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## Abstract

Synergistic enhancement of anti-tumor effects through the combined use of natural human interferon-alpha (nHuIFN-alpha) and natural human tumor necrosis factor-alpha (nHuTNF-alpha) enabled us to decrease the effective dose of each cytokine and consequently to reduce side effects. One hundred and twenty patients with advanced or recurrent solid cancer were entered in the trial from April 1985 to January 1988, of whom 112 patients were evaluable. A mixture of nHuINF-alpha and nHuTNF-alpha was injected intravenously as the maintenance dose 1 x 10(6)U or more/day for over 8 weeks. There was no response in 40 patients injected with the maintenance dose of 1 x 10(6)U/day, but of 72 patients receiving more than 2 x 10(6)U/day (10 micrograms of nHuIFN-alpha and 3 micrograms of nHuTNF-alpha), 4 had complete responses, 10 had partial responses, and 4 had minor responses. The overall response rate was 12.5% (14/112) and the rate was 19.5% in 72 patients with more than 2 x 10(6)U/day. Positive responses were as follows: hepatoma 3/8), renal cell cancer (4/11), breast cancer (4/17), ovarian cancer (1/2), malignant thymoma (1/1) and liposarcoma (1/1). Serious adverse effects like hypotension, oliguria and severe hepatobiliary toxicity were never experienced. The effective and adequate dose of the mixed preparation was considered 2 to 4 x 10(6)U/day/body.

**KEYWORDS:** interferon-?, tumor necrosis factor-?, cancer, combination therapy, early phase ?

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# Early Phase II Study of Interferon-$\alpha$ and Tumor Necrosis Factor-$\alpha$ Combination in Patients with Advanced Cancer

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**Synergistic enhancement of anti-tumor effects through the combined use of natural human interferon-$\alpha$ (nHuIFN-$\alpha$) and natural human tumor necrosis factor-$\alpha$ (nHuTNF-$\alpha$) enabled us to decrease the effective dose of each cytokine and consequently to reduce side effects. One hundred and twenty patients with advanced or recurrent solid cancer were entered in the trial from April 1985 to January 1988, of whom 112 patients were evaluable. A mixture of nHuINF-$\alpha$ and nHuTNF-$\alpha$ was injected intravenously as the maintenance dose $1 \times 10^6$ U or more/day for over 8 weeks. There was no response in 40 patients injected with the maintenance dose of $1 \times 10^6$ U/day, but of 72 patients receiving more than $2 \times 10^6$ U/day (10 $\mu$g of nHuIFN-$\alpha$ and 3 $\mu$g of nHuTNF-$\alpha$), 4 had complete responses, 10 had partial responses, and 4 had minor responses. The overall response rate was 12.5% (14/112) and the rate was 19.5% in 72 patients with more than $2 \times 10^6$ U/day. Positive responses were as follows: hepatoma 3/8), renal cell cancer (4/11), breast cancer (4/17), ovarian cancer (1/2), malignant thymoma (1/1) and liposarcoma (1/1). Serious adverse effects like hypotension, oliguria and severe hepatobiliary toxicity were never experienced. The effective and adequate dose of the mixed preparation was considered 2 to $4 \times 10^6$ U/day/body.**

*Key words :* **interferon-$\alpha$, tumor necrosis factor-$\alpha$, cancer, combination therapy, early phase II**

A large number of cytokines have been discovered, massproduced, and applied clinically. Among them, interferon (IFN) and tumor necrosis factor (TNF) have been expected to become useful as antitumor therapies. Interferons are classified into $\alpha$, $\beta$, and $\gamma$ types, but they are clinically similar in both their antitumor spectra and degree of effect. In solid cancers receiving systemic IFN therapy, the efficacy is high in renal cell cancer (1-2), Kaposi's sarcoma (3-4), malignant melanoma (5), carcinoid (6-7), and brain tumors (5). A good response has also been seen in a few cases of breast and ovarian cancer (5). Interferons are reported to be almost ineffective for gastric cancer, hepatoma, colon cancer and other tumors of the digestive organs. IFN-$\alpha$, which was the first interferon to be applied clinically, is classified into IFN-$\alpha$ derived from human leukocytes (leukocyte interferon, Cantell-type interferon, LIF), IFN-$\alpha$ derived

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from human lymphoblastoid cells (HLBI), and recombinant IFN-$\alpha$ (rIFN-$\alpha$). The crude LIF used in the initial trials was reported to produce a response rate of 20% in patients with breast cancer, ovarian cancer and renal cancer (8–9), but later more highly purified preparations of either natural HLBI or rIFN-$\alpha$ proved to be almost ineffective for breast, ovarian and other tumors (5, 10–11).

