The Journal of Laryngology & Otology
September 2004, Vol. **118,** pp. 681–687

Interferon alpha-2b as adjuvant treatment of recurrent respiratory papillomatosis in Cuba: National Programme (1994–1999 report)

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Abstract

Respiratory papillomatosis is a life-spoiling disease due to its high recurrence rate. Interferon (IFN) alpha-2b treatment, adjuvant to surgery, was assessed for its contribution to disease control and patient quality of life improvement. One hundred and sixty-nine patients (85 children and 84 adults) were included after surgical removal of the lesions followed by intramuscular IFN alpha-2b (Heberon alfa R, Heber Biotec), starting with 10^5 IU/Kg weight in children or 6 x 10^6 IU in adults, three times per week. The dose was reduced monthly, if no relapses occurred, until a monthly maintenance with 5 x 10^4 IU/Kg of weight in children or 3 x 10^6 IU in adults up to two years. In case of relapse, it was surgically removed and the patient returned to the higher dose level. The relapse frequency decreased significantly in 77 per cent (69/90) of the recurrent patients both in children (34/46, 74 per cent) and adults (35/44, 79 per cent). Among patients included after their first papilloma, 67 per cent (44/66) had complete (no relapses) or partial (only one relapse) responses (children: 15/33, 45 per cent; adults 29/33, 88 per cent). One hundred and eighteen patients (73 per cent) concluded the treatment without lesions (children: 58 per cent; adults 82 per cent), while the rest showed a significant reduction in the number and size of lesions. IFN was well tolerated. Sixty-two patients (38 per cent) did not have adverse events. The main adverse reactions were fever (59 per cent), chills (24 per cent), arthralgias and myalgias (14 per cent) and headache (10 per cent). One patient developed anti-IFN alpha neutralizing antibodies and became resistant to treatment with recombinant IFN alpha-2b; he responded to natural leucocyte IFN alpha. Treatment with IFN alpha-2b, as an adjuvant to surgery represents a favourable and safe therapeutic alternative for patients with recurrent respiratory papillomatosis.

Key words: IFN Alpha-2b; Respiratory Tract Diseases; Papilloma; Laryngeal Diseases, Surgery

Introduction

Recurrent respiratory papillomatosis (RRP) is a disease characterized by lesions, mostly at the laryngeal level, which can lead to respiratory distress and airway obstruction. Respiratory papillomas are epithelial, benign, neoplastic growths, mostly caused by types 6, 11, and sometimes by types 16 or 18 human papillomavirus (HPV).[1,2] This severe disease can occur at any age but starts more frequently in early childhood (under five years old) with an unpredictable evolution: some tumours grow very slowly while others have a more severe course.[3,4]

The larynx is the most frequent localization of RPP, but the lesion can spread along the entire respiratory and upper digestive tracts. Due to its natural tendency to recur, it impacts notably on the patients' quality of life, their families, and on the health system. At the moment, 80 per cent of relapses are reported in the younger age groups and 36 per cent for adults.[5]

The use of the interferons as adjuvant of the surgery in the treatment of RRP was first reported two decades ago.[6] Several studies have demonstrated the effectiveness of this medication for the decrease and control of papilloma recurrences in 50 to 70 per cent of the patients as well as a partial resolution in 20 to 42 per cent of them.[7–13]

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Accepted for publication: 11 May 2004.

A programme for the treatment of RRP with leucocyte interferon as an adjuvant to surgery started in Cuba in 1983. Its results up to 1992, which included only laryngeal lesions, have been published already.[14] This work has continued as a phase IV clinical trial, for all RRP locations, using recombinant interferon alpha-2b. This paper reports the results obtained for patients included from 1994 to 1997 and followed for up to two years afterwards.

Materials and methods

This multicentre, phase IV study represents a continuation of the National Programme for the control of RRP developed in Cuba since 1983.[14] It included all patients with RRP (both sexes and all age groups) living in any municipality of the Republic of Cuba and was carried out at 17 otorhinolaryngology (ORL) services of General clinical-surgical and paediatric hospitals throughout the whole country (see appendix). This report comprises patients included from May 1994 to December 1997 that were followed up to December 1999. The study was monitored by the National Centre for the Co-ordination of Clinical Trials (CENCEC).

