Acta Medica Okayama

Antitumor effect of combined intraperitoneal administration of human recombinant interferon-beta and interferon-gamma against intraabdominal carcinomatosis in nude mice.

Hidenori Kondo*   Noriaki Tanaka[†]

Yoshio Naomoto[‡]   Kunzo Orita**

*Okayama University,
[†]Okayama University,
[‡]Okayama University,
**Okayama University,

Antitumor effect of combined intraperitoneal administration of human recombinant interferon-beta and interferon-gamma against intraabdominal carcinomatosis in nude mice.*

Hidenori Kondo, Noriaki Tanaka, Yoshio Naomoto, and Kunzo Orita

Abstract

The development of useful therapy for intraabdominal carcinomatosis originating from gastrointestinal cancer is an important theme in cancer therapy. We developed recently an experimental model of intraabdominal carcinomatosis in nude mice by intraperitoneal transplantation of human colon cancer cells (RPMI 4788). Using this model, we investigated the antitumor effects of recombinant human interferon (rIFN)-beta and rIFN-gamma administered singly or in combination. Treatment was initiated 2 days after CD-1 nude mice were inoculated intraperitoneally with 5 X 10(6) RPMI 4788 cells. Intraperitoneal administration for 10 consecutive days of either rIFN-beta (2.5 X 10(5) IU/mouse/day) or rIFN-gamma (2.5 X 10(5) JRU/mouse/day) resulted in a significant prolongation of survival compared with the saline control group [survival in the control: 41.8 +/- 5.6 days (mean +/- SD)]. Combined administration of rIFN-beta and rIFN-gamma for 10 days yielded a marked synergistic effect on the prolongation of survival (114.0 +/- 8.2 days). However, combined administration of rIFN-beta and rIFN-gamma in a single dose equal to the total dose given fractionally over 10 days did not yield a synergistic effect. These results suggest that daily administration of rIFN-beta and rIFN-gamma combined may provide a highly potent antitumor effect against human peritoneal carcinomatosis.

KEYWORDS: antitumor effect, human recombinant interferon, synergistic effect, intrabdominal carcinomatosis, mude mice

---

Antitumor Effect of Combined Intraperitoneal Administration of Human Recombinant Interferon-β and Interferon-γ against Intraabdominal Carcinomatosis in Nude Mice

Hidenori Kondo*, Noriaki Tanaka, Yoshio Naomoto and Kunzo Orita

First Department of Surgery, Okayama University Medical School, Okayama 700, Japan

The development of useful therapy for intraabdominal carcinomatosis originating from gastrointestinal cancer is an important theme in cancer therapy. We developed recently an experimental model of intraabdominal carcinomatosis in nude mice by intraperitoneal transplantation of human colon cancer cells (RPMI 4788). Using this model, we investigated the antitumor effects of recombinant human interferon (rIFN)-β and rIFN-γ administered singly or in combination. Treatment was initiated 2 days after CD-1 nude mice were inoculated intraperitoneally with 5×10^6 RPMI 4788 cells. Intraperitoneal administration for 10 consecutive days of either rIFN-β (2.5×10^5 IU/mouse/day) or rIFN-γ (2.5×10^5 JRU/mouse/day) resulted in a significant prolongation of survival compared with the saline control group [survival in the control: 41.8 ± 5.6 days (mean $\pm$ SD)]. Combined administration of rIFN-β and rIFN-γ for 10 days yielded a marked synergistic effect on the prolongation of survival (114.0 ± 8.2 days). However, combined administration of rIFN-β and rIFN-γ in a single dose equal to the total dose given fractionally over 10 days did not yield a synergistic effect. These results suggest that daily administration of rIFN-β and rIFN-γ combined may provide a highly potent antitumor effect against human peritoneal carcinomatosis.