Recombinant TNF-$\alpha$ (rTNF-$\alpha$) has generally been used only in phase I trials (12–13), and there are few reports of phase II trials (14). The administration and dosage schedules for rTNF-$\alpha$ have varied widely, and a common consensus has not been obtained from the phase I trials. In addition to minor side effects, such as fever and chills, serious adverse effects have been reported, including hypotension and hepatobiliary damage. In phase I and II trials, TNF has been evaluated as only slightly effective for renal, colonic, gastric and hepatic cancer.

Thus, the clinical evaluation of TNF and IFN as single agents has shown only limited antineoplastic activity in man. Recent studies have suggested a synergism between these two proteins in both tumor cell lines and tumor-bearing animals (15–17). However, clinical application of the concomitant use of IFN and TNF has been reported once, in the phase II trial, without clinical response (18).

In 1984, we conducted a phase I trial using a mixture of high-purity natural human IFN-$\alpha$ (nHuIFN-$\alpha$) and natural human TNF-$\alpha$ (nHuTNF-$\alpha$) derived from the BALL-1 cell line at a 1:1 by activity ratio. This mixture, provisionally called OH-1, possessed a far stronger synergistic antitumor activity and a broader antitumor spectrum when compared with the individual cytokines both *in vitro* (16,19–20) and *in vivo* (17), and it showed extremely low toxicity in small animals. In the phase I trial (21), the dose-limiting factor for the concurrent administration of nHuIFN-$\alpha$ and nHuTNF-$\alpha$ was not clear, but the maximum tolerated dose (MTD) for this intravenous infusion combination appeared to be over $20 \times 10^6$ U/body. The usual adverse effects reported for IFN-$\alpha$ administration such as fever and chills were seen, but there were no serious side effects, such as the hypotension and hepatobiliary damage which are usually found with TNF administration. Based on this encouraging preclinical profile and the acceptable toxicity level found in the phase I trial, we conducted an early phase II trial of OH-1 in patients with advanced solid tumors.

## Materials and Methods

*Patients.* The subjects were patients with solid cancers satisfying the conditions set out below (a–h). Informed consent was obtained from all the patients and/or their families before they entered the trial.

a) Malignant tumor was confirmed histologically and/or cytologically. b) Measurable and/or evaluable lesions were present. c) More than 4 weeks had elapsed since the previous therapy had been completed and the effects of the previous therapy were considered to be negated. d) The patient had a life expectancy of 8 weeks or longer. e) The performance status by Karnofsky's classification was $\geqq 50\%$, or it was $\leqq 3$ by the ECOG calssification. f) The patient was free from advanced organ dysfunction. g) The patient was free from serious complications or superimposed cancers. h) The age was under 76 years.

*Drug and administration protocol.* The supernatant obtained by stimulating the B-cell human lymphoblastoid cell line (BALL-1, Hayashibara Biochemical Laboratories Inc., Okayama City, Japan) with hemagglutinating virus of Japan (HVJ) was passed through a corresponding monoclonal antibody-immobilized column, and was separated and purified to obtain nHuIFN-$\alpha$ and nHuTNF-$\alpha$ (22). nHuIFN-$\alpha$ is a glycoprotein having 166 amino acids composed of $\alpha$-2, 7, 8 with a molecular weight of 10,000, while nHuTNF-$\alpha$ is a glycoprotein having 159 amino acids with a molecular weight of 17,000. The amino acid structure of the N-terminal has a homology of 98.7% to rTNF-$\alpha$ (35). The separated and purified nHuIFN-$\alpha$ and nHuTNF-$\alpha$ were mixed 1:1 by the biological activity ratio. This mixture, provisionally called OH-1 preparation, was used in the present trial. The activity of nHuIFN-$\alpha$ is expressed in international units (IU) on the basis of the IFN-$\alpha$ standard of the NIH, and its specific activity is $2 \times 10^8$ IU/mg protein.

