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Three Schedules of Recombinant Human Interleukin-2 in the Treatment of Malignancy: Side Effects and Immunologic Effects in Relation to Serum Level

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Recombinant human interleukin-2 (rIL-2) was administered to 34 patients with advanced malignancy. Three schedules of rIL-2 administration employed were as follows: (A) 2-hr iv infusion of 6.7×10^5 U/m^2/day (A_1, 6 cases) or 2.2×10^6 U/m^2/day (A_2, 8 cases) for five consecutive days; (B) 24-hr continuous iv infusion of 3.3×10^5 U/m^2/day (B_1, 3 cases), 6.7×10^5 U/m^2/day (B_2, 7 cases) or 1.1×10^6 U/m^2/day (B_3, 5 cases) for 28 consecutive days; and (C) 24-hr continuous iv infusion of 6.7×10^5 U/m^2/day (C, 5 cases) for 5 consecutive days per week for four weeks. The common side effects were fever (79%), eosinophilia (61%), malaise (56%), erythema or rash (50%), chills (38%) and nausea or vomiting (35%), with the dose-limiting toxicities being hypotension in group A, and renal dysfunction with fluid retention in groups B and C. In the case of 2-hr iv infusion, rIL-2 was rapidly cleared from the plasma, with a half life of about 30 min, while in the case of 24-hr continuous infusion, more than 1 U/ml serum IL-2 activity was maintained for 14 days in group B_3. Natural killer (NK) and lymphokine-activated killer (LAK) activities were augmented by rIL-2 administration in patients of groups A, B_3 and C. In eight patients of group B, NK and LAK activities transiently decreased after rIL-2 administration, and recovered by day 3. The percentage of IL-2 receptor and Leu HLA-DR positive cells reached the peak level on day 7 in group B. In patients of group C, the percentage of Leu HLA-DR positive cells as well as NK and LAK activities increased upon rIL-2 administration and decreased during an intermission of two days. However, the percentage of rIL-2 receptor positive cells increased during the intermission of rIL-2. The most effective schedule of rIL-2 administration was considered to be the schedule of group C on the basis of this study.

Key words: Recombinant human interleukin-2 — Adverse effects — Immunologic responses

Interleukin-2 (IL-2) is an immunologically active glycoprotein which is produced by helper/inducer T cells following activation with mitogen or antigen. IL-2 has been reported to augment proliferative and cytotoxic T cell responses and natural killer (NK) cell activity, to induce lymphokine-activated killer (LAK) cells and to produce interferon-γ (IFN-γ) *in vitro*.[1-6] Mice receiving IL-2 have demonstrated augmentation of NK and LAK activities as well as proliferative T cell responses to alloantigens *in vivo*.[7-10] Regression of established pulmonary and hepatic metastases was induced by administration of high doses of rIL-2 alone or in combination with LAK cells.[11-14] We had previously demonstrated that a high dose of human recombinant IL-2 (rIL-2) was capable of mediating the regression of established pulmonary and hepatic metastases of B-16 melanoma cells by multiple sc injections of human rIL-2.[13] Activation of human peripheral blood lymphocytes or murine splenocytes by IL-2 *in vitro* can kill a variety of fresh tumor cells,[15] including fresh autologous, syngeneic, and allogeneic tumor cells.[2, 16, 17]

In 1983, the gene coding for human interleukin 2 was cloned and expressed in *Escherichia coli*.[18] The nucleotide and amino acid sequences of rIL-2 were determined to be identical with those of the native IL-2.[19] The availability of a large amount of pure rIL-2, produced by recombinant DNA technology, makes it possible to conduct extensive clinical

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trials for a variety of cancers. So far, however, only a few reports have appeared on clinical trials with rIL-2, demonstrating that tumor regression can be obtained by administration of a high dose of rIL-2 alone or with LAK cells in some cancer patients. This study was conducted to evaluate the response rate, side effects and immunologic effects in relation to serum level of IL-2 for the determination of the most effective schedule of rIL-2 administration.

Materials and Methods

Human Recombinant Interleukin-2 Lyophilized human rIL-2 (Shionogi Pharmaceutical Company, Osaka), stored at $-20°$, was reconstituted in sterile water for injection, and diluted with 5% dextrose in water to the appropriate concentration just before administration. The activity was stable for at least 24 hr in this fluid. The purity of the rIL-2 was >99% and the specific activity was 1.4×10^7 U/mg protein. The units of rIL-2 were determined by ^{3}H-thymidine incorporation using CTLL-2 cells according to the method of Gillis *et al.*[20] and were expressed in international units.