Key words : antitumor effect, human recombinant interferon, synergistic effect, intraabdominal carcinomatosis, nude mice

Interferons (IFNs) are classified into three major classes based on their antigenic and physicochemical properties : the leukocyte-derived IFN-α, fibroblast-derived IFN-β, and T-lymphocyte-produced IFN-γ. IFNs have antiviral, antitumor, and immunomodulating activities (1, 2). The cloning of IFN genes has led to a number of forms of recombinant human IFN (rIFN)-α as well as rIFN-β and rIFN-γ (3-5). rIFNs are now available in preparations of greater than 95% purity and can be produced in quanties large enough for clinical trials. rIFNs were expected to have antitumor effects, but efficacy has been limited to only certain malignant tumors when the rIFNs were administered systemically (6). Hence, local administration of IFNs has been gaining

---

* To whom correspondence should be addressed.

attention as a new approach to IFN therapy (6). Furthermore, many authors have reported the synergistic effect of combined IFN therapy, particularly the combinations of IFN-α and IFN-γ, or IFN-β and IFN-γ (7-15).

At the present time, the prognosis of peritoneal carcinomatosis from gastrointestinal cancer is poor, and a definitive therapy has not yet been developed. In addition, peritoneal recurrence is the most common form of post-operative recurrence of gastrointestinal cancer (16). Therefore, the development of a prophylaxis and treatment for peritoneal carcinomatosis is greatly awaited.

Recently, we developed an experimental model of intraabdominal carcinomatosis using human colon cancer cells in nude mice (17). In the present study, we examined the antitumor effect of intraperitoneal administration of rIFN-β and rIFN-γ in combination using this *in vivo* nude mouse model in order to study new strategies of IFN therapy.

Materials and Methods

Tumor cells. RPMI 4788 cells derived from a human colon cancer were kindly supplied by Roswell Park Memorial Institute (Buffalo, N.Y.). The tumor cells were maintained in RPMI 1640 medium (Nissui Pharmacological Co., Tokyo, Japan) supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, N.Y.) at 37°C in a humidified 5% CO_2 atmosphere. The characteristics of this tumor cell line have been described in detail previously (18). Cells in the exponentially proliferating phase were harvested for the experiments. Histological examination showed that the tumors transplanted subcutaneously into nude mice were poorly differentiated adenocarcinomas.

Animals. Six- to 7-week-old CD-1 (ICR) nu/nu male nude mice were obtained from Charles River Japan, Inc., Kawasaki, Japan. Throughout the experiment, the mice were maintained under specific pathogen-free conditions using laminar flow

racks and were fed sterile food and water in our experimental animal center.

Interferons. Recombinant human interferon-β (rIFN-β) produced by *Escherichia coli* (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) had a purity of over 99.5% and a specific activity of over 5×10^7 international units (IU)/mg protein (19). Recombinant human interferon-γ (rIFN-γ) produced by *E. coli* (Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan) had a purity of over 99% and a specific activity of over 5×10^6 Japanese reference units (JRU)/mg protein (20). The titer of rIFN-β was expressed as IU/ml of reference IFN-β (J-Ref-02 ; National Institute of Health, Tokyo), which had been established based on the IU of WHO IFN-β reference (G 023-902-527). The titer of rIFN-γ was expressed as JRU/ml of Japanese rIFN-γ reference (J-Rr-40101 ; National Institute of Health), which had been established based on the IU of WHO IFN-γ reference (Gg 23-901-530). rIFNs were dissolved in physiological saline for the experiments.

Establishment of intraabdominal carcinomatosis with ascites in nude mice. The details of this nude mouse model have been described previously (17). Briefly, RPMI 4788 cells were harvested with a solution of 0.25% trypsin in PBS and suspended in PBS. CD-1 nude mice were inoculated intraperitoneally with 5×10^6 viable RPMI 4788 cells/mouse in 0.5 ml of PBS using a 27-gauge needle (day 0). Peritoneal dissemination of tumor cells progressed in the abdominal cavity of the nude mice after the inoculation. All nude mice had ascites accumulation, and died of cachexia and remarkable retention of ascites in a relatively short time. In the abdominal cavity, bloody ascites and peritoneal dissemination of tumor on the greater omentum, mesocolon and diaphragma were common findings. Histological examination of the peritoneal tumors showed poorly differentiated adenocarcinomas.

