steroid receptor determination for protocol B-09 of the national Surgical Adjuvant Breast Project. In: Sarfaty GA, Nash AR, Keightly DD, eds. *Estrogen Receptor Assays in Breast Cancer. Laboratory Discrepancies and Quality Assurance*, New York, Masson, 1981, 27–39.
6. Raam S, Gelman R, Cohen JL. Estrogen receptor assay: interlaboratory and intralaboratory variation in the measurement of receptor using dextran-coated charcoal technique: a study sponsored by E.C.O.G. *Eur J Cancer Clin Oncol* 1981, **17**, 643–649.
7. Klijn JGM, Foekens JA. Prognostic factors in breast cancer: a review. In: Goldhirsch A, Veronesi W, eds. *Endocrine Therapy of Breast Cancer IV*, Monographs of the European School of Oncology, Springer-Verlag, Berlin, 1990, 17–30.
8. Rochefort H, Augereau P, Briozzo P, *et al.* Structure, function, regulation and clinical significance of the 52K pro-cathepsin D secreted by breast cancer cells. *Biochimie* 1988, **70**, 943–949.
9. Thorpe SM, Rochefort H, Garcia M, *et al.* Association between high concentrations of Mr 52,000 cathepsin D and poor prognosis in primary human breast cancer. *Cancer Res* 1989, **49**, 6008–6014.
10. Spyratos F, Maudelonde T, Brouillet JP, *et al.* Cathepsin-D: an independent prognostic factor for metastasis of breast cancer. *Lancet* 1989, **ii**, 1115–1118.
11. Tandon AK, Clark GM, Chamness GC, Chirgwin JM, McGuire WL. Cathepsin D and prognosis in breast cancer. *N Engl J Med* 1990, **322**, 297–302.
12. Koenders A, Thorpe SM, on behalf of the EORTC Receptor Group. Standardization of steroid receptor assays in human breast cancer—I. Reproducibility of oestradiol and progesterone receptor assays. *Eur J Cancer Clin Oncol* 1983, **19**, 1221–1229.
13. Rogier H, Freiss G, Besse MG, *et al.* Two-site immunoenzymometric assay of the 52-kDa-cathepsin D cytosols of breast cancer tissues. *Clin Chem* 1989, **35**, 81–85.
14. Garcia M, Capony F, Derocq D, Simon D, Pau B, Rochefort H. Monoclonal antibodies to the estrogen-regulated M_r 52,000 glycoprotein: characterization and immunodetection in MCF7 cells. *Cancer Res* 1985, **45**, 709–716.
15. Sachs L. *Angewandte Statistik*, Berlin, Springer, 1984.
16. EORTC Breast Cancer Cooperative Group. Revision of the standards for the assessment of hormone receptors in human breast cancer. *Eur J Cancer Clin Oncol* 1980, **16**, 1513–1515.
17. Jeffcoate SL. *Efficiency and Effectiveness in the Endocrine Laboratory*. London, Academic Press, 1981.
18. Benraad Th, Koenders A. Estradiol receptor activity in lyophilized calf uterus and human breast tumor tissue. *Cancer* 1980, **46**, 2762–2764.
19. Koenders A, Benraad Th. Preparation of lyophilized reference samples for quality control of steroid receptor measurements. *Ligand Rev* 1981, **3**, 32–39.
20. Yonezawa S, Takahashi T, Wang XJ, *et al.* Structures as the proteolytic processing region of cathepsin-D. *J Biol Chem* 1988, **263**, 16504–16511.
21. Westley B, Rochefort H. A secreted glycoprotein induced by estrogen in human breast cancer cell lines. *Cell* 1980, **20**, 352–362.
22. Garcia M, Lacombe MJ, Duplay H, *et al.* Immunohistochemical distribution of the 52-kDa protein in mammary tumors: a marker associated with cell proliferation rather than with hormone responsiveness. *J Steroid Biochem* 1987, **27**, 439–446.
23. Rochefort H. Cathepsin-D in breast cancer. *Breast Cancer Res Treatm* 1990, **16**, 3–8.