Patient Selection Thirty-four patients, hospitalized at the Japan National Cancer Center Hospital from July 1985 to July 1986, with advanced malignancy, were entered into the study. Criteria for patient entry into the study inculuded the following: (a) histologically proven malignancy; (b) a minimum life expectancy of six weeks; (c) recovery from any apparent side effects of prior treatments including chemotherapy, radiotherapy or operation; (d) Eastern Cooperative Oncology Group performance score of 0–3; (e) no anticancer therapy for at least four weeks prior to entry; (f) preserved hematologic function (while blood cell count $\geq$4,000/mm^3 and platelet count $\geq$100,000/mm^3); and (g) adequate hepatic and renal function

Table I. Patient Characteristics

	A_1	A_2	B_1	B_2	B_3	C	Total
No. of patients (Male)	6 (3)	8 (5)	3 (3)	7 (4)	5 (4)	5 (2)	34
Median age (Range)	58(36–83)	54(40–77)	65(55–77)	58(32–77)	56(46–70)	58(51–62)	85(32–83)
Diagnosis							
Primary lung cancer	5	6	1	5	1	0	18
Primary gastric cancer	0	0	0	1	0	0	1
Metastatic tumor of the lung	1	2	2	1	4	5	15
Stage							
II	2	0	0	0	0	0	2
III	0	0	0	1	1	0	2
IV	4	8	3	6	4	5	30
Prior therapy							
no prior therapy	3	1	0	2	0	0	6
CT alone	1	0	0	2	1	0	4
CT and RT	1	3	0	1	0	1	6
OP alone	1	0	2	1	1	2	7
OP and CT	0	2	1	1	3	2	9
OP, CT and RT	0	2	0	0	0	0	2
Performance status (ECOG)							
0	1	0	2	2	1	0	6
1	3	4	0	4	3	4	18
2	1	1	0	1	0	0	3
3	1	3	1	0	1	1	7

A. 2-hr iv infusion for 5 consecutive days: A_1, 6.7×10^5 U/m^2/day; A_2, 2.2×10^6 U/m^2/day.
B. 24-hr continuous iv infusion for 28 consecutive days: B_1, 3.3×10^5 U/m^2/day; B_2 6.7×10^5 U/m^2/day; B_3, 1.1×10^6 U/m^2/day.
C. 24-hr continuous iv infusion of 6.7×10^5 U/m^2/day for 5 consecutive days, 4 weekly cycles.
Abbreviations: CT, chemotherapy; RT, radiotherapy; OP, Operation; ECOG, Eastern Cooperative Oncology Group.

(serum bilirubin $\leq$1.5 mg/dl, serum creatinine $\leq$2.0 mg/dl). Written informed consent was obtained from all patients prior to initiation of the therapy.

Study Design Patient characteristics are shown in Table I. Twenty-eight patients who had received prior antitumor therapy were entered in this trial because prior standard chemotherapy had failed for their underlying malignancy. RIL-2 was administered by one of the three schedules: (A) 2-hr iv infusion of 6.7×10^5 U/m^2/day (A_1, 6 cases) or 2.2×10^6 U/m^2/day (A_2, 8 cases) for five consecutive days, (B) 24-hr continuous iv infusion of 3.3×10^5 U/m^2/day (B_1, 3 cases), 6.7×10^5 U/m^2/day (B_2, 7 cases) or 1.1×10^6 U/m^2/day (B_3, 5 cases) for 28 consecutive days; and (C) 24-hr continuous iv infusion of 6.7×10^5 U/m^2/day (C, 5 cases) for five consecutive days per week, four cycles. RIL-2 was administered by an automatic infusion pump.

The doses of rIL-2 were determined from the phase I study reported by Lotze *et al.*,[21] in which the maximum tolerated dose of rIL-2 was 3,000 U/kg/day by continuous infusion or 1,000,000 U/kg/day by bolus infusion, suggesting that administration of 1,000 U/kg/hr is generally tolerable. One unit of rIL-2 in Lotze's regimen corresponded to approximately 0.4 international unit. Doses of rIL-2 administered in this study were determined on the basis of this phase I study.

