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Interferons Alpha and Gamma Differ in Their Ability to Cause Tumour Stasis and Regression *In Vivo*

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Abstract—*We have studied the antitumour activity of human lymphoblastoid interferon-α [HuIFN-α(N)] and human recombinant interferon-γ (rHuIFN-γ) on 12 early passage (passages 2–7) human tumour xenografts derived from primary malignancies. Systemic daily therapy of established (approx. 0.5 cm diameter) subcutaneous xenografts with HuIFN-α(N) resulted in significant tumour stasis, and occasionally regression, in nine of 12 breast, bowel, and ovarian cancers studied. A significant decrease in tumour mitotic index was seen in three HuIFN-α(N) sensitive breast tumours. In contrast, none of nine of the same tumours responded significantly to rHuIFN-γ therapy. Direct administration of rHuIFN-γ into the tumour did not improve its therapeutic efficacy. However, when tumour cells from xenografts were dissociated and grown as colonies in soft agar, both IFNs, used at doses that are found circulating* in vivo *after therapy, inhibited colony development in three of three lines tested. Combinations of rHuIFN-γ and HuIFN-α(N) had no synergistic benefit in four of four xenografts studied.*

INTRODUCTION

THE ANTITUMOUR ACTIVITY of interferon-α (IFN-α), predicted from murine tumour models and small studies in human cancer, has been confirmed in extensive clinical trials over the past 10 years [1, 2]. However, responses have mainly been seen in haematologic malignancies and no clinical benefit has been observed in the common solid tumours such as breast, bowel, and lung. It may be that the growth of such tumours cannot be regulated by IFN-α, that insufficient IFN-α reaches the tumour site, or that therapy has not been carried out for a sufficient time or with a sufficient dose. In the light of these possibilities we have been studying the response of human tumour xenografts derived from common solid tumours to human IFN-α, and also to IFN-γ, an IFN that is currently undergoing Phase I/II clinical assessment. In previous papers we reported that two human tumour xenografts responded to a range of IFN-αs but not to IFN-γ [3] and that the tumour growth inhibition was due to a direct effect of the IFNs on the human tumour [4]. We have now studied a more representative sample of human tumours at early passage in the nude mice. We report that a majority of tumours will respond to IFN-α but not to IFN-γ when growing subcutaneously in nude mice. However, three of three tumours tested were sensitive to the direct antiproliferative effect of both IFNs when grown in soft agar.

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MATERIALS AND METHODS

Mice

Female nu/nu nude mice of mixed genetic background were bred by A. Sebesteny and J. Menzies (I.C.R.F. breeding unit Clare Hall South Mimms, U.K.) and maintained as described in Ref. [3].

Tumours

All tumours were derived from primary human tumour material which was implanted subcutaneously (s.c.) into nude mice in 1 mm cubes using a Bashford needle. The tumours were maintained only by passage in the nude mouse. The histological diagnosis of the tumours studied is shown below:

GFC	adenocarcinoma of the caecum
GFW	adenocarcinoma of the caecum
GFDe	adenocarcinoma of the caecum
NCM	infiltrating ductal carcinoma of the breast, Grade III
GFL	adenocarcinoma of the caecum
GFS	adenocarcinoma of the colon
GFH	adenocarcinoma of the colon
OS	moderately differentiated serous cystadenocarcinoma of the ovary
EF3	adenocarcinoma of the caecum
NCH	infiltrating ductal carcinoma of the

	breast, Grade III
NCB	infiltrating ductal carcinoma of the breast, Grade II
1068	mucoid carcinoma of the breast.

Tumour therapy

Mice were implanted with tumour as described previously [3] and therapy began when tumours reached an average diameter of about 0.4–0.5 cm (this took 7–21 days depending on the tumour). Mice were divided into groups of four and therapy was given at daily intervals. Control mice were injected with diluent (calcium and magnesium free phosphate buffered saline containing 3 mg/ml bovine serum albumin, Sigma, U.K.). The IFNs in this diluent were stored at −70°C in single dose aliquots. Lymphoblastoid IFN-α, kindly supplied by Dr G. Lewis, Wellcome Research Laboratories, Beckenham, Kent, was derived from Namalwa cells, had a specific activity of 2×10^8 U/mg and was greater than 99% pure. Human recombinant IFN-γ, also greater than 99% pure, had a specific activity of 2×10^7 U/mg and was kindly supplied by Dr A. Galatzka, Biogen, Geneva, Switzerland. All injections were made in volumes of 0.1 or 0.2 ml.

