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HeberFERON, a new formulation of IFNs with improved pharmacodynamics. Perspective for cancer treatment

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ABSTRACT

The rational combination of recombinant IFN-α2b and IFN-γ resulted in a new formulation of interferons (HeberFERON) with improved pharmacodynamics. In basal cell carcinomas HeberFERON produces a more rapid antitumor effect and results in a larger number of complete responses. In patients with glioblastoma multiforme, the administration of HeberFERON after surgery and radiotherapy results in an estimated overall survival of 19 months. Patients with stage III or IV renal cell carcinoma also appear to benefit from the intravenous administration of HeberFERON, with prolongation of survival and good quality of live. HeberFERON offers a promising alternative formulation of interferons for the treatment of cancer with a very favorable safety profile.

1. Interferons: discovery and clinical uses

1.1. Discovery of interferons

As early as the 1930s, the process of viral interference where primary infection with an avirulent or inactivated virus renders cells resistant to a second distinct virus infection was a well-known phenomenon in virology. One of the earliest reports of viral interference, the Magrasi phenomenon, involved the inoculation of a non-encephalitogenic strain of herpes simplex virus into the cornea of rabbits to prevent encephalitis by an encephalitogenic strain inoculated intracerebrally [1].

In 1954, Japanese virologists Yasu-ichi Nagano and Yasuhiko Kojima of the Institute for Infectious Disease at the University of Tokyo reported an inhibitory factor that was derived from tissue suspensions of rabbits infected with an inactivated vaccinia virus [2,3]. These authors inferred that the interfering virus had induced a soluble substance that made other cells resistant to subsequent viral infection and defined the existence of a factor that conferred the property of viral interference.

Then in 1957 the British virologist, Alick Isaacs, and Jean Lindenmann, a Swiss research fellow working at the National Institute for Medical Research in London, reported their results obtained by incubating chick chorioallantoic membranes with inactivated influenza virus A of Melbourne for varying times at 4^0C and 37^0C [4]. By pre-exposing cells to inactivated virus at 4^0C or 37^0C they hoped to define the nature of the agent that protected cells from subsequent infection with live or virulent virus at 37^0C. Their results confirmed the phenomenon of viral interference. Furthermore, inhibition of influenza infection was not achieved by pre-exposure at 4^0C, indicating that tissue metabolic events were necessary for inhibition of viral replication [5].

The inhibitor substance responsible for the phenomenon of viral interference was named interferon (IFN) by Nagano and Kojima [2,3] and by Isaacs and Lindenmann [4].

Then in 1965 Frederick Wheelock [6] reported an IFN-like antiviral activity in the supernatant fluid of cultures of human leukocytes after incubation with the mitogen phytohemagglutinin (PHA). Like virus-induced IFN, the PHA-induced inhibitor was macromolecular and soluble and acted on human cells only [7]. However,

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the activity of the mitogen-induced leukocyte-derived factor, designated IFN-γ, was less resistant to heat and to acid, two criteria that distinguished it from other IFNs.

The IFNs are actually a family of polypeptides with pleiotropic functions that are produced by diverse cells under different stimuli. The IFNs are classified in three major categories, Type I (α, β, ω, ε, τ, κ, ν); Type II (γ); and Type III (λ) IFNs [8–11]. All type I IFN subtypes bind to and activate the Type I IFN receptor (IFNR), while Type II and III IFNs bind to and activate the Type II and III IFNRs, respectively [12].

In addition to powerful antiviral property, it was quickly realized that IFNs had anti-proliferative and anti-cancer activities. IFN directly inhibits the proliferation of tumor cells, and generally has a stronger growth inhibitory effect on tumor cells than on normal cells. IFN is also known to induce apoptosis in some cells. Thus, IFN not only directly inhibits the proliferation of tumor cells and destroys them, but also indirectly inhibits tumor cells by stimulating the immune system. IFN is known to enhance the activity of killer T cells, NK cells, and ADCC, and to stimulate macrophages and neutrophils to destroy tumor cells [13,14]. The clinical utility of IFN-α2b for the treatment of patients with melanoma, renal cell carcinoma (RCC), and chronic myelogenous leukemia (CML), has been demonstrated.