Patients were included after indirect and/or direct endoscopic examination, lesion exeresis and histopathological confirmation of papillomatous diagnosis. The criteria taken into account were a) macroscopic: soft, friable nodules or pieces, not larger than 1 cm in diameter, ulcerated or not, and b) microscopic digitiform papillae composed of a fibrous tissue axis covered by a more or less regularly stratified squamous epithelium. Lesions could be anywhere in the respiratory tract. Only multiple or recurrent lesions were considered eligible if located in the nasal cavities. For the rest of the locations both recurrent and first-onset lesions were taken into account for inclusion. In cases with recurrent disease, the data on relapse frequency, before inclusion, were taken from their clinical records of previous lesion excisions. All patients (parents or legal guardians for children under 18 years old) gave their written, informed consent to participate in the programme. Exclusion criteria were any evidence of malignancy, non-obstructive cardio-respiratory failure, or severe sepsis.

Patients were included after the surgical excision of the lesion. This was done by microsurgery of the larynx under general anaesthesia. Then, they received treatment with recombinant human IFN alpha-2b (Hebron alfa R, Heber Biotec, Havana), for one year using a dose-reduction schedule (Table I). This was the same schedule as in the previous report of this programme.[14] An additional year's maintenance treatment (50 000 IU/Kg and 6 x 10^6 IU monthly in children and adults, respectively) was added and then up to five years further follow up. If during the treatment a new papillomatous lesion appeared, the immediately higher dose level was restarted after lesion removal. Afterwards, the scheme was continued. Prophylactic use of antipyretic medications was prescribed.

Cases were seen monthly as out-patients during the treatment period. They were evaluated by endoscopy every six months or whenever symptoms of relapse appeared. The main endpoint was the occurrence of relapses. Their frequencies (RF) before and after IFN treatment were compared in patients with recurrent disease at inclusion. In those cases, complete response was considered if no relapses occurred during or after treatment; partial response if the RF diminished after treatment; and no response if it did not change or increased. For patients who were included upon their first occurrence of papilloma, complete response was considered if they did not have relapses at all, partial response if they had only one relapse after treatment, and no response when there were two or more relapses after treatment. This latter classification was arbitrary, looking for the strictest criteria of efficacy. The recurrence-free interval was also calculated as the time elapsed from surgical excision of the papilloma to the appearance of the first new lesion.

A disease score was obtained before, and after, treatment in an attempt to quantify the effect.[15] Briefly, it took into account the number, size, and whether the lesions occluded the lumen. The patient had one point for each anatomical localization affected (each nasal fossa, oral cavity, nasopharynx, oropharynx, hypopharynx, epiglottis, ventricle, false folds, actual folds, subglottis, trachea, carina, and each bronchus), one point if the papilloma occupied more than one third of the anatomical location's surface, and one point if it occluded more than one third of the site lumen. Therefore the highest possible score was 45 points.

The clinical examination looked for adverse reactions to treatment as well. They were classified according to their intensity as mild (did not require treatment), moderate (required and responded to intervention), severe (did not respond to treatment; required hospitalization or prolongation of

TABLE I

TREATMENT SCHEDULE

Period (months)*	Children (up to 15 years old)	Adults (> 15 years old)
1	100 000 IU/ Kg, 3 times/ week	6 x 10^6 IU 3 times/ week
2	75 000 IU/ Kg, 3 times/ week	3 x 10^6 IU 3 times/ week
3	50 000 IU/ Kg, 3 times/ week	3 x 10^6 IU 3 times/ week
4	50 000 IU/ Kg, twice/ week	3 x 10^6 IU 3 times/ week
5 to 12	50 000 IU/ Kg, once/ week	3 x 10^6 IU twice/ week

* If relapse return to the immediately upper level

https://doi.org/10.1258/0022215042244741 Published online by Cambridge University Press

TABLE II

DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF THE PATIENTS

Characteristic		Children	Adults	Total *
N		85	84	169
Gender	Male	40 (48%)	53 (63 %)	93 (56 %)
	Female	43 (52%)	31 (37 %)	74 (44 %)
Race	White	54 (65%)	59 (70%)	113 (68%)
	Non white	27 (35%)	25 (30%)	52 (32%)
Age in years (median and range)		7 (1–15)	34 (16–72)	15 (1–72)
Disease	First occurrence	33 (41%)	36 (43%)	69 (42%)
characteristic	Recurrent	47 (59%)	47 (57%)	94 (58%)

* The total for some characteristics does not match 169 because of missing data

hospitalization) and very severe (put the patient's life in danger; required intensive care). Patients were also monitored for haemoglobin, white blood cells and platelet counts, and alanine-aminotransferase by regular clinical laboratory procedures. Anti-interferon antibodies were monitored at least twice during treatment by a sandwich-type ELISA test and verified for anti-IFN antiviral activity neutralization.[16]

All data were included in double-entry validated databases. Statistics were calculated using the SPSS© version 8.0 package. Variable distributions were checked for normality using the Kolmogorov–Smirnov test. Since most of them did not have normal distributions, median and ranges were preferred for descriptive statistics. Comparative analyses were performed using Wilcoxon's non-parametric paired test. Significance level was $p < 0.05$.