Eur J Cancer, Vol. 28, No. 1, pp. 75–78, 1992.
Printed in Great Britain

0964–1947/92 $3.00 + 0.00

A Dose-escalation Study of Recombinant Interferon-alpha in Patients with a Metastatic Carcinoid Tumour

Cees H.N. Veenhof, Ronald de Wit, Barbara G. Taal, Luc Y. Dirix, John Wagstaff, Arie Hensen, Anneke C. Huldij and Piet J.M. Bakker

The efficacy of interferon alpha-2b in doses up to 12 × 10^6 IU three times weekly was studied in 21 patients with a metastatic carcinoid tumour. Of these 21 patients, 19 were evaluable for response. Patients were treated with escalating dosages of interferon alpha-2b: 3 × 10^6 IU, 6 × 10^6 IU and 12 × 10^6 IU. The escalation was performed every 8 weeks when no objective tumour regression was observed. Patients were also evaluated for biochemical response and symptomatic improvement. One objective tumour regression was observed. Of the 15 patients with elevated 5-hydroxyindole acetic acid (5-HIAA) excretion, 5 (33%) had a more than 50% decrease in 5-HIAA excretion. Relief of symptoms occurred in 11 patients (58%). This improvement was already apparent during the initial 8 weeks of treatment. Increasing the dose to 6 or 12 × 10^6 IU interferon alpha-2b did not result in further symptomatic improvement. In contrast toxicity was considerable with the higher dosages of interferon alpha-2b. It is concluded that low dose interferon alpha-2b (3 × 10^6 IU) three times weekly is as effective as higher dosages of interferon alpha-2b at ameliorating symptoms of the carcinoid syndrome.
Eur J Cancer, Vol. 28, No. 1, pp. 75–78, 1992.

INTRODUCTION

CARCINOID TUMOURS are uncommon neoplasms thought to arise from basogranular argentaffin cells in the base of the intestinal crypts [1]. Carcinoids of truly malignant behaviour most commonly originate in the small bowel [2, 3]. These tumours most commonly metastasise to the liver, with bone and lung being the next most frequent sites [4]. Carcinoids are often slow growing tumours and even in the presence of metastatic disease survival can be several years without treatment [5].

Treatment options for metastatic carcinoid should always include surgical procedures [1, 2], hepatic artery occlusion [6], or chemotherapy [7–9]. Chemotherapy has produced only minimal evidence of benefit. Response rates with the most favourable regimens are in the 20–30% range, and these

responses have only been partial and of short duration [9]. Treatment has also been directed at the release and the actions of substances released by the tumour which cause the symptoms of diarrhoea and flushes. A wide variety of agents have been used for this purpose, including antidiarrhoeal agents, adrenergic blocking agents, kinin antagonists, serotonin antagonists, corticosteroids and somatostatin [1, 10]. Although these drugs may result in good palliation of symptoms, they do not alter the natural course of the disease.

In 1983 Öberg *et al.* [11] reported on the activity of low dose ($3–6 \times 10^6$ IU daily) leucocyte alpha interferon ($IFN\alpha_{2b}$) on clinical symptoms and hormone levels in patients with mid-gut carcinoid tumours. This study was updated in 1986 with a total of 36 patients [12]. Of these 36 patients, 4 (11%) had objective reduction in tumour size, with 2 achieving a complete and 2 a partial response. Of 30 patients with elevated urine 5-hydroxyindolacetic acid (5-HIAA) excretion, 16 (53%) had a reduction of 50% or more. Approximately two thirds of the patients had subjective improvements, primarily related to a lessening in the incidence of flushing and diarrhoea.

These results together with the availability of recombinant human $IFN\alpha_{2b}$ and the expected low level of toxicity from low-dose recombinant IFN-alpha, prompted us to perform this study for patients with carcinoid tumours. The aim of the study was to further define the objective and biochemical response rate, time to response and duration of response, and the subjective improvement especially with regard to the carcinoid syndrome.

PATIENTS AND METHODS

25 patients entered this multi-centre trial between February 1988 and December 1989.