IFNs are not effector molecules in their own right. After binding to specific receptors on the surface of target cells that are coupled to intracellular signal transduction and second messenger pathways, they confer pleiotropic activities.

1.2. Pharmacokinetic and pharmacodynamics of IFNs

The pharmacokinetics (PK) of intramuscular IFN is characterized by absorption of IFN-α and IFN-γ >80%, and 30% to 70%, respectively [15], with maximal serum or plasma concentrations occurring after 1 to 8 hours, followed by measurable concentrations for 4 to 24 hours after injection for both IFN-α and IFN-γ [16]. Commonly, the pharmacodynamic (PD) consequence of IFN administration is characterized by changes in known IFN targets including (a) neopterin, a marker of response to type I and II IFNs [17,18]; (b) β -2 microglobulin, a protein induced by both IFNs [19,20]; and (c) 2′-5′ oligoadenylate synthetase (2′–5′OAS), an enzyme induced by both IFN-α and IFN-γ, and involved in IFN-mediated viral RNA degradation [21,22]. The PDs of IFN-β at doses of 18×10^6 IU in healthy volunteers includes a five-fold increase relative to baseline of neopterin levels [23]. Subcutaneous administration of $27–36 \times 10^6$ IU of IFN-α2a to healthy volunteers resulted in a four-fold increase in plasma neopterin concentrations [24]. In a separate study, administration of PEG-IFN-α to patients or healthy volunteers resulted in approximately a three-fold induction of neopterin 48 hours after injection [25–27]. In the case of IFN-γ, no PD evaluation for neopterin, beta 2 microgloabulin, or 2′–5′OAS could be found in a search of the literature.

IFN-α and IFN-γ have relatively short serum half-lives. Consequently, one potential approach to enhance efficacy is to improve their PK and/or PD properties. Approaches to improve the PK properties of therapeutic proteins include modifications by various approaches including pegylation. Alternatively, attempts are often made to improve the PDs by combining drugs that may be synergistic. These approaches offer the possibility of enhancing activity without additional toxicity, but possibly with less frequent injections, which can lead to better compliance and quality of life.

1.3. Clinical uses of IFNs in oncology

Clinical investigations with IFN began in the late 1970s. Once IFN-α2 was identified, it was expressed in E. coli, rapidly purified, and used for research and clinical trials. The first clinical trial was initiated on January 15, 1981 [16,28]. Clinical studies demonstrated that stimulating the antitumor immune response with IFN could mediate the tumor regression [29]. The first indication approved for IFN-α2 was for hairy cell leukemia (HCL) in 1986 [30].

For many years, IFNs alone or combined with chemotherapy were used as first-line treatments for various malignancies, including HCL, some non-Hodgkin lymphomas, RCC, and melanoma. IFN-α2a and IFN-α2b as their pegylated IFN variants have been approved by various regulatory agencies for the treatment of multiple cancers (Table 1) [31,32]. Treatment with IFNs also leads to predictable toxicity [16].

Beginning in 1986 in Cuba, the Center for Genetic Engineering and Biotechnology (CIGB) in Havana has produced recombinant human IFN-α2b, marketed as Heberon Alfa R [33]. The therapeutic use of this product in the Cuban public health network has led to the accumulation of a large amount of data. In Cuba, Heberon Alfa R is indicated for the treatment of neoplasms of the lymphatic and hematopoietic systems and for solid tumors. Neoplasms of the lymphatic and hematopoietic systems include: (a) HCL; (b) multiple myeloma as maintenance therapy for patients in remission after induction treatment; (c) non-Hodgkin's lymphoma specifically follicular lymphoma with a high tumor mass in combination with doxorubicin, cyclophosphamide, teniposide, and prednisolone; (d) mycosis fungoides; and (e) CML. b) Solid malignancies include (a) Kaposi's sarcoma in patients with AIDS without a history of opportunistic infections; (b) RCC; and (c) malignant melanoma [34].

Over time the IFNs have been displaced in many cases by other comparatively more effective therapies [31]. Novel targeted therapies and immune-checkpoint inhibitors have replaced the use of IFNs in the treatment of several malignancies, including HCL [35], CML [36], and melanoma [37]. However, IFNs are still used in the treatment of oncologic diseases in several countries [38,39].