Results

One hundred and sixty-nine patients (85 children and 84 adults) from 12 of the 14 provinces of Cuba were included. Table II shows the demographic and baseline characteristics of the patients. Gender and race distributions correspond approximately to the Cuban population. Most patients had recurrent disease. The larynx was the most frequent localization but 38 (22 per cent) of the patients had extralaryngeal lesions. Multiple localizations occurred in 62 per cent of the patients (Table III). The main symptoms were dysphonia (77 per cent), dyspnoea (30 per cent), dry cough (20 per cent), stridor (17 per cent), and nasal obstruction (14 per cent). Bleeding was present in 14 patients (eight per cent). Cyanosis (two per cent), dysphagia (two per cent) and nasal itching (one per cent) were rare. Symptoms were more frequent and intense among children than adults.

The general course of the study is shown in Table IV. Out of the 169 patients included, 130 (77 per cent) completed the basic one-year treatment schedule and 95 (56 per cent) the two-year maintenance period. Five of the withdrawals were due to adverse events. The rest were voluntary abandoners. Sixty-nine patients had more than two years of treatment due to recurrences during treatment that made them restart the schedule. Nevertheless, all patients (except for seven that never started and two where information on the relapse frequency before treatment was missing) were evaluated. Twenty-eight of the 32 withdrawals during the first year (88 per cent) received benefit from the treatment.

One hundred and seventeen patients (73.1 per cent) were classified as complete (45 per cent) or partial (28.1 per cent) responders (Table V). The other 43 patients (26.9 per cent) were considered as non-responders to treatment with IFN since their RF increased after therapy or had more than one relapse if they were included upon the onset of the disease. The overall response rate was larger among the recurrent patients than among the first occurrence ones (76.7 *vs*. 66.6 per cent), although the latter had a more complete response (53.0 *vs*. 36.7 per cent). Adults showed a better overall (78.4 *vs*. 63.6 per cent) but smaller complete (16.9 *vs*. 28.9 per cent) response rates.

TABLE III

RESULTS OF CLINICAL MEASUREMENTS

	Children		Adults		Total	
	Before	After	Before	After	Before	After
Relapse frequency [a]: median (range)	1.7 (0.2–22.1)	0.6 (0–9.5)[b]	0.7 (0.1–10)	0 (0–4.4)[b]	1.0 (0.1–22.1)	0.4 (0–9.5)[b]
Disease score: median (range)	2.5 (1–13)	0 (0–9)[b]	1 (1–6)	0 (0–3)[b]	2 (1–13)	0 (0–9)[b]
Patients with lesions	85	30 (35%)	84	21 (25%)	169	51[c] (30%)
Lesions on the larynx	67 (86%)	26 (31%)	57 (68%)	11 (13%)	124 (73%)	37 (22%)
Lesions on other sites	11 (14%)	3 (4%)	27 (32%)	4 (5%)	38 (22%)	7 (4%)
Single lesions	19 (24%)	11 (13%)	38 (45%)	4 (5%)	57 (34%)	15 (9%)
Multiple lesions	59 (76%)	18 (21%)	46 (55%)	11 (13%)	105 (62%)	29 (17%)
> 1/3 of site area occupied	36 (46%)	9 (10%)	30 (36%)	6 (7%)	66 (39%)	15 (9%)
> 1/3 of site lumen occluded	27 (35%)	8 (9 %)	10 (12%)	1 (1%)	37 (22%)	9 (5%)

a) Only for patients with recurrent disease at inclusion
b) $p < 0.05$ with respect to before treatment (Wilcoxon's paired test)
c) Includes the seven patients who did not begin the treatment and where taken as the worse case

https://doi.org/10.1258/0022215042244741 Published online by Cambridge University Press

TABLE IV

GENERAL COURSE OF THE STUDY

Period	Children	Adults	Total
Included in the study	85	84	169
Did not begin treatment	1	6	7
Began treatment	84	78	162
Received ≤ 12 months of treatment	13	19	32
(Complete / Partial / No response)	(6 / 3 / 4)	(15 / 4 / 0)	(21 / 7 / 4)
Continued treatment	71	59	130
Received 12 – 23 months of treatment	12	23	35
(Complete / Partial / No response)	(4 / 5 / 3)	(14 / 3 / 6)	(18 / 8 / 9)
Received ≥ 24 months of treatment	59	36	95
(Complete / Partial / No response)	(14 / 21 / 23)	(19 / 9 / 7)	(33 / 30 / 30)
Non evaluated (missing information)	1	1	2