The activity of nHuTNF-$\alpha$ was measured according to the method of Eifel (23), with the concentration of nHuTNF-$\alpha$ showing 50 % killing of L-929 cells treated with actinomycin D (Pharmacia, Sweden) being determined as 50 units/ml. The titer of nHuTNF-$\alpha$ was expressed in Hayashibara's house reference units, and the specific activity was $7 \times 10^8$ U/mg protein. Therefore, $1 \times 10^6$ U of OH-1 preparation refers to a mixture of $1 \times 10^6$ IU of nHuIFN-$\alpha$ and $1 \times 10^6$ U of nHuTNF-$\alpha$ dissolved in 5 % human albumin solution. Vials containing $100 \times 10^3$ U, $500 \times 10^3$ U, and $1 \times 10^6$ U in 1 ml, were prepared, stored frozen, thawed before use, and immediately dissolved in 20 ml of physiological saline or 20 % glucose solution. The solution was slowly injected intravenously over several minutes. Generally, the first injection was $100 \times 10^3$ U, the second injection $500 \times 10^3$ U, and the maintenance dose $1 \times 10^6$ U or more/day. The preparation was administered in two divided portions consecutively for over 8 weeks, or for as long as the tumor was stable or responsive. In patients with hepatoma who had an indwelling catheter in the hepatic artery, the OH-1 was directly infused through the catheter. If fever higher than 38 °C was induced by OH-1 administration, indomethacin or aspirin was given prior to the next administration of OH-1.

*Clinical investigations.* Following the side effects investigation format of the Japan Society of Cancer Therapy, clinical symptoms were checked every day, while general hematological tests (RBC, WBC, Hb, Ht, platelets, etc.), serum biochemical tests (ALP, GOT, GPT, LDH, protein, BUN, creatinine, bilirubin, AFP, CEA, electrolytes, etc.), and urinalysis were performed before administration and every week during administration. Antibody levels for OH-1 were measured before administration, 2 weeks after starting treatment, and 3 days after the end of the trial. The measurement was done by radioimmunoassay which detected both neutralizing and nonneutralizing antibodies to nHuIFN-$\alpha$ and nHuTNF-$\alpha$.

*Evaluation of the response.* Responses were evaluated at 4-week intervals. They were defined as complete responses (CR) when all measurable tumors disappeared for more than 4 weeks; as partial responses (PR) when lesions were reduced in size by more than 50 % for more than 4 weeks; and as minor responses (MR) when lesions were reduced by more than 50 % for less than 4 weeks, or by 25 % to 50 % for more than 4 weeks. No change (NC) meant that lesions were reduced in size by less than 50 % or increased in size by less than 25 %, and freedom from occurrence of new lesions was maintained for at least 4 weeks. Progressive disease (PD) meant that lesions increased in size by more than 25 %, or new lesions appeared. CR and PR cases were regarded as effective ones for calculation of the efficacy rate. The period from the achievement of CR or PR until the time of recognition of an obvious increase of the same lesions or the development of new lesions was regarded as the effective period.