IFN-type II, composed solely of IFN-γ, promotes host responses for antitumor immunity. IFN-γ is known to play a significant role in all three phases of cancer immunoediting, including elimination, equilibrium, and escape [40]. IFN-γ has direct antiproliferative, apoptotic, and antiangiogenic effects on tumor cells, in addition to indirect effects on antitumor immunity [41,42]. However, despite demonstrated antitumor properties of IFN-γ, to date there is no approved indication reported for the treatment of cancer with this cytokine. In contrast to Type I IFNs, IFN-γ has not reached the stage of an antitumor clinically useful drug.

The IFN-γ produced in Cuba at CIGB (Heberon gamma) has been used in the treatment of patients with juvenile rheumatoid arthritis, chemoresistant pulmonary tuberculosis, idiopathic pulmonary fibrosis, and atypical pulmonary mycobacteriosis [43]. Sufficient IFN must be used to result in an effective protective response, but the quantity and duration of the IFN's response must be limited so as to minimize damage and toxicities. Thus, a better understanding of IFNs signaling mechanisms will help to optimize the IFN's treatment of cancer patients [44].

IFNs have been widely used in the treatment of human solid and hematologic malignancies. However, despite the well-known antitumor activity of IFNs, major advances have not been achieved in the last few years. One potentially hopeful option could be the combination of IFN-α and IFN-γ, two molecules with recognized synergistic antiproliferative effects on several cancer.

2. The HeberFERON concept

While interest in the clinical application of IFNs increased in the mid 1970s, this was followed by a decline in the early 1980s because of its apparent limited success in the treatment of cancer. However, again in the 1990s, interest increased because of its value in the management of a range of conditions, including malignant melanoma and Kaposi's sarcoma [45]. Enthusiasm for IFNs,

Table 1
Clinical uses of IFNs type I (2012).