Patients were eligible in the case of histologically or cytologically confirmed metastatic carcinoid tumour. Patients were required to have either measurable tumour or an elevated 5-HIAA excretion. Measurable tumour was defined as a lesion demonstrable on physical examination, chest X-ray, computed tomography or ultrasound that permitted direct bidimensional measurement. Previous treatments were allowed provided that treatment was withdrawn at least 4 weeks before the start of interferon therapy. Patients had to have a WHO performance status of 0–2 and were not to be older than 75 years of age. Pretreatment assessment included routine investigations of blood and urine, haemoglobin content, leucocyte and platelet counts, serum creatinine, albumin, liver function tests and electrolytes. The urinary 5-HIAA excretion was determined as an average of two 24 h samples. All patients had to give informed consent.

Patients were ineligible for this study if they had one of the following conditions: a life expectancy of less than 8 weeks, leukopenia (leucocytes $< 3.5 \times 10^9$/l) or thombocytopenia (platelets $< 100 \times 10^9$/l), previous radiotherapy to indicator lesions, any known co-existent malignant disease or pre-existent malignant disease other than adequately treated basal or squamous cell carcinoma of the skin or stage I carcinoma of the cervix, clinical signs of brain involvement or leptomeningeal disease, a serious psychiatric disorder, active heart disease, uncontrolled bacterial infection, concurrent therapy with corticosteroids or previous exposure to interferon or other cytokines. Of the 25 patients entered in this study, 21 patients were eligible of whom 19 were evaluable for response. Early progression leading to death before 8 weeks occurred in 2 patients. The patient characteristics of the 21 eligible patients are shown in Table 1. 14 patients had symptoms of the carcinoid syndrome.

Table 1. Patients' characteristics

Characteristiscs	No.
Male/female	8/13
Median age years (range)	56 (33–70)
WHO performance status	
0	4
1	9
2	8
Site of metastasis	
Liver	16
Bone	5
Lung	6
Subcutaneous	1
Other	3
Symptoms	
Flushing	13
Diarrhoea	13
Wheezing	4
Prior chemotherapy	3

3 patients had received prior chemotherapy. The pretreatment condition of most patients was good; 13 patients had a WHO performance status of 0 or 1. The median time from diagnosis of carcinoid tumours to the start of interferon therapy was 13 months (range 1–68). Of the 19 evaluable patients, 17 had a measurable tumour mass, and 13 also had an elevated 5-HIAA excretion. 2 further patients had an elevated 5-HIAA alone.

Treatment

Patients were treated with recombinant $IFN\alpha_{2b}$ (INTRON-A, Schering-Plough, Amstelveen, the Netherlands), which was administered subcutaneously three times weekly with a starting dose of 3×10^6 IU during the first 8 weeks (Fig. 1). For those patients who had objective tumour regression after 8 weeks of treatment, therapy was continued as long as this favourable response lasted. For those patients who had stable or progressive disease at the end of the first 8 weeks, irrespective of changes in

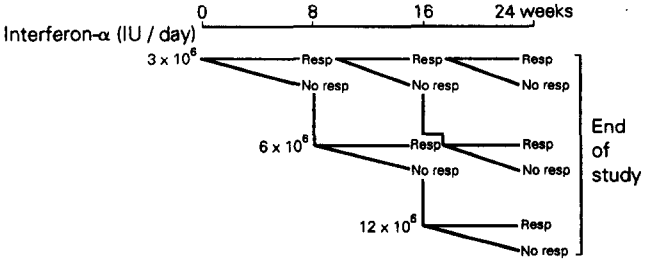

Fig. 1.