Experimental schedule.
Experiment 1. From day 2 through day 11, mice were given daily intraperitoneal injections (0.2 ml/mouse) of rIFN-β (2.5×10^5 IU/mouse/day) and rIFN-γ (2.5×10^5 JRU/mouse/day), singly or in combination. Saline (0.2 ml/mouse) in place of rIFNs was injected from day 2 for 10 consecutive days into mice of one control group (negative control group). Mitomycin C (MMC, Kyowa Hakko

Kogyo Ltd., Tokyo, Japan) was administered at a dose of 2 mg/kg/day on day 2 to mice of another control group (positive control group).

Experiment 2. From day 2 through day 11, mice were given daily intraperitoneal injections (0.2 ml/mouse) of rIFN-β (5.0×10^4 IU/mouse/day) and rIFN-γ (5.0×10^4 JRU/mouse/day), singly or in combination. Saline (0.2 ml/mouse) was injected from day 2 for 10 consecutive days in the control group.

Experiment 3. On day 2, mice were given a single intraperitoneal injection (0.2 ml/mouse) of rIFN-β (2.5×10^6 IU/mouse/day) and rIFN-γ (2.5×10^6 JRU/mouse/day), singly or in combination. Saline (0.2 ml/mouse) was injected on day 2 in the control group.

Statistical analysis. In all experiments, five mice were included in each treatment group. The survival time of the mice was observed for 120 days after the treatment. The significance of differences in the mean survival time between groups was determined by the Mann-Whitney U test.

Results

Antitumor effect of daily intraperitoneal administration of rIFN-β and rIFN-γ singly

or in combination. Because rIFN-β and rIFN-γ exhibited direct antiproliferative activity against RPMI 4788 cells *in vitro* in a dose-dependent manner (21), we designed studies to determine whether they were also active *in vivo* against the same tumor cells.

As shown in Fig. 1-(A) and Table 1, rIFN-β and rIFN-γ significantly prolonged the survival time when administered individually (Experiment 1). The mean survival time was 87.0 ± 33.8 days (mean $\pm$ SD, $p < 0.01$ vs control) in the rIFN-β group and 72.2 ± 23.6 days ($p < 0.05$ vs control) in the rIFN-γ group, while it was 41.8 ± 5.6 days in the saline control group. The survival time in the MMC group (positive control) was 95.6 ± 33.9 days ($p < 0.01$). In the combined treatment group, the survival time was 114.0 ± 8.2 days ($p < 0.01$ vs control).

As shown in Fig. 1-(B) and Table 1, daily administration of rIFN-β and rIFN-γ individually at a dose one-fifth of that in Experiment 1 did not result in significantly prolonged survival (rIFN-β, 62.4 ± 9.8 days; rIFN-γ, 58.6 ± 12.2 days) compared

Table 1 Antitumor effect of rIFN-β and rIFN-γ, singly or in combination, against experimental intraabdominal carcinomatosis in nude mice

Treatment	Mean survival time (days)	
	Mean $\pm$ SD[c]	Range
Experiment 1[a]		
Control (saline)	41.8 ± 5.6[d]	$36 - 49$
rIFN-β	87.0 ± 33.8[e]	$50 - > 120$
rIFN-γ	72.2 ± 23.6[f]	$46 - 109$
rIFN-β + rIFN-γ	114.0 ± 8.2[g]	$104 - > 120$
MMC	95.6 ± 33.9[h]	$51 - > 120$
Experiment 2[b]		
Control (saline)	47.8 ± 11.9[i]	$36 - 66$
rIFN-β	62.4 ± 9.8[j]	$49 - 75$
rIFN-γ	58.6 ± 12.2[k]	$45 - 71$
rIFN-β + rIFN-γ	71.1 ± 14.5[l]	$54 - 88$

a: The doses of recombinant human interferons, rIFN-β and rIFN-γ, used in this experiment was 2.5×10^5 IU or JRU/mouse/day. Details of the experimental conditions are described under Materials and Methods.
b: The daily dose of rIFNs administered was one-fifth of that in Experiment 1. Details of the experimental conditions are described in Materials and Methods.
c: Statistical significance of differences (Mann-Whitney U test) d vs e, g, h: $p < 0.01$; d vs f: $p < 0.05$; f vs g: $p < 0.05$; i vs j, k: not significant; i vs l: $p < 0.05$; j, k vs l: not significant.