A: On label	
IntronA / I*nterferon alfa-2*	
European Medicines Agency (EMA) Approvals	
Hairy-cell leukaemia (HCL)	Treatment of patients with hairy cell leukaemia.
Chronic myelogenous leukaemia (CML)	*Monotherapy:* Treatment of adult patients with Philadelphia-chromosome- or bcr/abl-translocation-positive chronic myelogenous leukaemia. Clinical experience indicates that a haematological and cytogenetic major / minor response is obtainable in the majority of patients treated. A major cytogenetic response is defined by <34% Ph+ leukaemic cells in the bone marrow, whereas a minor response is ≥34 %, but <90 % Ph+ cells in the marrow *Combination therapy:* The combination of interferon alfa-2b and cytarabine (Ara-C) administered during the first 12 months of treatment has been demonstrated to significantly increase the rate of major cytogenetic responses and to significantly prolong the overall survival at three years when compared to interferon alfa-2b monotherapy
Multiple myeloma	As maintenance therapy in patients who have achieved objective remission (more than 50% reduction in myeloma protein) following initial induction chemotherapy. Clinical experience indicates that maintenance therapy with interferon alfa-2b prolongs the plateau phase; however, effects on overall survival have not been conclusively demonstrated
Follicular lymphoma	Treatment of high-tumour-burden follicular lymphoma as adjunct to appropriate combination induction chemotherapy such as a CHOP-like regimen. High tumour burden is defined as having at least one of the following: bulky tumour mass (> 7 cm), involvement of three or more nodal sites (each > 3 cm), systemic symptoms (weight loss > 10 %, pyrexia > 38°C for more than eight days, or nocturnal sweats), splenomegaly beyond the umbilicus, major organ obstruction or compression syndrome, orbital or epidural involvement, serous effusion, or leukaemia
Carcinoid tumour	Treatment of carcinoid tumours with lymph node or liver metastases and with 'carcinoid syndrome'
Malignant melanoma	As adjuvant therapy in patients who are free of disease after surgery but are at high risk of systemic recurrence, e.g. patients with primary or recurrent (clinical or pathological) lymph-node
United States Food and Drug Administration (USFDA) Approvals	
HIV/AIDS-related Kaposi sarcoma	On November 21, FDA licensed Intron A and Roferon A (human interferon alpha injection) for the treatment of Kaposi's Sarcoma, a cancer resulting from HIV
Hairy-cell leukaemia (HCL)	The use of IFN-α in the treatment of HCL is limited. However, IFN-α may still have a place in the treatment of HCL in pregnancy. It can also be used in patients presenting with very severe neutropaenia (neutrophil count $<0.2 \times 109$/l) to increase the neutrophil count prior to nucleoside analogue therapy
Malignant melanoma	The approval was based on a single, open-label, multicenter trial enrolling 1,256 patients. After surgical resection, patients were randomized (1:1) to either PEG-IFN or observation for 5 years. PEG-IFN, 6 μg/kg per week, was administered s.c. for eight doses, followed by 3 μg/kg per week for up to 252 weeks. The relapse-free survival (RFS) interval, the primary efficacy endpoint, was significantly longer in PEG-IFN–treated patients. The median RFS times were 34.8 months and 25.5 months, respectively. There was no statistically significant difference in the overall survival time.
Non-Hodgkin lymphoma	Intron A (interferon alfa-2b, recombinant) for injection in conjunction with anthracycline-containing combination chemotherapy has been approved for the initial treatment of patients with clinically aggressive non-Hodgkin's lymphoma. The addition of Intron A to chemotherapy increased median progression-free survival from 1.5 years in patients with chemotherapy alone to 2.9 years in patients in the chemotherapy plus Intron A group. Moreover, patients in the chemotherapy + Intron A group experienced a significant prolongation of overall survival as compared to patients in the chemotherapy alone group (median not yet reached vs. 5.6 years).
Metastatic renal cell carcinoma	The approval was primarily based on results from a randomized, double-blind, placebo-controlled clinical trial. In total, 649 patients (bevacizumab plus IFN, 327; placebo plus IFN, 322) were enrolled. The median PFS times, by investigator determination, were 10.2 months for the bevacizumab plus IFN arm and 5.4 months for the placebo plus IFN arm. The IRC analysis of 569 patients with available radiographs yielded similar results (median PFS time, 10.4 months versus 5.5 months. There was no survival advantage. Support for the above results was provided by summarized results of a North American cooperative group study of bevacizumab plus IFN-alpha2b versus IFN-alpha2b alone. The median PFS times were 8.4 months versus 4.9 months in favor of the bevacizumab combination. There was no survival advantage. Serious adverse events were reported more frequently in bevacizumab-treated patients (31% versus 19% and 63% versus 47%, respectively). The most common bevacizumab-related toxicities were bleeding/hemorrhage, hypertension, proteinuria, and venous or arterial thromboembolic events

B: Proposed indications	
Indication	**IFN**
Acute myeloid leukemia	PegIFN-α2a
Castration-resistant prostate cancer	IFN-α2b
Chronic lymphocytic leukemia	IFN-α2b
Cutaneous lymphoma	IFN-α2b
Polycithemia Vera	PegIFN-α2a
Relapsed follicular lymphoma	IFN-α2b
Systemic mastocitosis	IFN-α2b
Testicular teratoma	IFN-α2b

Recombinant Interferon Alfa-2b	
Synonym:	IFN alpha-2B Interferon alfa-2B Interferon alpha-2b
US brand name:	Intron A
Foreign brand name:	Alfatronol Glucoferon Heberon Alfa Urifron Viraferon

initially driven by the view that they represented a potential miracle cure for cancer, declined in part because of failures that may have been the result of an incorrect immune system approach, but also declined as enthusiasm shifted to apparently more novel therapeutic agents, initially agents targeting angiogenesis, then the tyrosine kinase inhibitors, and more recently immune check point inhibitors, which appear more effective and much better biologically characterized. The relative inactivity of IFN in clinical trials might be explained in part by its rapid clearance, with a short half-life in human subjects [46]. Attempts at delivering larger doses of IFN to maintain plasma levels have been limited by significant systemic toxicity [47].

In 1983 Karol Siroka and Howard Smedle wrote: *Clearly interferon is not to cancer what penicillin was to bacterial infection. Nevertheless, it may still have a part to play in the treatment of cancer and only careful clinical and laboratory research in centres familiar with the rigours of such investigation can determine its eventual role in clinical oncology* [48].