Correspondence to C.H.N. Veenhof.
C.H.N. Veenhof, R. de Wit and P.J.M. Bakker are at the Division of Medical Oncology, F4-223, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam; B.G. Taal is at the Netherlands Cancer Institute, Amsterdam, The Netherlands; L.Y. Dirix is at the Department of Oncology, Antwerp University Hospital, Antwerp, Belgium; J. Wagstaff is at the Department of Medical Oncology, Free University Hospital, Amsterdam; A. Hensen is at the Elisabeth Gasthuis, Haarlem; and A.C. Huldij is at the Comprehensive Cancer Centre Amsterdam, Amsterdam, The Netherlands.
Revised 18 Sep. 1991; accepted 16 Oct. 1991

5-HIAA excretion, the dose of interferon was escalated to 6×10^6 IU three times weekly. For those patients who had stable or progressive disease after another 8 weeks of treatment with 6×10^6 IU interferon, a final escalation was made to 12×10^6 IU interferon three times weekly. In patients where the 5-HIAA excretion was the only tumour parameter, the dose of interferon was escalated until a complete biochemical response was observed. The dosages were escalated every 2 months to study a possible dose–response relationship. If stable or progressive disease was documented after 24 weeks of interferon treatment, treatment was stopped, because it was believed that further treatment would not result in either an objective nor a further subjective improvement. Treatment was also discontinued in the case of intolerable toxicity (WHO grade 3–4), any disease complication necessitating an interruption of interferon treatment for more than 4 weeks or when patients did not tolerate scheduled treatment due to influenza-like fatigue symptoms. The use of acetaminophen was allowed when necessary for palliation of fever or myalgia. Patients were seen every 2 weeks during the first 8 weeks, and thereafter every 4 weeks during the course of treatment. Complete blood counts and chemistry were obtained every two weeks during the first 8 weeks, urine 5-HIAA excretion every 4 weeks. Patients were evaluated every 8 weeks for the assessment of indicator lesions and urine 5-HIAA.

Response and toxicity evaluation

Patients were judged evaluable if they had completed at least 8 weeks of treatment. Objective response was defined according to standard WHO criteria [13]. If hepatomegaly was used as an indicator, the sum of the measurements below the xiphoid process and each costal margin at the midclavicular line had to be reduced by at least 30%. For a biochemical response it was required that there had to be at least a 50% reduction in the pretreatment 5-HIAA excretion for at least 4 weeks (partial remission). A complete biochemical remission was defined as a return of 5-HIAA excretion to normal values. For symptomatic improvement it was required that there had to be at least a 25% reduction in flushing, stool frequency, nausea or vomiting episodes, or wheezing episodes. Patients were evaluated for toxicity according to the WHO toxicity grading system.

RESULTS

The median duration of interferon therapy was 16 weeks (range 8–24). At 8 weeks all 19 patients were evaluable for objective and/or biochemical response. After 16 weeks, data for response were available for only 15 patients. After 24 weeks, 11 patients had completed 8 weeks of the highest dose of interferon treatment and were therefore evaluable for objective and/or biochemical response. 1 patient had a partial remission after 24 weeks of treatment; in the others no objective tumour regression was seen. Stable disease was documented in 13 of the patients at 8 weeks, in 9 patients at 16 weeks and in 3 patients at 24 weeks. All other patients were progressive during interferon therapy at 8, 16 and 24 weeks, respectively.

Of the 15 patiens with elevated 5-HIAA excretion, 5 had more than a 50% decrease in 5-HIAA excretion (33%). All five biochemical responses occurred within 8 weeks of the start of therapy. For 3 of the 5 patients this response was sustained up to the 24th week of treatment. Symptomatic improvement occurred in 11 of the 19 patients (58%). Of the 13 patients with flushing, 8 had an improvement in their symptoms, 7 of 13 had a lessening in the frequency of diarrhoea, and of the 4 patients with complaints of wheezing 2 had symptomatic improvement. In 3 of the 5 patients where there was a more than 50% decrease in 5-HIAA excretion relief of flushing and/or diarrhoea was also observed. One patient had symptomatic deterioration in spite of a more than 50% decrease in 5-HIAA excretion. The performance status improved in only 4 patients.

All patients were evaluable for toxicity. During 3×10^6 IU of interferon treatment, 11 patients complained of mild flu-like symptoms with chills, fever and myalgia, which did not necessitate interruption of treatment. Two patients had WHO grade 1 haematological toxicity. During the 6 and 12×10^6 IU of interferon treatment notable side effects developed, which consisted mainly of flu-like symptoms, and were the eventual reason for stopping treatment in most patients. 4 patients developed grade 1 haematological toxicity, and grade 1 gastrointestinal toxicity was observed in another 5 patients. Mild anorexia was mentioned by 6 patients. Although response criteria may somewhat differ between the different studies, the results are within the 90% confidence intervals for response of data found in the literature (see Table 2).