The tumour size index was assessed weekly and calculated as the multiple of the two largest tumour diameters at right angles to one another.

Mitotic index

Mice were given 50 μg colchicine (BDH Ltd, Poole, U.K.) in 0.1 ml water s.c. 2 h before killing to arrest metaphases. Tumours were fixed in formal saline, and 5 μm paraffin wax sections were stained with haematoxylin and eosin. Six sections were taken at random through each block, and the M.I. (%) was obtained from counts of four fields in each section; the total number of cells counted was approx. 7×10^3 per tumour.

Preparation of solid tumour for clonogenic assay

Three tumours were used: 1068, GFH, and GFW. The solid tumour was digested in hyaluronidase/collagenase solution (Sigma, U.K.) [5]. The single cell suspension obtained after filtration was then prepared for a Courtney assay [6] at a concentration of 10^5 cells/ml. For each IFN-α or -γ concentration (10, 10^2, 10^3 U/ml), three tubes were set up. These were fed weekly with fresh medium containing IFN at the appropriate dilution. At 4–5 weeks, colonies were scored as containing less than or more than 50 cells.

RESULTS

Response of established early passage tumours to HuIFN-α(N) and rHuIFN-γ

Mice were treated with 2×10^5 U of HuIFN-α(N) s.c. or rHuIFN-γ intraperitoneally (i.p.) once their tumours reached an approximate diameter of 0.5 cm, and therapy continued for 28 days. These routes of administration resulted in optimal serum levels for the different IFNs with a peak level of 10^4 U IFN-α and 3×10^4 U IFN-γ occuring 8 h after injection [3]. As Fig. 1 shows, nine of 12 xenografts treated with HuIFN-α(N) exhibited a significant tumour stasis (as assessed by Student's *t* test), and in three of these (see GFW, NCM and OS), tumour regression occurred. In the three xenografts that did not show a significant response (GFC, GFH, NCB), the sizes of tumours in the treated groups were generally lower than control tumours but the individual variation between tumour sizes was too great for statistical significance. In three xenografts the s.c. route of administration was compared to intratumoural (i.t.). This did not offer a therapeutic advantage (data not shown).

In nine of the xenograft lines, rHuIFN-γ therapy was also evaluated. None of these nine showed a statistically significant tumour stasis ($P > 0.07$) and only three of the nine mean tumour size indices were lower than control at the end of therapy. IFN-γ given by s.c. or i.t. injection was also ineffective (data not shown).

Although the results shown here are from an early passage of the tumours, the majority were retested at least one more time at a later passage and responses were consistent, with the exception of the tumour GFW, which became less sensitive to HuIFN-α(N) (tumour stasis as opposed to tumour regression) after 10 more passages. The 1068 tumour, which was not treated with rHuIFN-γ at an early passage, was consistently sensitive when tested at later passages causing a tumour stasis.

Effect of IFN therapy on tumour mitotic index

The effects of HuIFN-α(N) therapy on the tumour mitotic index were studied in three breast tumours: NCM, NCH and NCB. As shown in Table 1, 7–8 days therapy with HuIFN-α(N) resulted in a decrease in the tumour mitotic index in two xenografts. Similar results were seen when these xenografts were studied at 14 days (data not shown) and in a third xenograft a significant reduction was seen at the 35 day time point.

Table 1. *Effects of HuIFN-α(N) on xenograft mitotic index*

Tumour	Days of therapy	M.I.% (S.E.M.) Diluent	HuIFN-α(N)	
NCH	8	3.85 (0.23)	3.01 (0.11)	$P < 0.02$
NCM	7	4.14 (0.08)	2.40 (0.18)	$P < 0.001$
NCB	35	3.74 (0.24)	2.12 (0.36)	$P < 0.02$

Results shown here from NCH passage 15 and NCM and NCB passage 8.