At CIGB, where both IFNs are produced, and where several investigators had accumulated more than a decade of experience with their clinical use and their mechanisms of action, we began in 1998 to evaluate how to rationally combine both molecules to obtain a more potent antitumor effect. Two characteristic of types I and II were important to achieve this goal: the synergistic effects on the activation and expression of several genes regulated by both IFNs [49] and similar PKs with maximum IFN blood concentrations achieved approximately 6 to 10 hours after their administration [15].

Using recombinant IFN-α2b and IFN-γ produced at CIGB, preclinical studies exploring combinations were conducted to define the optimum proportions that would lead to synergistic inhibition of cell growth inhibition using the HEp-2, human cervical adenocarcinoma cell line and the GL-5 malignant glioma cell line established in our laboratory [50], along with primary cell cultures established from biopsies obtained from patients with basal cell carcinoma (BCC). The data obtained in these studies were analyzed building isobolograms. From these studies three combinations of IFNs were identified with different proportions of IFN-α2b and IFN-γ, based on the sensitivity of growth in tissue cultures to the various combinations [51].

In murine xenograft models of HEp-2 and U87MG, a glioblastoma cell line, we compared CIGB-128 (HeberFERON) with cisplatin or temozolomide (TMZ), respectively, as controls. The inhibitory effect of HeberFERON on HEp-2 growth was significant with respect to placebo and similar to cisplatin. The means of maximal area of tumors was 366.6 $\pm$ 61.7 mm^3 in control animals treated with placebo, 30.6 $\pm$ 41.8 mm^3 in animals treated with HeberFERON, and 63.7 $\pm$ 0 mm^3 animals treated with cisplatin. Similarly, a significant reduction of tumor growth was achieved with HeberFERON in malignant glioma cells in mice. The means of maximal volume of tumors was 267.8 $\pm$15 mm^3 in control animals treated with placebo compared with 74 $\pm$ 6.5 mm^3 in animals treated with HeberFERON, and 90.2 $\pm$ 19 mm^3 in animals treated with TMZ.

After the first successful clinical trials provided evidence of superiority of HeberFERON over the individual IFNs, a patent was secured for the combination [51]. HeberFERON is a pharmaceutical formulation that contains IFN-α2b and IFN–γ in a ratio that maximally inhibits tumor cell growth.

3. Clinical research with HeberFERON

3.1. Non-melanoma skin cancer

BCC was initially selected as a model for the clinical development of HeberFERON. As the most frequent tumor, rapid recruitment of patients was guaranteed. Additionally, it is an easy-to-treat tumor that grows slowly, has a low propensity to metastasize, infrequently leads to death, and responds in a short time to therapy with IFN [52,53]. During the clinical development of the HeberFERON formulation, several clinical trials were conducted.

Pharmacodynamic studies of HeberFERON in patients with mycosis fungoides [54] and healthy volunteers [55] found a six- to nine-fold increase in serum neopterin, respectively; values that were higher than those in the literature with any subtype or variant of IFN. Concerning ß-2-microglobulin, the increments observed in healthy volunteers with HeberFERON were superior (100%) to those reported for of PEG-IFN-α (60%) [25,27]. Additionally, 2–5 OAS1 serum levels were also markedly increased in healthy volunteers after administration of HeberFERON [55].

The first efficacy study that provided evidence of the superiority of HeberFERON over IFN-α2b was conducted in 40 patients with surgically resectable BCC, with a mean age of 67 years and 57.9% females. Treatment with HeberFERON (n=19) achieved a 95% overall response rate compared with 90% for IFN-α2b (n=21). Complete responses (CR) were 42.1% and 33.3% in the HeberFERON and IFN-α2b groups, respectively. CRs occurred 1 month earlier in the HeberFERON group than in the IFN-α2b or IFN–γ groups [56]. No tumor progressions were observed during the study. Duration of CR was at least 1 year with HeberFERON.