72   Kondo *et al.*

Fig. 1   Survival curves of nude mice with experimental intraabdominal carcinomatosis following intraperitoneal administration of rIFN-β and rIFN-γ, singly or in combination. Mice were inoculated intraperitoneally (i. p.) with 5×10^6 RPMI 4788 cells/mouse (day 0). Each group included 5 mice. Treatment was initiated from day 2.
(A) Experiment 1 : rIFN-β (2.5×10^5 IU/mouse/day) and rIFN-γ (2.5×10^5 JRU/mouse/day) were administered i. p., singly or in combination, for 10 consecutive days from day 2. Control (saline) ($\bullet$), rIFN-β ($\triangle$), rIFN-γ ($\square$), rIFN-β+rIFN-γ ($\bigcirc$). MMC (2 mg/kg/day) ($\blacktriangledown$) was injected i. p. on day 2.
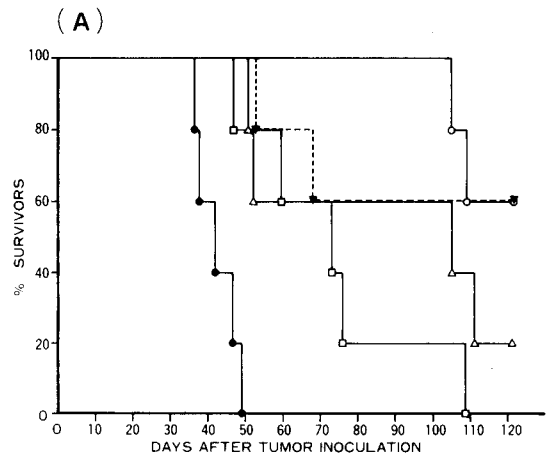
(B) Experiment 2 : rIFN-β (5.0×10^4 IU/mouse/day) and rIFN-γ (5.0×10^4 JRU/mouse/day) were administered i. p., singly or in combination, for 10 consecutive days from day 2. Control (saline) ($\bullet$), rIFN-β ($\triangle$), rIFN-γ ($\square$), rIFN-β+rIFN-γ ($\bigcirc$).
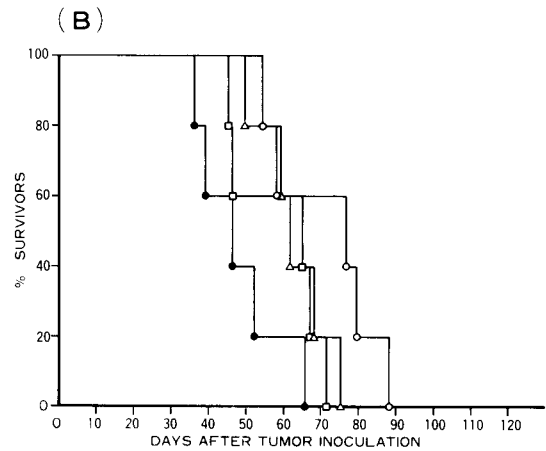
(C) Experiment 3 : rIFN-β (2.5×10^6 IU/mouse/day) and rIFN-γ (2.5×10^6 JRU/mouse/day) were administered i. p., singly or in combination, on day 2. Control (saline) ($\bullet$), rIFN-β ($\triangle$), rIFN-γ ($\square$), rIFN-β+rIFN-γ ($\bigcirc$).
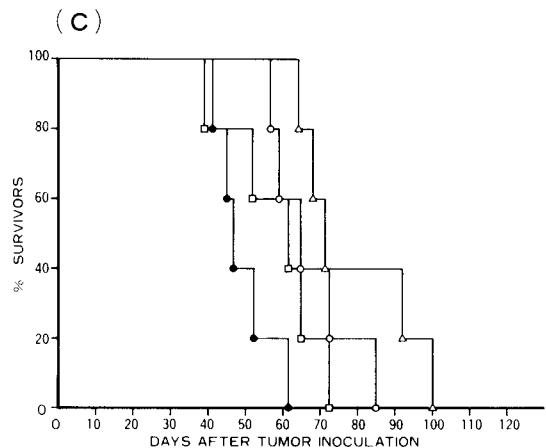
with the control group (47.8±11.9 days) (Experiment 2). However, daily combined administration of rIFN-β and rIFN-γ yielded a significant antitumor effect (71.0±14.5 days, p < 0.05) compared with the control group.