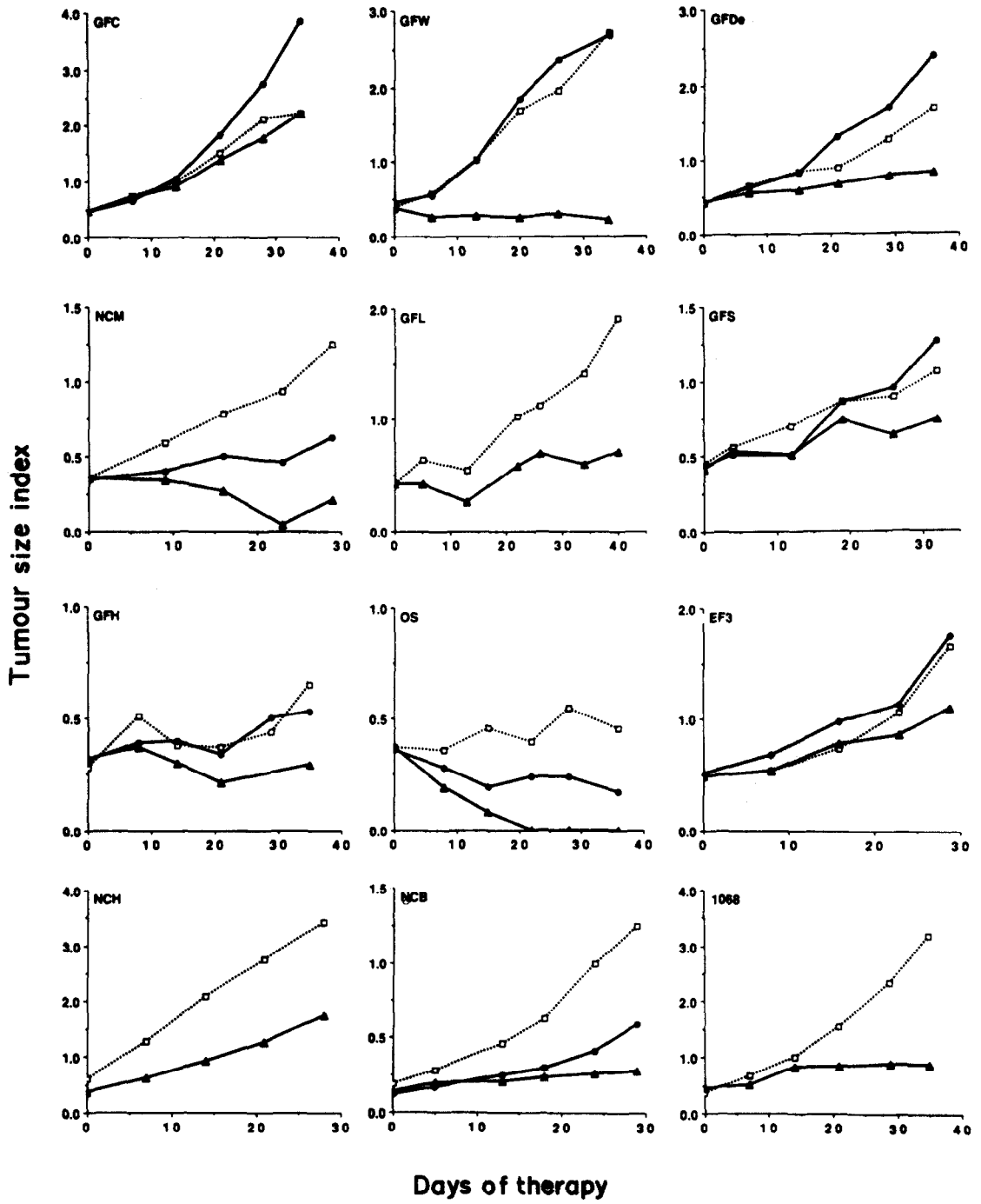

Fig. 1. The effect of human IFNs α and γ on the growth of early passage human tumour xenografts. Mice were treated daily with s.c. HuIFN-α(N), i.p. rHuIFN-γ, or s.c. diluent, after tumours reached 0.4–0.5 cm *average diameter. There were four mice in each treatment group. Differences between mean tumour size indices in control and HuIFN-α(N) treated tumours at the end of therapy were assessed for significance by Student's* t *test as shown below. Differences between control and rHuIFN-γ treated tumours at this time were not significant,* P > 0.07. *GFC passage* 2 P = 0.991, *GFW passage* 3 P = 0.001, *GFDe passage* 6 P = 0.004, *NCM passage* 4 P = 0.001, *GFL passage* 2 P = 0.000, *GFS passage* 6 P = 0.05, *GFH passage* 6 P = 0.147, *OS passage* 4 P = 0.000, *EF3 passage* 7 P = 0.04, *NCH passage* 5 P = 0.03, *NCB passage* 5 P = 0.157, 1068 *passage* 7 P = 0.000. ---□--- *Control;* —▲— *HuIFN-α(N)* 2 × 10^5 *U s.c. daily;* —●— *rHuIFN-γ* 2 × 10^5 *U i.p. daily.*

In vitro *sensitivity to HuIFN-α(N) and rHuIFN-γ*

Tumour cells from three of the xenograft lines, 1068 (mucoid carcinoma of the breast), GFH (adenocarcinoma of the colon), and GFW (adenocarcinoma of the caecum) could be grown *in vitro* as colonies in soft agar. As shown in Fig. 2, rHuIFN-γ and HuIFN-α(N) caused colony inhibition in all three tumours when they were tested at later passages. This colony inhibition occurred at doses 1–3 logs lower than the peak level of these IFNs in the serum of treated mice [3]. The inhibition by the two IFNs was manifest primarily as a significant