Table 2. The results of IFN therapy in patients with metastatic carcinoid tumour

	No. of patients	Objective responses (%)	Biochemical responses (%)	Symptomatic responses (%)
Öberg *et al.* [12]	36	4/36 (11)	16/30 (53)	Overall 23/36 (64)
Smith *et al.* [14]	14	0/14	5/14 (36)	Overall 6/9 (67)
Moertel *et al.* [15]	27	4/20 (20)	9/23 (39)	Flushing 13/20 (65)
				Diarrhoea 5/15 (33)
Välimäki *et al* [16]	8	1/8 (13)	2/8 (25)	Overall 2/7 (29)
This study	21	1/17 (6)	5/15 (33)	Overall 11/19 (58)
				Flushing 8/13 (62)
				Diarrhoea 7/13 (54)
Total	106	10/95 (11)	37/90 (41)	Overall 40/64 (63)
				Flushing 21/33 (64)
				Diarrhoea 12/28 (43)

DISCUSSION

In this study we have demonstrated that IFNα_{2b} therapy produces biochemical and symptomatic responses in 33 and 58% of patients, respectively. Our study and those of Smith *et al* [14] and Moertel *et al.* [15] demonstrate that escalating the dose of IFNα_{2b} does not improve the likelihood of obtaining a response. Indeed escalating the dose may be counter productive firstly because it results in more toxicity which frequently precludes prolonged administration of the IFNα_{2b} and secondly the higher doses may exceed the optimum biological active dose. The former point is born out by the fact that the median duration of therapy in the study reported by Moertel *et al.*, was only 8 weeks and the reported toxicity appeared to exert a markedly deleterious effect on the quality of the patients life.

The duration of therapy with IFN has been observed to be important with regard to the probability of obtaining and maintaining measurable responses by Öberg *et al.* [12]. This group gave fixed daily doses (3–6 $\times$ 10^6 IU) of leucocyte IFN for a mean period of 22.4 months. Although subjective improvement and significant reductions in tumour markers were observed during the first 3 months of therapy measurable responses of solid tumours only occurred much more slowly. The Swedish group also reported that 39% of their patients had stable disease during IFN treatment and that these patients together with the responding patients progressed rapidly once the drug was stopped. All these data taken together imply that for IFN therapy to be successful in patients with malignant carcinoid tumours the drug must be administered in moderate, and therefore tolerable doses, for prolonged periods. Whether therapy with IFN results in longer survival times for treated patients has not been determined by any of the studies reported to date. However by reducing the symptoms of the carcinoid syndrome this treatment has the potential of improving the quality of life of the patients provided that toxicity of the drug is not excessive. In our study a dose of 3 $\times$ 10^6 IU three times a week subcutaneously satisfied this demand. Of the other treatment modalities for symptomatic metastatic carcinoid only the somatostatin analogues are comparable with interferon. Although objective remissions are rare [17], treatment with somatostatin results in approximately two thirds of the patients in a prolonged symptomatic improvement [18, 19]. The addition of IFNα_{2b} to somatostatin did not have any benefit, in one report [20]. However, the number of patients treated in this study was small, so that no firm conclusion can be drawn about the effect of the combination.

For the near future more objective studies are required in order to determine the impact that IFN therapy, whether or not combined with a somatostatin analogue, has on the quality of life and survival of these patients, when used at a fixed daily dose for a prolonged time.