## Results

One hundred and twenty patients were entered

**Table 1** Background data of 120 eligible patients

| Item | No. of cases | Item | No. of cases |
|---|---|---|---|
| [Sex] | | [Cancer stage] | |
| Male | 69 | I | 0 |
| Female | 51 | II | 6 |
| [Age] | | III | 27 |
| < 20 | 2 | IV | 84 |
| 20 ~ | 1 | Unkown | 3 |
| 30 ~ | 9 | [Peformance status] | |
| 40 ~ | 23 | 0 | 0 |
| 50 ~ | 34 | 1 | 52 |
| 60 ~ | 34 | 2 | 43 |
| 70 ~ 75 | 15 | 3 | 23 |
| 76 | 2 | 4 | 2 |
| [Diagnosis] | | Unknown | 0 |
| Breast cancer | 20 | [Metastasis] | |
| Colon cancer | 19 | None | 36 |
| Stomach cancer | 18 | Yes | 84 |
| Hepatoma | 16 | [Preceding treatment] | |
| Lung cancer | 11 | None | 15 |
| Renal cell cancer | 11 | Yes | 105 |
| Pancreas cancer | 8 | (Type of treatment) | |
| Esophagus cancer | 2 | Surgery | 80 |
| Uterus cancer | 2 | Radiotherapy | 22 |
| Ovarian cancer | 2 | Chemotherapy | 75 |
| Rhabdomyosarcoma | 2 | Hormonotherapy | 4 |
| Cholangioma | 1 | Immunotherapy | 14 |
| Malignant thymoma | 1 | TAE | 5 |
| Melanoma | 1 | Lymphocyte therapy | 2 |
| Liposarcoma | 1 | Gelmanium | 1 |
| Brain tumor | 1 | Thermotherapy | 1 |
| Thyroid cancer | 1 | SSM (Maruyama's vaccine) | 5 |
| Gall bladder cancer | 1 | | |
| Paraganglioma | 1 | | |
| Extradural tumor | 1 | | |

Average age: 55.1 Cancer staging: according to TNM Classification of Malignant Tumors (4th Edition, UICC, 1987)

in the trial from April 1985 to January 1988, in whom 8 patients were unevaluable ones, consisting of 4 with death within 1 month, 2 with short administration of the OH-1 mixture and 2 with insufficient follow-up. The clinical responses were evaluated in the remaining 112 patients, and the adverse reactions were evaluated in all 120 eligible ones. These follow-up statuses were finally assessed on December, 1989. Background data of 120 eligible patients were shown in Table 1.

*Clinical response.* Of the 112 evaluable patients, 4 had CR, 10 PR, and 4 MR (Table 2). Overall response rate was 12.5 %, or 14.4 % if MR cases were included. The mixture of $1 \times 10^6$ U/body/day produced no response in 40 patients. All positively responded cases had been given $2 \times 10^6$ U or more of the mixture, in which the response rate was 19.4 % (14/72), 25 % (18/72) if MR cases were included. The percentage and actual numbers of cases with CR, PR, MR, NC and PD were 5.6 % (4/72), 13.8 % (10/72), 5.6 % (4/72), 48.6 % (35/72), and 26.4 % (19/72), respectively. The types of cancers showing positive response were as follows: hepatoma (3/8, 37.5 %), renal cell cancer (4/11, 36.4 %), breast cancer (4/17, 23.5 %), ovarian cancer (1/2), malignant thymoma (1/1) and liposarcoma (1/1).

Tumor regression occurred not only at the primary lesions but also at metastatic sites such as lungs, liver, bones, subcutaneous tissues, and lymph nodes (Table 3).

The duration of response was over 15.5 months on the average in the patients with CR,