Median recurrence-free intervals were 173 (range: 21–3032) and 285 (7–2771) days in children and adults, respectively. The extreme cases were, on one side, patients with very aggressive disease. A one-year-old boy who had had three relapses in three months before inclusion had the first relapse after the onset of treatment on day 21 and continued with one relapse every seven months under treatment. The adult was a 25-year-old male who, after the first relapse one-week post-surgery, continued under IFN treatment with nine relapses in one year. A 14-year-old boy, who had been treated with IFN during the first phase of the programme, relapsed after eight years without disease or treatment, and a 46-year-old man who had received IFN during the first phase, did not have relapses for 7.5 years, received IFN again after this late recurrence, and has been disease-free for three additional years. After the end of IFN treatment, 74 patients (32 children and 42 adults), have been at least one additional year without relapses (15 of them more than four years).

Other quantitative results are shown in Table III. The RF in recurrent patients decreased significantly, both for children and adults, according to the non-parametric Wilcoxon's paired test. At the end of treatment 118 patients (69.8 per cent) did not have lesions. The disease intensity score showed a very significant decrease as well, from 2.43 to 0.59 points after treatment. This was because of the reduction of the number of lesions and their size as can be seen in Table III.

Of the 169 included patients, 162 (96 per cent) were exposed at least once to IFN. Adverse events are shown in Table VI. The most frequent were those related to the flu-like syndrome characteristic of IFN application. It is noteworthy to mention that toxicity due to IFN was much more frequent among adults. Approximately 50 per cent of the children did not report any adverse event at all. One patient developed high titres of anti-IFN alpha neutralizing antibodies.[17] He became resistant to treatment and received natural leucocyte IFN alpha, with which further relapses were prevented.

Discussion

This study included 169 RRP patients, who represent the great majority of such cases in the country, corresponding to a prevalence rate of 1.5/100 000 inhabitants, quite similar to the one previously reported in the first phase of this programme.[14] The aim of the study was to evaluate the use of IFN alpha-2b in this population, within usual clinical practice, as part of a patient management programme. Therefore, selection criteria were wide and the sample represents the clinical variability of the disease. Their gender and race distribution did not show any clear predominance of a particular group, except for a slight male predilection in adults, as has been previously reported.[18] Cases were equally distributed between children and adults. Forty-two per cent of them were included upon disease onset. The rest showed a quite varied relapse frequency (0.1 to 22.1 per year, before treatment).

Although the inclusion was extended to patients with lesions on the entire respiratory tract, the larynx was still the most frequent localization in 68 per cent of adults and 86 per cent of children. The latter had a more aggressive disease, as shown by a higher

TABLE V

FINAL EVALUATION OF THE PATIENTS

Final evaluation		Children	Adults	Total
First occurrence patients	Complete response	11 (33.3%)	24 (72.7%)	35 (53.0%)
	Partial response	4 (12.1%)	5 (15.1%)	9 (13.6%)
	No response	18 (54.5%)	4 (12.1%)	22 (33.3%)
Recurrent patients	Complete response	9 (19.6%)	24 (54.5%)	33 (36.7%)
	Partial response	25 (54.3%)	11 (25.0%)	36 (40.0%)
	No response	12 (26.1%)	9 (20.5%)	21 (23.3%)
Total	Complete response	24 (28.9%)	48 (16.9%)	72 (45.0%)
	Partial response	29 (34.7%)	16 (61.5%)	45 (28.1%)
	No response	30 (36.1%)	13 (20.5%)	43 (26.9%)

https://doi.org/10.1258/0022215042244741 Published online by Cambridge University Press

TABLE VI
ADVERSE REACTIONS TO INTERFERON TREATMENT

Reaction	Children		Adults		Total	
Fever	42	50.0%	54	69.2%	96	59.3%
Chills	12	14.3%	26	33.3%	38	23.5%
Myalgias	13	15.5%	10	12.8%	23	14.1%
Arthralgias	10	11.9%	13	16.7%	23	14.1%
Headache	11	13.1%	6	7.7%	17	10.5%
Asthenia	6	7.1%	5	6.4%	11	6.8%
Weight loss	5	6.0%	5	6.4%	10	6.2%
Alopecia	3	3.6%	3	3.8%	6	3.7%
Allergy	3	3.6%	0	0	3	1.9%
Anaemia	1	1.2%	0	0	1	0.6
Patients without AR	41	48.8%	21	26.9%	62	38.3%

proportion of patients with multiple lesions, larger ones, and more than one third lumen occlusion. This correlates with the fact that symptoms were also more intense and frequent in children, particularly dysphonia, as an index of larynx involvement. These findings agree with the previous reports that RRP is more severe in younger ages.[18–20]