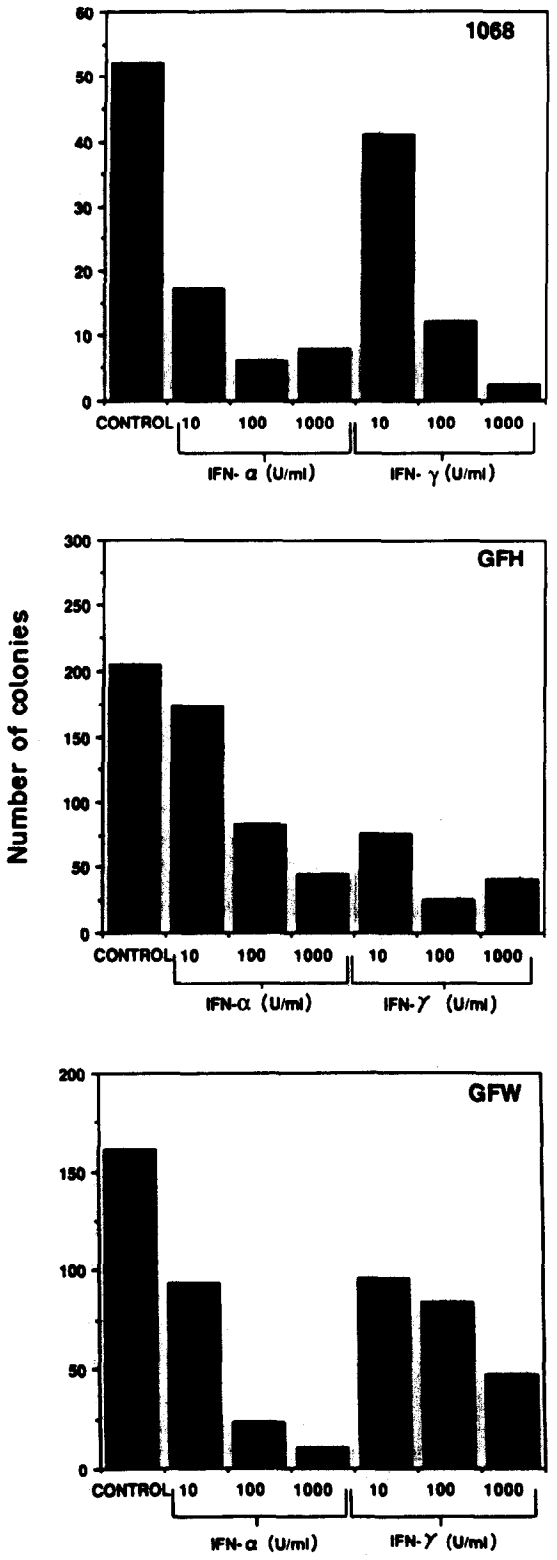

Fig. 2. The effect of human IFNs α and γ on growth of xenograft cells in soft agar. Data shown for colonies of more than 50 cells. Differences between control and HuIFN-α(N) or rHuIFN-γ were significant by Student's t test for all groups (P < 0.008) except for 10 U/ml HuIFN-α(N) and at 10 U/ml rHuIFN-γ in the 1068 tumour.

reduction in colonies containing more than 50 cells. The number of small colonies of 10–50 cells was approximately the same in control and treated cultures.

The efficacy of in vivo combinations of rHuIFN-γ and HuIFN-α(N)

Four of the xenograft lines, 1068, GFC, EF3, and NCH were tested for their response to combinations of HuIFN-α(N) and rHuIFN-γ. This therapy did not result in a significant improvement in tumour response in any of these. Figure 3 shows results from two of the xenografts.

DISCUSSION