**Table 2** Clinical efficacy of OH-1 against 112 evaluable patients with various solid cancers

| Diagnosis | Evaluable patients | Anti-tumor effect | | | | | Response rate (CR + PR/Evaluable cases) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CR | PR | MR | NC | PD | $< 1 \times 10^6$ U | $\geqq 2 \times 10^6$ U | Total |
| Stomach cancer | 16 ( 8) | | | 1 (1) | 5 ( 4) | 10 ( 3) | | | |
| Colon cancer | 17 ( 9) | | | | 10 ( 4) | 7 ( 5) | | | |
| Hepatoma | 14 ( 8) | 1 (1) | 2 ( 2) | | 7 ( 4) | 4 ( 1) | | 37.5 % ( 3/ 8) | 21.4 % ( 3/ 14) |
| Breast cancer | 19 (17) | 1 (1) | 3 ( 3) | 1 (1) | 10 ( 9) | 4 ( 3) | | 23.5 % ( 4/17) | 21.1 % ( 4/ 19) |
| Lung cancer | 11 ( 6) | | | 1 (1) | 4 ( 2) | 6 ( 3) | | | |
| Pancreas cancer | 7 ( 2) | | | | 2 ( 1) | 5 ( 1) | | | |
| Renal cell cancer | 11 (11) | | 4 ( 4) | | 5 ( 5) | 2 ( 2) | | 36.4 % ( 4/11) | 36.4 % ( 4/ 11) |
| Esophagus cancer | 2 ( 1) | | | | 2 ( 1) | | | | |
| Uterus cancer | 2 ( 0) | | | | 2 ( 0) | | | | |
| Ovarian cancer | 2 ( 2) | | 1 ( 1) | | 1 ( 1) | | | 50.0 % ( 1/ 2) | 50.0 % ( 1/ 2) |
| Rhabdomyosarcoma | 2 ( 1) | | | 1 (1) | | 1 | | | |
| Gall bladder cancer | 1 ( 1) | | | | 1 ( 1) | | | | |
| Malignant thymoma | 1 ( 1) | 1 (1) | | | | | | 100.0 % ( 1/ 1) | 100.0 % ( 1/ 1) |
| Liposarcoma | 1 ( 1) | 1 (1) | | | | | | 100.0 % ( 1/ 1) | 100.0 % ( 1/ 1) |
| Melanoma | 1 | | | | | 1 | | | |
| Brain tumor | 1 ( 1) | | | | 1 ( 1) | | | | |
| Thyroid cancer | 1 ( 1) | | | | 1 ( 1) | | | | |
| Cholangioma | 1 ( 1) | | | | | 1 ( 1) | | | |
| Paraganglioma | 1 | | | | | 1 | | | |
| Extradural tumor | 1 ( 1) | | | | 1 ( 1) | | | | |
| Total | 112 (72) | 4 (4) | 10 (10) | 4 (4) | 52 (35) | 42 (19) | 0 % (0/40) | 19.4 % (14/72) | 12.5 % (14/112) |

CR: complete response, PR: partial response, MR: minor response, NC: no change, PD: progressive disease
( ): patients with daily dose of $\geqq 2 \times 10^6$ U

**Table 3** Clinical efficacy of OH-1 against primary lesions and metastatic lesions

| Tumor location | Tumor response | | | | | Response rate CR + PR/Evaluable cases | |
|---|---|---|---|---|---|---|---|
| | CR | PR | MR | NC | PD | | |
| **Metastatic lesion (M)** | | | | | | | |
| Liver | | 1 (1) | 1 (1) | 12 (4) | 15 (9) | 3.4 %, 1/ 29 | ( 6.7 %, 1/15) |
| Lung | 1 (1) | 3 (3) | 1 (1) | 9 (6) | 8 (5) | 18.2 %, 4/ 22 | (25.0 %, 4/16) |
| Bone | | 7 (7) | 1 (1) | 8 (6) | 5 (2) | 33.3 %, 7/ 21 | (43.8 %, 7/16) |
| Lymph node | 1 (1) | | | 6 (4) | 6 (1) | 7.7 %, 1/ 13 | (16.7 %, 1/ 6) |
| Abdominal cavity | | 1 (1) | | 3 (2) | 4 (1) | 12.5 %, 1/ 8 | (25.0 %, 1/ 4) |
| Thoracic cavity | | 1 (1) | 1 (1) | 1 | 2 (1) | 20.0 %, 1/ 5 | (33.3 %, 1/ 3) |
| Skin | | | | 2 (2) | 2 | | |
| Brain | | | | 3 (1) | | | |
| Urether | | | | | 1 | | |
| Adrenal gland | | 1 (1) | | | | | |
| Ovarium | | | | 1 (1) | | | |
| Others | | | | 2 (1) | 1 | | |
| Primary lesion (P) | 3 (3) | 2 (2) | 1 (1) | 25 (14) | 17 (6) | 10.4 %, 5/ 48 | (19.2 %, 5/26) |
| Total (P + M) | 5 (5) | 16 (16) | 5 (5) | 72 (41) | 61 (25) | 12.5 %, 20/159 | (21.7 %, 20/92) |
| Total (M only) | 2 (2) | 14 (14) | 4 (4) | 47 (27) | 44 (19) | 14.4 %, 16/111 | (24.2 %, 16/66) |

CR, PR, MR, NC, and PD: See Table 2. ( ): Cases with daily dose of $\geqq 2 \times 10^6$ U