The frequent recurrences and disease exacerbation of RRP together with the possible adverse events of IFN are factors that could affect the permanency of the patients during the whole two-year study period. Nevertheless, treatment compliance can be considered good, since 77 per cent completed the basic first year of treatment and most of the dropouts during the second year, had good response and probably abandoned the study because they did not feel the necessity to continue.

The treatment was well tolerated. IFN-related adverse reactions were present in 62 per cent of the patients. The fact that antipyretic medication was given prophylactically, and the relatively low dose of IFN used, has influenced the large proportion of patients without adverse events. The flu-like syndrome was the more frequent reaction. These events occurred during the first administrations of the IFN and disappeared after two weeks of treatment as has been reported previously.[21] Interestingly, adults suffered more than children due to adverse reactions. This experience has been found previously in other paediatric uses of IFN.[22]

One patient developed high titre anti-IFN alpha neutralizing antibodies that were associated to resistance to treatment. Immunogenicity of recombinant IFNs is a well-known phenomenon.[23, 24] It differs among the various preparations.[25–27] Larger proportions of IFN-treated RRP patients that develop neutralizing antibodies have been reported with other IFN alpha preparations.[27, 28] As in this case, anti-IFN alpha neutralizing antibodies can be related to resistance to treatment, or loss of response, as has been reported for patients with hairy cell or chronic myelogenous[29] leukaemia, chronic hepatitis C,[30, 31] and other causes.[32, 33] The fact that the patient further responded to natural IFN treatment is not a surprise since this kind of experience has been previously reported for other indications.[29, 34, 35]

- **This paper describes the use of Interferon alpha-2b (IFN alpha-2b) as an adjuvant to surgery in 169 cases with respiratory laryngeal papillomatosis**
- **IM IFN alpha-2b was administered in a dose of 10^5 IU/Kg weight in children or 6 x 10^6 IU in adults three times/week**
- **Each post-operative month, if there was no evidence of relapse, the dose was reduced until a monthly maintenance dose of 5 x 10^4 IU/Kg of weight in children or 3 x 10^6 in adults was maintained for up to two years**
- **The authors conclude that treatment with IFN alpha-2b as an adjuvant to surgery represents a favourable and safe therapeutic alternative for patients with recurrent respiratory tract papillomatosis**

The results indicate the efficacy of recombinant IFN alpha-2b in RRP. The relapse rate was reduced significantly, as compared to surgically removed lesion frequency before treatment. Disease severity, as measured by the score previously used by others,[15] points in the same direction. There were 118 patients free of lesions after treatment and fewer and smaller lesions in the rest. At least 70 per cent of the patients, who were considered as responders, received benefit from treatment. However, the criterion for a non-responder was quite strict, following the 'worse case basis'. Probably some of the patients who were included after their first occurrence of papilloma and had few relapses during treatment benefited from it, even if they were considered as 'non-responders'. The fact that an important number of patients did not relapse during one or more years after the end of treatment is encouraging. These results obtained with the use of recombinant IFN alpha-2b are essentially similar to those previously achieved in this same programme using natural leucocyte interferon alpha.[14] However, it is not possible to establish comparisons between both products, since the reports differ in time and data collection methodology, which was more rigorous on this second phase.

There were more responders among adults than children. It is known that the disease is generally more aggressive among the latter.[18, 19] At the same time there were more complete responders among children. This could be due to a larger proportion of spontaneous regression in children that can influence the final result.

The non-responder patients deserve particular attention. They represent more than 20 per cent of the population and still bear very aggressive, life-limiting disease. Studies on HPV infection–IFN

https://doi.org/10.1258/0022215042244741 Published online by Cambridge University Press

relationship have identified several factors that can be involved in resistance. Besides the appearance of anti-IFN neutralizing antibodies, HPV subtype can be an important feature. Some of them can impair IFN action through the action of their oncogenic E6 and E7 proteins.[36, 37] A thorough characterization of these patients is needed in order to find out a better way of helping them.