1. Brennan MF, MacDonald JS. Carcinoid tumours. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer, Principles and Practice of Oncology*. Philadelphia, JB Lippincott Company, 1985, 1223–1228.
2. Moertel CG. Treatment of the carcinoid tumour and the malignant carcinoid syndrome. *J Clin Oncol* 1983, **1**, 727–740.
3. Herbsman H, Wetstein L, Rosen Y, *et al.* Tumours of the small intestine. *Curr Probl Surg* 1980, **17**, 121–182.
4. Mengel HE, Shaffer RD. The carcinoid syndrome. In: Holland JF, Frei E, eds. *Cancer Medicine*, Philadelphia, Lea & Febiger, 1973, 1584–1594.
5. Norheim I, Öberg K, Theodorsson-Norheim E, *et al.* Malignant carcinoid tumour, an analysis of 103 patients with regard to tumour localization, hormone production and survival. *Ann Surgery* 1987, **206**, 115–125.
6. Moertel CG. Sequential hepatic artery occlusion and chemotherapy for metastatic carcinoid tumour and islet cell carcinoma. *Proc Am Soc Clin Oncol* 1985, **4**, 80.
7. Engstrom PF, Lavin PT, Moertel CG, Polsch E, Douglas Jr HO. Streptozocin plus fluorouracil versus doxorubicin therapy for metastatic carcinoid tumour. *J Clin Oncol* 1984, **2**, 1255–1259.
8. Kvols LK, Buck M. Chemotherapy of metastatic carcinoid and islet cell tumours. *Am J Med* 1987, **82**, 77–83.
9. Moertel CG. An Odyssey in the land of small tumours. *J Clin Oncol* 1987, **5**, 1503–1522.
10. Kvols LK, Moertel CG, O'Connell MJ, Schutt AJ, Rubin J, Hahn RG. Treatment of the malignant carcinoid syndrome. Evaluation of a long-acting somatostatin analogue. *N Engl J Med* 1986, **315**, 663–666.
11. Öberg K, Funa K, Alm G. Effects of leukocyte interferon on clinical symptoms and hormone levels in patients with mid-gut carcinoid tumours and carcinoid syndrome. *N Engl J Med* 1983, **309**, 129–133.
12. Öberg K, Norheim I, Lind E, *et al.* Treatment of malignant carcinoid tumours with human leukocyte interferon: Long-term results. *Cancer Treat Rep* 1986, **70**, 1297–1304.
13. Miller AB, Hoogstraten B, Stagnet M, *et al.* Reporting results of cancer treatment. *Cancer* 1981, **7**, 201–214.
14. Smith DB, Scarffe JH, Wagstaff J, Johnston RJ. Phase II trial of rDNA alpha 2b interferon in patients with malignant carcinoid tumour. *Cancer Treat Rep* 1987, **71**, 1265–1266.
15. Moertel CG, Rubin J, Kvols LK. Therapy of metastatic carcinoid tumour and the malignant carcinoid syndrome with recombinant leucocyte A interferon. *J Clin Oncol* 1989, **7**, 865–868.
16. Välimäki M, Järvinen H, Salmela P,Sane T, Sjöblom SM, Pelkonen R. Is the treatment of metastatic carcinoid tumor with interferon not as successful as suggested? *Cancer* 1991, **67**, 547–549.
17. Kvols LK. Therapeutic considerations for the malignant carcinoid syndrome. *Acta Oncol* 1989, **28**, 433–438.
18. Vinik AI, Thompson N, Eckhauser F, Moattari AR. Clinical features of carcinoid syndrome and the use of somatostatin analogue in its management. *Acta Oncol* 1989, **28**, 389–402.
19. Vinik AI, Moattari AR. Use of somatostatin analogue in management of carcinoid syndrome. *Dig Dis Sci* 1989, 34 Suppl, 14S–27S.
20. Linkesch M, Kuzmits R, Geyer G. Therapie des metastasierenten karzinoids mit dem somatostatin analogon octreotid und mit rekombinanten interferon alpha 2b. *Wein Klin Wschr* 1989, **101**, 455–457.

Acknowledgements—We thank Mr W.P.M. Breed, Mr A. Dolman, Mrs M.A. ten Haaft, Mr H.A. Lammers, Mr J.H. Schornagel, Mr T.A.W. Splinter for their cooperation in this study and Mrs R. Groot for typing the manuscript. The IFNα_{2b} was kindly provided by Schering-Plough Inc. The data management was supported by the Dutch Cancer Society (CKVO 87/13).