A randomized, controlled, double blind phase 2 dose-finding study of perilesional HeberFERON in BCC, conducted in one center, enrolled 70 patients with tumors <4 cm in size. Seventy-five patients received one dose (0.875, 1.75, 3.5, 7.0, or 10.5 MIU) 3 times a week for 3 weeks. Patients were predominantly men (53.3%), between 29 and 82 years of age, 89.2% were white, and 68% had a comorbid disease (primarily arterial hypertension and cardiac insufficiency). The overall response rate was 93% (60% CR) and 85% (64% CR) at a dose of 7.0 and 10.5 MIU, respectively [56]. The 5-year follow-up of patients who achieved a CR found no recurrences in patients who had received 3.5 and 10.5 MIU doses of HeberFERON. Moreover, the appearance of second BCC in patients treated with HeberFERON (data from all groups) was reduced to half when compared with reports in the literature. Other clinical trials in patients with advanced non-Melanoma Skin Cancer [57] and periocular BCC or squamous cell skin cancer [58] have also demonstrated impressive anti-growth activity of this IFN formulation.

3.2. High-grade gliomas

High-grade gliomas (HGG) are the most aggressive and lethal primary tumors of the brain. Treatment of patients with HGG still remains primarily palliative, with a goal of improving quality of life using surgery, radiotherapy, and chemotherapy. The use IFNs in the treatment of HGG has been shown useful [59–66].

HeberFERON was initially tested in the U87MG glioma cell line. *In vitro* studies demonstrated that this novel formulation of IFNs achieved greater suppression of mRNA expression of STAT-1/STAT-3 compared with the individual IFNs. Also observed in these experiments was up-regulation of TP53, bax, bad, casp3, casp8, and casp9, and down-regulation of bcl-2, by HeberFERON, changes consistent with a pro-apoptotic effect [67]. *In vitro* combination of HeberFERON with radiation therapy and TMZ resulted in a potentiation of its anti-proliferative effects consistent with the radiosensitization effects seen with IFNs and the down regulation of MGMT expression by HeberFERON in several models (classic, proneural) [68].

Genomic and proteomics studies in U87MG cells showed a distinctive genes and protein expression pattern when compared with IFN-α2b and IFN-γ as single agents (see Figure 1). These results provided evidence that HeberFERON is a distinct kind of IFN in terms of its genomic and proteomics properties. We would argue these findings, and support the conduct of new clinical studies in

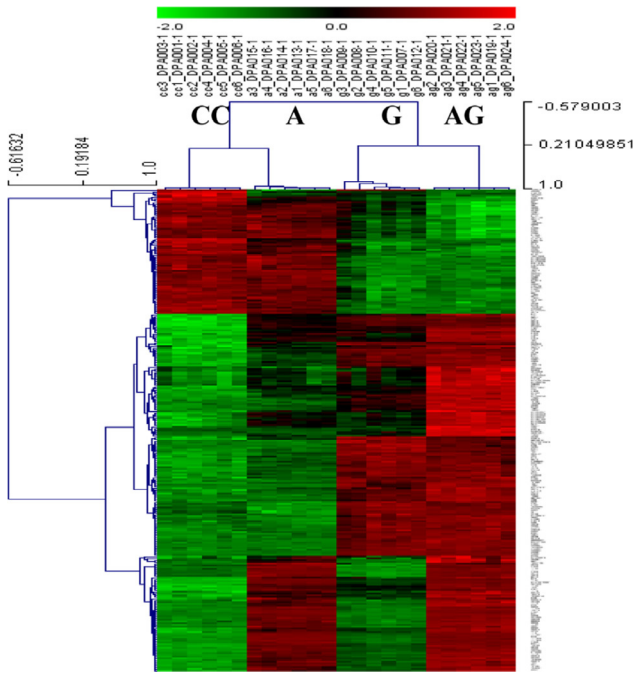

Fig. 1. Proteomic (left) and genomic (right) expression patterns in U87MG cells incubated with IFN-α2b (A), IFN-γ (G), or HeberFERON (AG) for 72 hours and non-treated control cell (CC).

patients with cancer to see where HeberFERON can produce robust antitumor effects.