This study was not a controlled one. Its aim was to evaluate an intervention in clinical practice. This kind of work has the sources of bias intrinsic to patients' condition and the variability of a multicentre programme where many hospitals and physicians take part. Its importance is to show how an intervention can impact on the course of a disease that otherwise would have been very relapsing and uncomfortable to patients and their families. The results of this national programme are highly important for the control of RRP infection in Cuba. Using a homemade biotechnological product it has been an effective and well tolerated therapeutic approach for this disease. This work continues as a pharmacosurveillance programme.

Acknowledgements

The authors are grateful for the help obtained from technicians Irayuma Alfaro (†) and Yuselis Dominguez and the Supply Group of CENCEC who took care of the preparation and distribution of the products used in this research. The public health authorities of the different provinces supported the work. The Centre for Genetic Engineering and Biotechnology, La Habana, provided all the interferon used in this work and the support for all the workshops, discussion meetings, and control visits it comprised.

References

1 Schneider A, Koutsky LA. Natural history and epidemiological features of genital HPV infection. In: Muñoz N, Bosch FX, Shah KV, Meheus A, eds., *The Epidemiology of Cervical Cancer and Human Papillomavirus*. International Agency for Research on Cancer, Lyon, 1992;25–52
2 Snoeck R, Wellens W, Desloovere C, Van Ranst M, Naesens L, De Clercq E, *et al*. Treatment of severe laryngeal papillomatosis with intralesional injections of cidofovir [(S)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl) cytosine]. *J Med Virol* 1998;**54**:219–25
3 Tucker HM. Laryngeal Neoplasia. In: *The Larynx*. New York: Thieme Medical Publishers, Inc., 1987;47–51
4 Steinberg BM, Meade R, Kalinowski S, Abramson AL. Abnormal differentiation of human papillomavirus induced laryngeal papillomas. *Arch Otolaryngol Head Neck Surg* 1990;**116**:1167–71
5 Kashima H, Mounts P, Leventhal B, Hruban RH. Sites of predilection in recurrent respiratory papillomatosis. *Ann Otol Rhinol Laryngol* 1993;**102**:580–3
6 Haglund S, Lundqvist PG, Cantell K, Strander H. Interferon therapy in juvenile laryngeal papillomatosis. *Arch Otolaryngol Head Neck Surg* 1981;**107**:327–32
7 Lundquist PG, Haglund S, Carlsoo B, Strander H, Lundgren E. Interferon therapy in juvenile laryngeal papillomatosis. *Otolaryngol Head Neck Surg* 1984; **92**:386–91
8 Lusk RP, McCabe, BF, Mixon JH. Three years experience of treating recurrent respiratory papilloma with interferon. *Ann Otol Rhinol Laryngol* 1987;**96**:158–62
9 Healy GB, Gelber RD, Trowbridge AL, Grundfast KM, Ruben RJ, Price KN. Treatment of recurrent respiratory papillomatosis with human leukocyte interferon. Results of a multicenter randomized clinical trial. *N Engl J Med* 1988;**319**:401–7
10 Kashima H, Leventhal B, Clark K, Cohen S, Dedo H, Donovan D, *et al*. Interferon alfa-N-1 (Wellferon) in juvenile onset recurrent respiratory papillomatosis: Results of a randomized study in twelve collaborative institutions. *Laryngoscope* 1988;**98**:334–40
11 Galloway DA, McDougall JK. Human papillomaviruses and carcinomas. *Adv Virus Res* 1989;**37**:125–71
12 Leventhal BG, Kashima HK, Mounts P, Thurmond L, Chapman S, Buckley S, *et al*. Long-term response of recurrent respiratory papillomatosis to treatment with lymphoblastoid interferon alfa-n1. *N Engl J Med* 1991; **325**:613–7
13 Phelps WC, Alexander KA. Antiviral therapy for human papillomaviruses: rationale and prospects. *Ann Intern Med* 1995;**123**:368–82
14 Deunas L, Alcantud V, Alvarez F, Arteaga J, Benitez A, Bopuza MC, *et al*. Use of interferon-α in laryngeal papillomatosis: eight years of the Cuban national programme. *J Laryngol Otol* 1997;**111**:134–40
15 Leventhal BG, Kashima HK, Weck PW, Mounts P, Whisnant JK, Clark KL, *et al*. Randomized surgical adjuvant trial of interferon alfa-n1 in recurrent papillomatosis. *Arch Otolaryngol Head Neck Surg* 1981; **114**:1163–9
16 González-Cabañas R, Ferrero J, Morales MG, Aguilera A, López-Saura P. Immunogenicity of recombinant interferon alpha-2b (Heberon alfa R). Detection of antibodies by means of an immunoenzymatic assay and antiviral activity neutralization. *Biotecnología Aplicada* 1998;**15**:71–6
17 Benito I, González-Cabañas R, Madrazo E, Quintana A, Bello I, Ferrero J, *et al*. Unusually high titer of anti-interferon alpha-2 neutralizing antibodies elicited by treatment in a patient with laryngeal papillomatosis. *J Interferon Cytokine Res* 1997;**17**:S108
18 Derkay CS, Darrow DH. Recurrent respiratory papillomatosis of the larynx: current diagnosis and treatment. *Otolaryngol Clin North Am* 2000;**33**:1127–42
19 Doyle DJ, Gianoli GJ, Espinola T, Miller RH. Recurrent respiratory papillomatosis: juvenile versus adults form. *Laryngoscope* 1994;**104**:523–7
20 Bauman NM, Smith RJ. Recurrent respiratory papillomatosis. *Pediatr Clin North Am* 1996;**43**:1385–401
21 Vial T, Bailly F, Descotes J, Trepo C. Effets secondaires del interféron alpha. *Gastroenterol Clin Biol* 1996;**20**:462–89
22 Garmendía G, Miranda N, Borroso S, Longchong M, Martinez E, Ferrero J, *et al*. Regression of infancy hemangioma with recombinant interferon alpha-2b. *J Interferon Cytokine Res* 2001;**21**:31–8
23 Itri LM, Campion M, Dennin RA, Palleroni AV, Gutterman JU, Groopman JE, *et al*. Incidence and clinical significance of neutralizing antibodies in patients receiving recombinant interferon alfa-2a by intramuscular injection. *Cancer* 1987;**59**:668–74
24 Antonelli G. In vivo development of antibody to interferons: an update to 1996. *J Interferon Cytokine Res* 1997;**17**:S39–46
25 Bertolotto A, Malucchi S, Milano E, Castello A, Capobianco M, Mutani R. Interferon beta neutralizing antibodies in multiple sclerosis: neutralizing activity and cross-reactivity with three different preparations. *Immunopharmacology* 2000;**48**:95–100
26 Hou C, Chuang WL, Yu ML, Dai CY, Chen SC, Lin ZY, *et al*. Incidence and associated factors of neutralizing anti-interferon antibodies among chronic hepatitis C patients treated with interferon in Taiwan. *Scand J Gastroenterol* 2000;**35**:1288–93
27 Nurmukhametov RKH, Onufrieva EK, Soldatskii IuL, Mezentseva MV, Kas'ianova NV, Tsvetnova MV, *et al*. Assessment of the formation of interferon neutralizing antibodies and their influence on the effectiveness of interferon therapy in children with juvenile respiratory papillomatosis. *Vestn Otorinolaringol* 2000;**4**:22–5