In the human tumour xenograft model, HuIFN-α(N) slowed tumour growth of a majority of early passage tumours derived from primary human malignancies, and in three cases caused tumour regression. The response was not immediate but took 2–3 weeks to develop in most cases. The reduction in tumour mitotic index in three tumours provides evidence for a direct antiproliferative effect of the IFN on the tumour and confirms our previous report that human IFNs have no demonstrable effect on the nude mouse host [4].

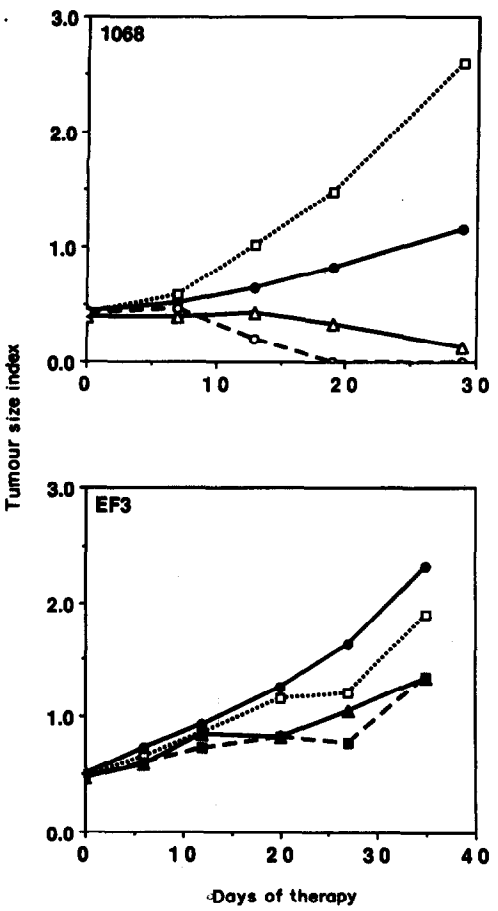

Fig. 3. Combinations of human IFNs α and γ in the xenograft models. In this experiment 1068 was used at passage 46, EF3 at passage 8. Mice were treated daily with s.c. HuIFN-α(N) and/or i.p. HuIFN-γ, or s.c. diluent, when their tumours reached 0.4–0.5 cm average diameter. The differences between the IFNs given singly or in combination were not significant as assessed by Student's t test. ---□--- Control; —▲— HuIFN-α(N) 2 × 10⁵ U daily; ---■--- HuIFN-α(N) 2 × 10⁵ U daily + rHuIFN-γ 2 × 10⁵ U daily; —△— HuIFN-α(N) 2 × 10⁴ U daily; ---○--- HuIFN-α(N) 2 × 10⁴ U daily + rHuIFN-γ 2 × 10⁵ U daily; —●— rHuIFN-γ 2 × 10⁵ U daily.