Antitumor effect of one intraperitoneal administration of rIFN-β and rIFN-γ singly or in combination (Experiment 3). The antitumor effect of a single dose of rIFN-β and rIFN-γ, which equaled the total dose used in Experiment 1, was examined. As shown in Fig. 1-(C) and Table 2, a single administration of rIFN-β alone resulted in significantly prolonged survival (mean survival time: 79.0±16.0 days, p < 0.01 vs control), while rIFN-γ alone (58.2±13.1 days) did not yield a significant antitumor effect compared with the control group (49.2±8.2 days). A single administration of rIFN-β and rIFN-γ in combination (67.4±11.5 days, p < 0.05 vs control) at the same total dose used in Experiment 1, did not afford a synergistic effect.

Table 2 Antitumor effect of a single administration of rIFN-β and rIFN-γ, individually or in combination, against experimental intraabdominal carcinomatosis in nude mice

Treatment[a]	Mean survival time (days)	
	Mean±SD[b]	Range
Control (saline)	49.2± 8.2[c]	41−62
rIFN-β	79.0±16.0[d]	64−100
rIFN-γ	58.2±13.1[e]	39−73
rIFN-β+rIFN-γ	67.4±11.5[f]	57−85

a: Experimental conditions are described under Materials and Methods (Experiment 3).
b: Statistical significance of differences (Mann-Whitney U test) c vs d: p < 0.01 ; c vs e: not significant; c vs f: p < 0.05 ; d, e vs f: not significant.

Discussion

Intraperitoneal administration of IFNs is a form of intralesional administration which is hoped to give a good antitumor effect against malignant ascites. In this study, we demonstrated that: 1) rIFN-β and rIFN-γ had dose-dependent antitumor activities *in vivo*. 2) Daily administration of either rIFN-β or rIFN-γ was more effective than a single administration of either rIFN at the same total dose. 3) Daily administration of rIFN-β and rIFN-γ in combination yielded synergistic (Experiment 1) or additive (Experiment 2) antitumor activity, while a single administration of the rIFNs in combination did not yield a synergistic effect. Kitahara *et al.* (22) reported that therapy with a single, large dose of IFNs was not effective against nude mouse-transplantable human tumors (ascites form), while long-term, multiple dose therapy (same total dose) was very effective. In our experiments, daily administration of either rIFN-β or rIFN-γ, provided a more potent antitumor effect than a single administration of either rIFN, although a single administration of rIFN-β provided a significant antitumor effect compared with the control. This difference is considered to be due to differences in the number of inoculated tumor cells and the sensitivity of the tumor cells *in vivo* to rIFN-β.

A synergistic increase in the antitumor effect of IFNs when administered in combination, particularly IFN-γ with IFN-α or IFN-β, has been reported by several authors. Fleischmann *et al.* (7, 8) showed that partially purified murine IFN-γ potentiated the antitumor effects of murine IFN-β both *in vivo* and *in vitro*. In the *in vivo* studies, IFN-γ (25 U/day), which alone had no effect on the growth of P388 lymphocytic leukemia tumors, significantly enhanced the growth-inhibitory capacity of IFN-β (7). Similar synergism between murine IFN-γ and murine IFN-α or IFN-β was reported in *in vitro* antiproliferative assays (8). De Clercq *et al.* (9) reported synergism in the antitumor effects of partially purified murine

IFN-β and IFN-γ against L1210 leukemia cells. These earlier studies, however, were performed with partially purified IFN preparations known to be contaminated with lymphokines which could potentiate the IFN activities. The synergism between IFNs has been confirmed with pure recombinant IFNs. Czarniecki *et al.* (11) and Denz *et al.* (12) reported that combining rIFN-γ with rIFN-α or rIFN-β resulted in synergistic potentiation of activity *in vitro*. Recently, Nosoh *et al.* (15) reported synergism between rIFN-α and rIFN-γ against human tumor xenografts transplanted subcutaneously in nude mice. To our knowledge, the present study is the first to demonstrate efficacy of intraperitoneal administration of rIFN-β and rIFN-γ in combination against intraabdominal carcinomatosis in nude mice. In this study, IFN-β was selected because pharmacokinetic experiments indicated that, compared with IFN-α, it tends to remain in tissues where it is inoculated (23).