28 Thurmond LM, Brand CM, Leventhal BG, Finter NB, Johnston JM. Antibodies in patients with recurrent respiratory papillomatosis treated with lymphoblastoid interferon. *J Lab Clin Med* 1991;**118**:232–40
29 Russo D, Candoni A, Zuffa E, Minisini R, Silvestri F, Fanin R, *et al*. Neutralizing anti-interferon-alpha antibodies and response to treatment of inpatients with Ph+ chronic myeloid leukaemia sequentially treated with recombinant (alpha 2a) and lymphoblastoid interferon-alpha. *Br J Haematol* 1996;**94**:300–5
30 Bonino F, Baldi M, Negro F, Oliveri F, Colombatto P, Bellati G, *et al*. Clinical relevance of anti-interferon antibodies in the serum of chronic hepatitis C patients treated with interferon-alpha. *J Interferon Cytokine Res* 1997;**17**:S35–8
31 Leroy V, Baud M, de Traversay C, Maynard-Muet M, Lebon P, Zarski JP. Role of anti-interferon antibodies in breakthrough occurrence during alpha 2a and 2b therapy in patients with chronic hepatitis C. *J Hepatol* 1998; **28**:375–81
32 Rajan GP, Seifert B, Prummer O, Joller-Jemelka HI, Burg G, Dummer R. Incidence and in-vivo relevance of anti-interferon antibodies during treatment of low-grade cutaneous T-cell lymphomas with interferon alpha-2a combined with acitretin or PUVA. *Arch Dermatol Res* 1996;**288**:543–8
33 McKenna RM, Oberg KE. Antibodies to interferon-alpha in treated cancer patients: incidence and significance. *J Interferon Cytokine Res* 1997;**17**:141–3
34 Milella M, Antonelli G, Santantonio T, Giannelli G, Currenti M, Monno L, *et al*. Treatment with natural IFN of hepatitis C patients with or without antibodies to recombinant IFN. *Hepatogastroenterol* 1995;**42**:201–4
35 Tefferi A, Grendahl DC. Natural leukocyte interferon-alpha therapy in patients with chronic granulocytic leukemia who have antibody-mediated resistance to treatment with recombinant interferon-alpha. *Am J Hematol* 1996;**52**:231–3
36 Garcia-Milian R, Hernández H, Panade L, Rodríguez C, Gonzalez N, Valenzuela C, *et al*. Detection and typing of human papillomavirus DNA in benign and malignant tumours of laryngeal epithelium. *Acta Otolaryngol* 1998; **118**:754–8
37 Perea SE, Massimi P, Banks L. Human papillomavirus type 16 E7 impairs the activation of the interferon regulatory factor-1. *Inter J Mol Med* 2000;**5**:661–6