Thus, in this model system we find that human breast, bowel, and ovarian cancers will 'respond' to IFN-α. However, over the past few years Phase I and II clinical trials with this IFN given systemically have shown very poor response rates in such solid tumours [1, 2]. There are several possible reasons for this discrepancy between response in humans and response in animals.

First, the responses seen in the animal models are primarily tumour static. Such a response would not be recorded positively in clinical trials in patients with advanced cancer. A second reason is that the human IFN-α given in the therapy of these human tumours growing in nude mice is essentially 'targeted'. We have no evidence from previous studies [4] that human IFN-α acts on any mouse tissue or binds to murine IFN-α/β receptors. Thus higher concentrations of IFNs may reach the xenografts than would reach their counterparts in humans. However, the blood supply to these subcutaneous tumours is not extensive. Using radiolabelled red cells we have found that the blood flow in subcutaneous tumours is essentially the same as mouse skin (unpublished data) and much lower than several other organs. A third reason relates to dose. The dose of human IFN-α that we have used in these studies would be equivalent to a dose of 20×10^6 U in humans if corrections are made to take into account different surface area to volume ratios between mouse and man [7]. In addition, the peak serum levels measured here were higher than those achieved by that dose in man. This may be because the human IFN failed to bind to ubiquitous murine IFN-α receptors. A human equivalent dose of 20×10^6 U, or more, is higher than the maintenance dose of $1–5 \times 10^6$ U given in long-term studies in the haematologic malignancies and some Phase I/II studies in solid tumours, but is a dose that has been used in clinical trials with Kaposi's sarcoma patients.

rHuIFN-γ was much less effective in the model than HuIFN-α(N) and the final tumour size index at the end of therapy was lower than control treated animals in only three of the nine different tumours treated. In none of these instances was the reduction statistically significant. The response could not be improved by local therapy, but the fact that two of the tumours unresponsive *in vivo* responded *in vitro* suggests that the lack of response may be due to inadequate quantities of IFN-γ reaching the tumour at the s.c. site. However, it is important to remember that the *in vitro* treatment with IFN-γ was carried out on small numbers of cells which may be more sensitive to IFN-γ than established tumours. We have evidence that low levels of rHuIFN-γ reach the tumours when they grow subcutaneously because previous studies have shown that tumour cell Class II MHC can be induced or enhanced by rHuIFN-γ given i.p. [8].

If the xenografts are grown at other sites, IFN-γ has some therapeutic activity. I.p. therapy of ovarian cancer xenografts growing i.p. resulted in a significant increase in lifespan of mice in two of three tumours tested [9]. Moreover, the development of experimental lung metastases of a human melanoma cell line in nude mice was also inhibited [10].

Human solid tumours have shown few, if any, responses to rHuIFN-γ in Phase I/II clinical trials [11]. However, IFN-γ is a more powerful immunomodulator than IFN-α and may be more useful when used in a way that optimizes its capacity for altering host responses. The nude mouse model cannot be used to investigate such immunomodulatory activity.

Combinations of IFNs α and γ have been reported to have synergistic antiproliferative and antiviral activity in a number of systems [12, 13] and a Phase I clinical trial of this combination has recently been reported [14]. In our model the addition of IFN-γ to the IFN-α therapy did not improve efficacy at the dosage regimes employed.

Thus a majority of human tumours were growth inhibited by HuIFN-α(N) in the xenograft model system. While a recent review of the response of several hundred xenografts to chemotherapy concluded that xenograft models overestimated the activity of most standard drugs as single agents, drugs that ranked high in xenograft response usually showed activity in the clinic [15]. When judged against results with chemotherapy, HuIFN-α(N) would be in the high activity category. Now that the clinical response to IFN-α after long-term administration in haematologic malignancies is well established, experimental studies such as those shown here may provide some rationale for further studies in solid tumours, particularly long-term therapy in patients with a small volume of disease, or as maintenance in patients in remission. Previous administration of IFN-α therapy in solid tumours has generally been of short duration. This study also suggests that novel IFNs or other peptide growth inhibitory molecules with greater antiproliferative activity and reduced toxicity may have clinical potential in the management of solid tumours.

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