Response and side effects were evaluated according to the WHO criteria.[22] In brief, complete response (CR) is defined as the complete disappearance of all evidence of the tumor for at least 4 weeks. Partial response (PR) is defined as a reduction of $\geq$50% in the product of the longest diameter and the diameter perpendicular to the longest (diameter product) in all measurable lesions for at least 4 weeks without appearance of a new lesion. No change (NC) required no decrease of $\geq$50% and no increase of $\geq$25% in the diameter product in all measurable lesions. Progressive disease is an increase of $\geq$25% in diameter product or the appearance of a new tumor lesion. Prior to therapy, all patients underwent a history taking, physical examination, complete blood counts, serum chemistry profiles, chest X-ray, ultrasonogram and radiologic studies to determine the extent of the disease. Following each injection of rIL-2, toxicity was carefully evaluated by repeated examinations including physical examination, complete blood counts and serum chemistry profiles. The tumor response was assessed just before the next scheduled administration and at the completion. Of the 34 patients, 24 were evaluable for response (seven patients dropped out due to severe side effects of rIL-2 administration).

Determination of Serum IL-2 Level All patients were monitored for levels of serum IL-2, using a modified two-step sandwich immunoradiometric assay. Serum samples were obtained in all patients before the infusion, 0.5, 2, 4 hr, 3 and 5 days after the start of rIL-2 injection in group A, and before the injection and on days 1, 2, 3, 5, 7, 14, 21, 28, 35 and 42 in groups B and C. In brief, polystyrene tubes pre-coated with a rabbit anti-rIL-2 polyclonal antibody (Shionogi Pharmaceutical Company, Osaka) were incubated with 100 μl of standard or test sample solution for 5 hr at 20 to 25°. After washing of the tube twice with 250 μl of buffer solution, 100 μl of ^{125}I-labeled rabbit anti-rIL-2 polyclonal antibody solution (affinity-purified $F(ab')_2$, fragment) (Shionogi Pharmaceutical Company) was added to each tube. The tubes were incubated for 16 hr at 20 to 25°, then washed out twice with 250 μl of the buffer solution. Radioactivity of the tubes was measured using an autowell gamma counter (Aloka, Tokyo); the limit of detection was 0.5 U/ml.

Assay with Anti-IL-2 Antibody Sandwich radioimmunoassay, non-inhibitory and inhibitory, was used to detect serum anti-IL-2 antibody. In non-inhibitory assay, serum samples were diluted with assay buffer (0.01*M* phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin; pH 7.4). Diluted serum (100 μl) was incubated in both a polystyrene tube coated with rIL-2 and a blank tube for 5 hr at 20 to 25°. These tubes were washed twice with 250 μl of wash buffer (0.01*M* PBS containing 0.5% Tween-20). The tubes were incubated with 100 μl of ^{125}I-protein A solution (10^6 cpm/ml) for 16 hr at 20 to 25°, and then washed again with the wash buffer three times. Radioactivity was determined with the autowell scintillation counter (Aloka), and the titer was expressed as the ratio (B/T) of the bound radioactivity (B) to the total radioactivity (T).

In inhibitory assay, 450 μl of diluted serum sample was preincubated with either 50 μl of 20 μg/ml rIL-2 or 50 μl of the assay buffer for 22 hr at 4°, and 100 μl of the content was transferred into the rIL-2 coated tube. The following procedure was the same as for the non-inhibitory assay.

Interferon-γ Assay Serum level of IFN-γ was determined by radioimmunoassay (Centocor, Malvern, PA) at the same time as serum IL-2 assay. Briefly, serum was incubated with beads coated with murine monoclonal anti-IFN-γ antibody for 2 hr at 20 to 25°. The beads were washed with distilled water three times and incubated with ^{125}I-anti-IFN-γ. After removal of unbound antibodies by washing with distilled water, the radioactivity of the beads was measured with the gamma scintillation counter (Aloka). The concentration of IFN-γ in the serum was determined from a curve generated from serial dilutions of the standard IFN-γ solution.

Cytotoxicity Assay K562, a human myeloid leukemia cell line, was used for the determination of the cytotoxicity of NK cells. Daudi cells derived from lymphoma, and PC-9 and PC-14 cells derived from adenocarcinomas of the lung (kindly donated by Professor Y. Hayata, Tokyo Medical College), which are NK-resistant cell lines, were used for determination of the cytotoxicity of LAK cells. All the cell lines were maintained in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Nissui, Tokyo) (RPMI-FBS).