The use of HeberFERON in 10 patients with HGGs (1 anaplastic astrocytoma and 9 glioblastoma multiform [GBM]) treated outside of a clinical trial has been reported [69]. Patients with poor life expectancy received the combination of intralesional IFNs three times per week over 1 month in doses up to 14.0 MIU. The treatment prolonged the survival of patients to a mean of 3 to 4 months after their diagnosis [69]. In addition, patients with grades I–IV tumors were treated with 7.0 MIU HeberFERON intravenously twice a week until death or the decision of the family and/or medical specialists. The average patient age was 49 years, ranging from 25 to 76 years, and was slightly higher in patients with stage IV cancer; 64.5% were male and 77.4% were white. The diagnosis was GBM in 38.7% and anaplastic astrocytoma in 22.6%. Median Karnofsky performance status (KPS) was similar in all groups, with KPS70 in patients with grades I–III tumors and KPS80 in patients with grade IV malignant gliomas. All patients with KPS100 had grade III tumors. Previous therapies consisted of surgery + radiotherapy in 64.5% and surgery alone in 22.6%. Three patients with GBM had been previously treated with nimotuzumab, an anti-EGF monoclonal antibody produced at the Center for Molecular Immunology in Havana, Cuba [70].

Six months after the initiation of treatment, 100% of patients with grade I and II tumors were alive. At 12, 18, and 24 months, 100% of patients with grade I tumors were alive, with the percent of patients with grade II tumors alive recorded as 85.7%, at 12 and 18 months and 71.4% at 24 months. Thirty-six of the HGG patients had grade III (15) or grade IV (21) tumors, with mean ages of 46 ± 12 and 51 ± 13 years, respectively. Seventy-two percent had been treated with surgery and radiotherapy prior to HeberFERON treatment with median tumor resection of 80%. Eighty-six percent and 57.1% of patients with grade III and grade IV tumors were alive 12 months after surgery plus radiotherapy and HeberFERON administration, respectively. The estimated overall survival for these GBM patients was 19.9 months. The prolonged survival of some patients with HGG receiving HeberFERON raises the possibility that further molecular characterization of responders and non-responders will permit the design of better treatment schedules in combination with other therapies as an option for this devastating and challenging form of cancer.

3.3. Solid tumors

IFNs exert anti-proliferative and apoptotic effects, promote anti-angiogenesis, and induce an immune response, thereby presenting as ideal anti-neoplastic therapeutics. As summarized above, in oncology, the IFNs provide important treatment options for a number of solid tumors, including melanoma, RCC, and AIDS-related Kaposi's sarcoma [16]. More recently, treatment of osteosarcoma, BCC, cervical intraepithelial neoplasia, and bladder cancer with IFN has reported some success when used either in first-line or as a salvage therapy [71,72].

Given the results with HeberFERON in the treatment of non-melanoma skin cancer, in 2006 we began a compassionate, exploratory, prospective, open label, non-randomized, uncontrolled multicenter program allowing for the use of HeberFERON in patients with advanced-stage solid tumors. HeberFERON was used either in combination with existing therapies or as a single agent when the indicated therapy was deemed contraindicated or impractical. A diverse group of malignancies were treated with compassionate HeberFERON, including RCC, colon, pancreas, bladder, breast, and lung carcinomas, advanced BCC, melanoma, lymphoma, and malignant gliomas.

Sixty patients from seven health institutions in Cuba were treated; 19 (31.7%) were from policlinic primary care institutions. The average age of patients was 62 years, ranging between 36 and 94 years. Most patients were white (65.0%) and 56.7% were male. The average weight was 67.9 kg. Thirty-four (56.7%) had metastatic disease, with one metastasis in 19 (55.9%), two in nine (26.5%), and multiple metastases in six (17.6%). The sites of metastatic disease were diverse.

The doses of HeberFERON administered ranged from 3.5 to 11.5 x 10^6 IU, and were adjusted based on the patient's age. Thirty-four patients (56.7%) received a dose of 3.5 MIU, while 16 (26.7%) and seven (11.7%) received 7 and 11.5 MIU, respectively. In some cases dose reductions were implemented as needed, with 14 of 16 who received 7 MIU having their dose reduced to 3.5 MIU. Several routes of administration were used. Most patients received treatment intramuscularly (50.0%), intravenously (25.0%), or intralesionally (16.7%). The median number of doses received per patient was 16. Seventy-six percent of patients received two doses of HeberFERON per week.

