-II, comparing HEBERPAG (IFN-a+g) vs separated IFN-α-2b or IFN-γ. In the figure, the comparisons between HEBERPAG and IFN-α-2b groups. The first CR with HEBERPAG was observed one month before with respect to IFN-α-2b. HEBERPAG group obtained more CR than IFN-α-2b group.

Duration of clinical response

Following treatment of a BCC, all patients are at some degree of risk of both local recurrence (treatment failure) and the development of further primary BCC at other sites (new lesions). The risk of local recurrence is an individual risk, based upon the tumor characteristics and the treatment used. However, for primary BCC treated appropriately by experienced practitioners, the recurrence

rate should be low. This is not true for recurrent BCC, where recurrence rates are universally higher than for primary BCC. Patients who have had recurrent (especially multiply recurrent) lesions treated are particularly worthy of follow up in view of their relatively high risk of further recurrence. The timing of follow-up visits should take into account the generally slow growth rate of BCC. Evidence suggests that recurrent disease may take up to 5 years to present clinically, and that up to 18% of recurrent BCC may present even later (Table 3) [24]. In a review of all studies published since 1947 looking at the treatment of primary BCC by various modalities, less than one third of all recurrences presented in the first year of follow up, 50% presented within 2 years, and 66% within 3 years [25].

Table 3 Primary and secondary efficacy end points of patients with BBC or SCCS.

Trail	*Outcome*	*BCC/SCCS*
InCCNM-I [21] 56% received doses between 3.5 – 10.5 MIU. Anasagasti-Angulo L et al. (2008)	OR–no. (%)	13 (83.3)
	SD–no. (%)	1 (13.0)
	P–no. (%)	2 (12.5)
	Duration of response–mo.	38
	95% CI	22.6 – 53.4
Retrospective study [22] 66.7% received doses ≤ 3.5 MIU. Garcia-Vega Y et al. (2013).	OR–no. (%)	15 (71.4)
	95% CI	52.3 – 89.0
	SD–no. (%)	5 (23.8)
	P–no. (%)	1 (4.8)
	Duration of response–mo.	22.6

The low recurrence rate in patients treated with HEBERPAG could be an expression of the more potent antitumoral effect of this new formulation of IFNs with synergistic antiproliferative properties.

The prolongation of antitumoral clinical response observed for patients treated with HEBERPAG could involve the immune activation starting early after first administration; the antitumor effects mediated by activated immune cells over weeks to months; and activation of innate and adoptive immune responses coordinated and stimulated by both IFNs [21].

Mohs surgery provides the best chance of cure for all BCCs arising on the face with 5-year recurrence rates of anything up to 6.5% [26]. However, due to time and cost limitations, it should be reserved for the treatment of high-risk primary or recurrent BCCs on the face.

Vismodegib, a small-molecule inhibitor of the Hedgehog signaling pathway, was approved by the US Food and Drug Administration (FDA) for the treatment of advanced BCC [27, 28]. The efficacy and safety of vismodegib (150 mg per day orally) was investigated in a single-arm, open-label, two-cohort trial involving 104 patients with either metastatic BCC (n = 33) or locally advanced BCC (n = 71). Of the 104 patients enrolled, 96 patients were evaluable for objective response rate (ORR). In the efficacy-evaluable metastatic BCC cohort (33 patients) the ORR was 30.3% with no CR, and in the efficacy-evaluable locally advanced BCC cohort (63 patients) the ORR was 42.9%, which included 13 patients (20.6%) with a CR response [29]. The median response duration was 7.6 months in both the metastatic BCC cohort and the locally advanced BCC cohort.

Patients with locally advanced NMSC treated with HEBERPAG were more beneficiated [21] as did vismodegib for advanced BCC. The OR of HEBERPAG was 87% vs 43.0% vismodegib [25], and induced a double of CR than vismodegib. The duration of response was also superior for patients treated with HEBERPAG (38 months) compared with vismodegib (7.6 months). Side effects associated with the use of vismodegib are generally considered to be minor to moderate, and include muscle spasms, altered taste perception, weight loss, fatigue, nauseas and hair loss. Although categorized as mild to moderate, cumulative toxicity of this agent over time has led to discontinuation of therapy in a substantial fraction of patients. Chronic adverse effects will limit the potential utility of vismodegib [30]. However, the data from HEBERPAG patients with advanced NMSC come from low number of patients; they are very encouraging with respect to vismogenib clinical outcome (Table 4).

Table 4 Comparative data between clinical responses of Vismodegib and HEBERPAG during the treatment of advanced NMSC.

Drug	*OR*	*Duration of responses*	*Duration of treatment*	*References*
Vismodegib	43%	7.6 months	3 months	Sekulic A et al. [25]
HEBERPAG	87%	38 months	8 weeks (maximum)	Anasagasti L et al. [21]

HEBERPAG was registered in 2008 by State Control Center for Drug, Medical Equipment and Devises in Cuba (CECMED), and is indicated for the treatment of BCC of any subtype (all BCCs), size and localization, and adjuvant to other treatments, surgical or not. However, perilesional injections of HEBERPAG may offer an alternative in cases where patients:

- are unwilling or too sick to undergo surgery;
- are immunosuppressed;
- have superficial but diffuse involvement of large areas of conjunctiva or eyelid skin;
- have to remove large lesions with adequate excision margins that can be disfiguring as a result of loss of tissue, grafting, and subsequent scarring;

- the location of the BCC on cosmetic important areas (periocular, perioral, and perinasal areas) or;
- need adjuvant therapy to help prevent recurrence;
- need neo-adjuvant therapy to reduce tumor size for more efficient surgery.

HEBERPAG may also be used for other tumor conditions as determined by the doctors with knowledge about the anti-tumoral effects of this drug. The formulation provided the medical community with a new more potent IFN formulation for the treatment of cancer.

Conclusions

Overall, HEBERPAG is a novel formulation of IFNs, more potent than separated IFN with more rapid and prolonged clinical effect, excellent cosmetic effect and safety profile. The relatively benign side-effect profile of HEBERPAG could help in expanding its use as a perilesional and/or intratumoral therapies for patients with advanced BCC or in difficult for surgery and aesthetic outcomes localizations (periocular tumors).

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Conflict of interest

The authors wish to express that they have no conflict of interest.

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