**Table 4** Duration of response in 14 responded patients

| Patients | Stage of disease | Duration of treatment (mo) | Response | Duration of response (mo) |
|---|---|---|---|---|
| Liposarcoma | III | 14 | CR | 16 |
| Malignant thymoma | III | 5 | CR | 36 |
| Breast cancer | IV | 10 | CR | 4 |
| Hepatoma | III | 10 | CR | 6 |
| Renal cell cancer | IV | 27 | PR | 4 |
| Renal cell cancer | IV | 5 | PR | 1 |
| Renal cell cancer | IV | 11 | PR | 2 |
| Renal cell cancer | IV | 5 | PR | 1 |
| Hepatoma | III | 6 | PR | 2 |
| Hepatoma | III | 9 | PR | 4 |
| Breast cancer | IV | 6 | PR | 2 |
| Breast cancer | IV | 10 | PR | 5 |
| Breast cancer | IV | 13 | PR | 6 |
| Ovarian cancer | IV | 3 | PR | 1 |
| Rhabdomyosarcoma | IV | 3 | MR | 1 |
| Stomach cancer | III | 5 | MR | 1 |
| Lung cancer | IV | 4 | MR | 1 |
| Breast cancer | IV | 7 | MR | 2 |

CR, PR, and MR: See Table 2.

and over 2.8 months in those with PR (Table 4). The complete responder in the malignant thymoma has now been alive for more than 4 years without having recurrence and without any therapy after the administration of the mixture for the first 5 months.

The period from initial administration of the mixture to the time of a tumor regression of 50 % or more was $14.4 \pm 8.7$ weeks. The cumulative dosage of the mixture before the appearance of a tumor regression of 50 % or more was $269.54 \pm 165.61 \times 10^6$ U/body, while the daily dosage was about $3 \times 10^6$ U/body/day, (or $267.0 \pm 224.4 \times 10^4$ U/m$^2$). The maximum dose of the mixture given was $8 \times 10^6$ U/body/day.

*Clinical toxicities.* The toxic effects associated with this trial are listed in Table 5. No serious toxicities were observed. Most toxic symptoms were mild and graded on the lower levels of 1 and 2 according to the criteria of the Japan Society of Cancer Therapy. Most frequent toxic symptoms were fever, chills, general fatigue, nausea and vomiting, but they were not serious in spite of long-term use of the mixture and were easily controlable by using the ordinary antipiretics. The toxicities against bone-marrow and he-

**Table 5** Graded adverse reactions and abnormal laboratory data in 120 eligible patients

| Type of adverse reaction | Grade | | | | Total | Appearance rate (%) |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| Adverse reactions | | | | | | |
| Fever | 17 | 27 | 9 | 0 | 53 | 44.2 |
| Chill | 14 | 10 | 7 | 0 | 31 | 25.8 |
| General malaise | 16 | 9 | 4 | 1 | 30 | 25.0 |
| Nausea/vomiting | 9 | 9 | 3 | 0 | 21 | 17.5 |
| Headache | 7 | 2 | 2 | 0 | 11 | 9.7 |
| Myalgia | 1 | 1 | 0 | 0 | 2 | 1.8 |
| Somnolence | 1 | 0 | 2 | 0 | 3 | 2.5 |
| Skin rash | 1 | 0 | 0 | 0 | 1 | 0.9 |
| Abnormal laboratory data | | | | | | |
| Leukopenia | 23 | 26 | 16 | 1 | 66 | 55.0 |
| Thrombocytopenia | 16 | 11 | 9 | 0 | 36 | 30.0 |
| Hyperbilirubinemia | 7 | 4 | 1 | 0 | 12 | 10.0 |
| Increase in ALP | 25 | 7 | 4 | 0 | 36 | 30.0 |
| Increase in GOT | 24 | 27 | 2 | 1 | 54 | 45.0 |
| Increase in GPT | 21 | 16 | 2 | 1 | 40 | 33.3 |
| Increase in LDH | 19 | 13 | 0 | 0 | 32 | 26.7 |
| Increase in BUN | 9 | 2 | 4 | 0 | 15 | 12.5 |
| Increase in Creatinine | 4 | 0 | 0 | 0 | 4 | 3.3 |

patobiliary organs associated with OH-1 therapy were not serious, and were transient. Those toxicities decreased promptly after stopping the OH-1 for a week or reducing the dose of OH-1 preparation.

The antibody to OH-1 was negative in all the cases.