All patients had serial clinical laboratory tests, including assessment of kidney and liver function, and in the majority changes caused by HeberFERON were not observed. The adverse events (AEs) that occurred were mainly related to a flu-like syndrome and in the majority were of mild or moderate severity. Thirty-four patients (56.6%) discontinued treatment. The reasons for discontinuation included a lack of medication (41.2%), death (32.4%), voluntary withdrawal (5.9%), bone metastases (5.9%), ischemic cerebrovascular disease (2.9%), uncontrolled hypertension (2.9%), surgical intervention (2.9%), and other AEs (2.9%). Clinical responses were as follows: no CR, six partial responses (10%), 22 stabile disease (36.7%), and eight (13.3%) progressive disease. For 24 patients, information on clinical responses was not available. The estimated survival by tumor is summarized in Table 2.

Seventeen patients with advanced RCC received twice-a-week injections at a dose of 7.0 MIU for 1 month, and then continuously twice a week at a dose of 3.5 MIU. Eleven were men and six were women, with an average age of 57.4 years. Sixteen had undergone a radical nephrectomy and one was inoperable. Histol-

Table 2
Compassionate use of HeberFERON in solid tumors.

Location	Alive/Total	Estimated mean survival (months)	CI (95%)
Kidney	7/13	56.8	(41.5; 72.1)
Skin	9/10	35.7	(29.6; 41.8)
Lung	3/5	32.4	(27.2; 37.6)
Brain	2/7	18.9	(5.4; 32.3)
Prostate	1/3	12.0	(0; 29.6)
Colon-Rectum	0/5	8.8	(0.45; 17.1)
Bladder	0/3	5.7	(4.4; 7.0)
Pancreas	0/4	5.0	(2.9; 7.1)
Others	1/7	8.7	(2.6; 14.8)
Global	25/57	36.1	(27.8; 44.4)

ogy identified clear cell renal carcinoma in 10 patients (three with the eosinophilic variant and one with sarcomatoid differentiation); two urothelial carcinomas, two chromophobe variants, one papillary and one unclassifiable oncocytic tumor. One patient presented with stage II disease, 12 with stage III, and four with stage IV. These patients started treatment with HeberFERON after surgery. Two patients died (both stage III) and the estimated survival, with optimal quality of life, is 41 months.

While the number of options for the treatment of RCC is increasing, IFNs have been used for decades in the treatment of infiltrating and metastatic RCC, often in first-line. A phase 3 trial of bevacizumab combined with IFN-α2a showed significant improvements in progression-free survival in metastatic RCC [32]. Our experience using HeberFERON after surgery in patients with advanced and metastatic presentations provides encouraging results for the prolongation of the survival of these patients. Phase 2 and 3 clinical studies comparing HeberFERON with target therapies should be conducted [73].

3.4. Global safety

The global safety of the patients of the trials reported in this review was analyzed. Prophylactic administration of analgesics and antihistamines were administered pre-treatment or to palliate the adverse reactions. One hundred and ten different AEs were reported in 259 patients (80%). The most frequent events ($\geq$10%) were fever, chills, malaise/general discomfort, arthralgia, headache, asthenia, anorexia, myalgia, perilesional edema and erythema, and sickness. Most AEs were mild (89.6%), and 12.0% were moderate. The treatment approach adopted for most events involved no change in dose (96.8%). Most AEs disappeared (87.9%). Seventy-eight percent of the events were classified as very probably related and 11.4% as probably related to drug.

4. Conclusions

HeberFERON administered intradermally, intravenously, or intramuscularly at doses from 1.75 to 10.5 MIU is safe and well tolerated with potent anti-tumor activity. The improved PD of HeberFERON could explain the stronger antitumor effects observed in BCC compared with IFN-α and in advanced non-melanoma skin cancer, as well as the encouraging prolongation of survival of patients with HGG without the use of chemotherapy or patients with advances stage III or IV RCC.

Co-administration of IFN-α2b and IFN-γ with their potent synergistic actions should allow for more favorable PDs and possibly allow for the use of IFNs with reduced PK interference or additional toxicity. Efficacy trials can be carried out to confirm these findings.

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