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Dr H Nodarse-Cuní takes responsibility for the integrity of the content of the paper.
Competing interests: Drs H Nodarse-Cuní and Pedro Lopez-Saura are employees of the 'Centro de Investigaciones Biológicas', which is part of the 'Centro de Ingenieria Genéetica y Biotecnologia, Havana' complex, where IFN is produced. Independent from the Production or the Commercial Divisions

Appendix

Cuban Group for the Study of Interferon in Recurrent Respiratory Papillomatosis (*) Steering and Data Quality Committee

Participating institutions (number of patients) and investigators
Havana City (91): **'William Soler' Paediatric Hospital** (27) Elisa Leyva-Montero, Hortensia Rodríguez-Gómez; **'Calixto García' Hospital** (19) Nora Iznaga-Marín (*); **'Juan Manuel Marquez' Paediatric Hospital** (13) Ditza Viera-Alvarez (*), Ana Teresa Montelongo (*); **'Joaquín Albarrán' Hospital** (10) Isabel Benito-Soler; **'Carlos J. Finlay' Hospital** (9) Nerys Z. Morales-Alfonso, Sixto Moreno-Roselló; **Centro Habana Paediatric Hospital** (4) María Josefa García-Ortiz; **'Salvador Allende' Hospital** (3) Mirtha Domínguez-Rodríguez; **'Enrique Cabrera' Hospital** (2) Tahamara Alcalá-Villalón; **'10 de octubre' Hospital** (1) Luisa Enrique Panadés, Teresa Rodríguez-Roche; **'Luis Díaz Soto' Hospital** (1) Leonor C Arias-Oliva; **Cerro Paediatric Hospital** (1) Jesús Alvarez-Montesino; **'10 de octubre' Paediatric Hospital** (1) Sonia Richelme-Fabré; **Santiago de Cuba Province** (15): **'Saturnino Lora' Hospital** (8) Francisca Santiesteban-Aguilera; **'Infantil Sur' Paediatric Hospital** (7) Hector Fernández-Fernández; **Matanzas Province** (10): **'Faustino Pérez' Provincial Hospital** (10) Elsa Boyero-Palenzuela, Leonor González-Hernández; **Guantánamo Province** (9): **'Pedro A Pérez' Paediatric Hospital** (7) Dania Nordet-Cardona; **'Agostino Neto' Provincial Hospital** (2) Lucía Carnegie-Squires; **Las Tunas Province** (9): **'Ernesto Guevara' Provincial Hospital** (9) Amalia Nieves Alvarez; **Villa Clara Province** (8): **'Juan L. Miranda' Paediatric Hospital** (6) Mercedes de la Caridad Fernández, Clara Machado Bermúdez; **'Arnaldo Milian' Provincial Hospital** (2) Pablo Ruíz Porras; **Camagüey Province** (8): **Military Hospital** (6) Jorge Santana Alvarez; **Provincial Paediatric Hospital** (2) Juan Miguel Iglesias Solís; **Pinar del Río Province** (8): **'Pepe Portilla' Paediatric Hospital** (8) Ileana Chávez García; **Holguín Province** (6): **'Vladimir Ilich Lenin' Provincial Hospital** (6) Julia Pérez-Fernández, Dalia T Góngora-Pérez; **Sancti Spiritus Province** (3): **'Camilo Cienfuegos' Paediatric Hospital** (3) Jorge Luis Arteaga; **Ciego de Avila Province** (2): **'Antonio Luaces' Provincial Hospital** (2): Freddy Cruz Hernández.

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