In the experimental model of intraabdominal carcinomatosis developed by the authors, intraperitoneal inoculation of a set number of tumor cells causes death by tumor in mice in a relatively short time, thereby allowing evaluation of antitumor efficacy of the drug from prolongation of mouse survival time. Moreover, as tumor cells derived from humans are used, it is an ideal model for studying the direct antitumor effect of human IFNs, which have high species-specificity, and for obtaining results which closely approximate actual clinical results.

Two mechanisms are thought to participate in the antitumor activity of IFN. One of these is direct antiproliferative activity against tumor cells, while the other is activation of NK cells or macrophages which act on the immune system, hence leading to lysis of the tumor cells. In the present *in vivo* assay system, the IFNs were considered to have direct antitumor activity and

not to have indirect activity via mouse immune systems. However, when used in a human system, action of IFNs via the host defense mechanism must be considered in addition to direct antitumor activity, as indicated by Berk *et al.* (24) and Rambaldi *et al.* (25), who found that intraperitoneal administration of IFN-α and IFN-β activates NK cells in malignant ascites. Saito *et al.* (26) characterized the antiproliferative supernatant from ascites-associated rIFN-γ-treated macrophages and indicated the possibility that a component of the supernatant responsible for antiproliferative activity has antigenic determinants similar to those of tumor necrosis factor (TNF).

In conclusion, our results suggest that daily, combined administration of rIFN-β and rIFN-γ may provide highly potent antitumor activity against peritoneal carcinomatosis originating from human gastrointestinal cancer. In clinical practice, this method of administration could be applied not only as therapy for terminal peritoneal carcinomatosis, but also as postoperative adjuvant therapy for cases of gastrointestinal cancer positive for Douglas cul-de-sac cells. In addition, IFN has few side effects and can thus be used repeatedly over long periods compared with conventional anticancer agents.

References

1. Friedman RM and Vogel SN: Interferons with special emphasis on the immune system. Adv Immunol (1983) **34**, 97-140.
2. Kirchner H: Interferons, a group of multiple lymphokines. Springer Semin Immunopathol (1984) **7**, 347-374.
3. Taniguchi T, Ohno S, Fujii-Kuriyama Y and Muramatsu M: The nucleotide sequence of human fibroblast interferon cDNA. Gene (1980) **10**, 11-15.

4. Taniguchi T, Guarente L, Roberts TM, Kimelman D, Douhan J III and Ptashne M: Expression of the human fibroblast interferon gene in *Escherichia coli*. Proc Natl Acad Sci USA (1980) **77**, 5230-5233.

5. Taniguchi T, Pang RHL, Yip YK, Henriksen D and Vilcek J: Partial characterization of γ (immune) interferon mRNA extracted from human lymphocytes. Proc Natl Acad Sci USA (1981) **78**, 3469-3472.

6. Goldstein D and Laszlo J: Interferon therapy in cancer: from imaginon to interferon. Cancer Res (1986) **46**, 4315-4329.

7. Fleischmann WR Jr, Kleyn KM and Baron S: Potentiation of antitumor effect of virus-induced interferon by mouse immune interferon preparations. J Natl Cancer Inst (1980) **65**, 963-966.

8. Fleischmann WR Jr: Potentiation of the direct anticellular activity of mouse interferons: mutual synergism and interferon concentration dependence. Cancer Res (1982) **42**, 869-875.

9. de Clercq E, Zhang Z and Huygen K: Synergism in the antitumor effects of type I and type II interferon in mice inoculated with leukemia L1210 cells. Cancer Lett (1982) **15**, 223-228.