## Discussion

The present early phase II trial of OH-1 was mainly intended to clarify three points : for which type of solid cancers was the OH-1 preparation effective, what dose was most appropriate, and what degree of toxicity would be observed.

On the basis of the data of the phase I trial of OH-1 (21), OH-1 was administered i.v. for consecutive days. Since the half-lives after intravenous injection of nHuIFN-$\alpha$ and nHuTNF-$\alpha$ are only about 30 min. and since OH-1 is a kind of time-dependent anticancer drug (24), it was administered to patients in two divided doses.

*Clinical response.* The maitenance dose was escalated from $1 \times 10^6$ U/patient/day to $2 \times 10^6$ U or $3 \times 10^6$ U or more up to $8 \times 10^6$ U with the range of $1 \times 10^6$ U. No tumor regression occurred in any case where OH-1 preparation was administered at a daily dose of $1 \times 10^6$ U. However, when 72 patients received increased daily doses of $2 \times 10^6$ U or more, clinical response of 19.4 % (14/72) was obtained. Even though OH-1 preparation was not checked on carcinoid in this trial, OH-1 preparation showed considerably high efficacy rate in the wide range of solid cancers and seemed to have the higher efficacy superior to the single administration of either IFN or TNF. To our knowledge, there are not previously published cases of response of hepotoma to either IFN or TNF alone. It is not too much to say that OH-1 therapy is useful at least for hepatoma, renal cell cancer and breast cancer that are resistant to conventional therapies.

In ovarian cancer, the efficacy rate of IFN was reported to be 5 % to 19 % (5), and also one partial response case was found in this trial. As

to carcinoid, one complete response, and one partial response were obtained with OH-1 therapy after the completion of this trial. On soft tissues sarcoma, partial response was reported in one fibrosarcoma patient (5). In this trial, the liposarcoma case showed partial response. For the reasons mentioned above, OH-1 preparation will also be effective against carcinoid, ovarian cancer and soft tissues sarcoma. High frequency and long-term duration of NC of disease are also the most striking thing about OH-1 therapy. OH-1 preparation has the efficacy for both advanced primary lesions and metastatic lesions.

*Clinical toxicity.* The maximum maintenance level of OH-1 was $8 \times 10^6$ U/day, without observing hypotension or serious hepatobiliary damage in any case. At $6 \times 10^6$ U or more/patient/day, the incidence of grade III or IV toxicity increased suddenly. With long-term administration of 4 to $5 \times 10^6$ U/patient/day, elevations of GOT, GPT, leukopenia and thrombocytopenia of grade I or II appeared. They resolved following a week off the drug or following reduction of the dose. As in the phase I trial of OH-1, toxicity attributable to TNF-$\alpha$ (12–13) was not seen in the early phase II trial, and the observed toxicity was compatible with those of conventional reports on toxicity of IFN-$\alpha$ (5,11, 25). Side effects such as fever and chills could be easily controlled by the prior administration of indomethacin or aspirin, and with long-term use of OH-1 these antipyretics became unnecessary in some cases.

The low toxicity of OH-1 may be explained by the low dose of TNF-$\alpha$ in the OH-1 preparation. For our nHuTNF-$\alpha$ preparation, 350 U is equal to 1 JRU. Therefore, 3 to $4 \times 10^6$ U of OH-1 preparation has a content of about $1 \times 10^4$ JRU of nHuTNF-$\alpha$. This is an extremely small dose, corresponding to the starting dose used in the phase I and II trials of rTNF-$\alpha$ (12–14). Another probable reason for the low toxicity is that both cytokines were of the natural type. It is important to note that Old, when administering TNF-$\alpha$ to mice, found that deaths were caused by recombinant TNF, but not by nonrecombinant TNF (26). Old considered that either structural differences between recombinant and nonrecombinant TNF or the presence of toxic impurities in recombinant TNF caused this difference.

*Adequate dosage regimen.* The dose producing CR or PR was $2 \times 10^6$ U or more/patient/day, and an average of $14.4 \pm 8.7$ weeks was required before CR or PR was recognized. From the viewpoint of clinical toxicity, long-term administration of OH-1 at $4 \times 10^6$ U or more/patient/day causes reversible bone marrow suppression which will be regarded as a dose-limiting toxicity. Therefore, considering both the aspects of tumor efficacy and clinical toxicity, in order to administer OH-1 i.v. safely for more than 3 months, the optimum dose is considered to range from 1.8 to $2.4 \times 10$ U/m$^2$/day. This was obtained by converting 3 to $4 \times 10^6$ U/patient/day into the dose for an adult subject 170 cm tall and weighing 60 kg. Our nHuIFN-$\alpha$ was obtained by stimulating human lymphoblastoid cells with HVJ, and it belongs to the HLBI class. Priestman (27) recommended 2.5 to $5.0 \times 10^6$ IU/m$^2$ for long-term administration of HLBI, and Sarna *et al.* (28) have reported that the MTD should be $30 \times 10^6$ IU/12-h interval/7 days. Kimura *et al.* (12) have reported that the MTD for 1-month administration should be $12 \times 10^6$ IU/patient/day. All these reports are from studies using i.m. administration. Concerning i.v. administration of HLBI, Rohatiner *et al.* (29) suggested that the MTD should be a 7-day prolonged drip infusion of $100 \times 10^6$ U/m$^2$. We have administered OH-1 at doses of 3 to $4 \times 10^6$ U/patient/day consecutively for 3 years and 8 months to a 42-year-old female with breast cancer and bone metastases. No side effects except for mild injuries of grade I or II on hepatic function and bone marrow were observed. The antibody to OH-1 was negative, and the blood triglyceride levels remained normal. For four years approximately, the bone metastases remained somewhere between MR and NC, and the PS was 1. As for

the IFN, its administration schedule has not been determined definitely yet. For single agent treatments, a high dose is recommended by some (30) and a low dose by others (10). There is also the opinion that the actual dose is not so closely related to the antitumor effect (31). It is also debatable whether consecutive administration or intermittent injection of IFN is superior. Our study of the mechanism of action of OH-1 blocks, both *in vitro* (16) and *in vivo* (24), the cell cycle of cancer cells in the S-phase and prevents them from advancing to the $G_2$ and M-phases. Therefore, OH-1 is considered to be a kind of time-dependent anticancer drug. By consecutive administration of OH-1, tolerance can be formed against symptoms such as fever and chills. For these reasons, it seems best to administer a low dose of OH-1 over a long time period. It has also been reported that it takes several weeks to several months before an effect is first observed using single-agent IFN (1, 30, 32).

*Combinations of cytokines.* In the initial clinical trials using crude Cantell-type leukocyte interferon (LIF), it was effective for a wide range of solid cancers (8, 33), including osteosarcoma, breast cancer and carcinoid. However, the subsequent high-purity HLBI and rIFN-$\alpha$ preparations were almost useless against solid tumors, except for renal cell cancer and carcinoid. It therefore appeared that certain cytokines mixed in the crude ILF and IFN-$\alpha$ had shown synergistic antitumor activity. In fact, when BALL-1 cells were stimulated with HVJ, TNF-$\alpha$, TNF-$\beta$, and two or three other unknown cytokines were simultaneously produced in addition to IFN-$\alpha$ (22). Increased antitumor effects with the concomitant use of cytokines have been reported in many *in vitro* and *in vivo* experiments, using IFN + TNF (16-17,20,34-35), TNF + IL-2 (36), IFN-$\alpha$ + IFN-$\beta$ (37), IL-1 + TNF (38), *etc.* We found that the antitumor effect was further enhanced when nHuTNF-$\beta$ was added to OH-1 (nHuIFN-$\alpha$ + nHuTNF-$\alpha$) (unpublished data).

Since the immunological surveillance system of the body greatly depends on the cell-to-cell interaction of immune cells, in other words the cytokine cascade, it may be easily supposed that a number of cytokines cooperate to enhance antitumor activity. The use of multiple drugs in cancer chemotherapy and of multiple immunosuppressants in organ transplantation contributes to the reduction of the doses of individual drugs and allows the combination of various different actions. This has brought about dramatic carcinostatic effects or immunosuppressive effects while reducing toxicity on the whole. Therefore, combined use of cytokines and also the simultaneous use of cytokines and conventional anticancer drugs seem to be important issues to study in the future.

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