10. Ratliff TL, Kadmon D, Shapiro A, Jacobs AJ and Heston WDW: Inhibition of mouse bladder tumor proliferation by murine interferon-γ and its synergism with interferon-β. Cancer Res (1984) **44**, 4377-4381.

11. Czarniecki CW, Fennie CW, Powers DB and Estell DA: Synergistic antiviral and antiproliferative activities of *Escherichia coli*-derived human alpha, beta, and gamma interferons. J Virol (1984) **49**, 490-496.

12. Denz H, Lechleitner M, Marth Ch, Daxenbichler G, Gastl G and Braunsteiner H: Effect of human recombinant alpha-2- and gamma-interferon on the growth of human cell lines from solid tumors and hematologic malignancies. J Interferon Res (1985) **5**, 147-157.

13. Oleszak E and Stewart WE II: Potentiation of the antiviral and anticellular activities of interferons by mixtures of HuIFN-γ and HuIFN-α or HuIFN-β. J Interferon Res (1985) **5**, 361-371.

14. Brunda MJ and Wright RB: Differential antiproliferative effects of combinations of recombinant interferons alpha and gamma on two murine tumor cell lines. Int J Cancer (1986) **37**, 287-291.

15. Nosoh Y, Toge T, Niimi K, Nishiyama M, Hirabayashi N, Nakanishi K, Niimoto M and Hattori T: Experimental studies on the combined effects of alpha and gamma interferons against human tumor xenografts transplanted into nude mice. Jpn J Surg (1987) **17**, 168-173.

16. Kusama S: Clinical pathology and natural history of metastases to the peritoneum. Gastroenterol Surg (1983) **6**, 1167-1173 (in Japanese).

17. Naomoto Y, Kondo H, Tanaka N and Orita K: Novel experimental models of human cancer metastasis in nude mice; lung metastasis, intraabdominal carcinomatosis with ascites and liver metastasis. J Cancer Res Clin Oncol (1987) **113**, 544-549.

18. Moore GE and Koike A: Growth of human tumor cells *in vitro* and *in vivo*. Cancer (1964) **17**, 11-20.

19. Gomi K, Morimoto M and Nakamizo N: Growth-inhibitory activity of recombinant human interferon-β against cultured human cells. Gann (1983) **74**, 737-742.

20. Gomi K, Morimoto M and Nakamizo N: Characteristics of antiviral and anticellular activities of human recombinant interferon-γ. Jpn J Cancer Res (Gann) (1985) **76**, 224-234.

21. Kondo H, Tanaka N, Naomoto Y and Orita K: Combined effect of human recombinant interferon α, β and γ on tumor cell growth and cell cycle *in vitro*. Jpn J Cancer Chemother (1987) **14**, 1234-1239 (in Japanese).

22. Kitahara T, Minato K and Shimoyama M: Antitumor activity of human fibroblast interferon (HuIFN-β) and human lymphoblastoid interferon (HuIFN-α) on the transplanted human tumors in nude mice; in Interferon, Kishida ed, Japan Convention Services, Inc., Osaka (1984) pp. 132-137.

23. Hanley DF, Wiranowska-Stewart M and Stewart WE II: Pharmacology of interferons 1. Pharmacologic distributions between human leukocyte and fibroblast interferons. Int J Immunopharmacol (1979) **1**, 219-226.

24. Berek JS, Hacker NF, Lichtenstein A, Jung T, Spina C, Knox RM, Brady J, Greene T, Ettinger LM, Lagasse LD, Bonnem EM, Spiegel RJ and Zighelboim J: Intraperitoneal recombinant α-interferon for "salvage" immunotherapy in stage III epithelial ovarian cancer: A gynecologic oncology group study. Cancer Res (1985) **45**, 4447-4453.

25. Rambaldi A, Introna M, Colotta F, Landolfo S, Colombo N, Mangioni C and Mantovani A: Intraperitoneal administration of interferon β in ovarian cancer patients. Cancer (1985) **56**, 294-301.

26. Saito T, Berens ME and Welander EC: Characterization of the indirect antitumor effect of γ-interferon using ascites-associated macrophages in a human tumor clonogenic assay. Cancer Res (1987) **47**, 673-679.