Peripheral blood lymphocytes (PBL) were obtained from the patients before, during and after administration of rIL-2 as follows: group A, days 1, 2, 3, 7, and 11; group B, days 1, 2, 3, 5, 7, 14, 21, 28, 35 and 42; group C, days 1, 2, 3, 5, 7, 12, 14, 19, 21, 26, 28, 35 and 42. Briefly, mononuclear cells were separated by collecting the interface cells following centrifugation on a Ficoll-Conray cushion (1080g) of peripheral blood diluted with Eagle's minimum essential medium (MEM), and by washing twice with MEM and once with RPMI-FBS. The mononuclear cells in RPMI-FBS were incubated in Falcon 3003 plastic dishes (Falcon, Oxnard, CA), in a humidified atmosphere of 5% CO_2 at 37° for 1 hr. Non-adherent cells were obtained by repeated washing of the dishes with MEM, and they were confirmed to be >95% lymphocytes by peroxidase staining and to show >95% viability by means of the trypan blue dye exclusion test.

^{51}Cr-release assay was performed to measure the cytotoxicity of PBL. Briefly, 2.5×10^6 target cells in 250 μl were incubated with 250 μl of 125 μCi of $Na_2{}^{51}CrO_4$ (Japan Radioisotope Association, Tokyo) for 1 hr, washed with RPMI-FBS three times to remove unbound ^{51}Cr and suspended in RPMI-FBS at a concentration of 1×10^5/ml. The amount of ^{51}Cr released spontaneously from the target cells during the incubation period ranged from 10 to 20% of the maximum ^{51}Cr-release. For the determination of cytotoxicity, 100 μl of ^{51}Cr-labeled target cell suspension (1×10^5 cells/ml) in RPMI-FBS was mixed with 100 μl of lymphocyte suspension (5×10^6 cells/ml) in RPMI-FBS in a microtiter plate (E/T ratio of 25/1 or 50/1).

After incubation of the plates for 5 hr in a humidified atmosphere of 5% CO_2 at 37°, the plates were centrifuged at 400g for 10 min, and 100

Table II. Toxicities of rIL-2 Therapy

	A_1	A_2	B_1	B_2	B_3	C	Total (%)
No. of patients	6	8	3	7	5	5	34
Fever	5	6	1	7	4	4	27 (79)
Malaise	5	4	0	4	4	2	19 (56)
Erythema or rash	1	4	1	4	4	3	17 (50)
Loss of appetite	0	3	0	5	3	2	13 (38)
Chills	3	6	0	1	2	1	13 (38)
Nausea or vomiting	0	4	1	2	3	2	12 (35)
Eosinophilia 10–20%	3	2	1	0	2	2	10 (29)
>20%	0	0	1	5	2	3	11 (32)
Headache	3	2	0	1	2	1	9 (26)
Pruritus	0	0	0	2	3	2	7 (21)
Myalgia or arthralgia	1	1	0	3	1	0	6 (18)
Fluid retention	0	0	0	2	1	2	5 (15)
Weight gain (>5%)	0	0	0	3	1	0	4 (12)
Hypotension	0	3	0	1	0	0	4 (12)
Diarrhea	0	0	0	1	2	1	4 (12)
Oliguria	0	0	0	0	3	0	3 (9)
Liver dysfunction	0	0	0	2	0	1	3 (9)
Renal dysfunction	0	0	0	0	1	1	2 (6)
Pleural effusion	0	0	0	0	0	1	1 (3)
Duodenal ulcer	0	0	0	0	1	0	1 (3)
Thrombocytopenia	0	0	0	0	0	1	1 (3)
No. of patients whose treatment was stopped due to side effect	0	1	0	1	3	2	7 (21)

A. 2-hr iv infusion for 5 consecutive days: A_1, 6.7×10^5 U/m^2/day; A_2, 2.2×10^6 U/m^2/day.
B. 24-hr continuous iv infusion for 28 consecutive days: B_1, 3.3×10^5 U/m^2/day; B_2, 6.7×10^5 U/m^2/day; B_3, 1.1×10^6 U/m^2/day.
C. 24-hr continuous iv infusion of 6.7×10^5 U/m^2/day for 5 consecutive days weekly, 4 cycles.

μl of the supernatant from each well was removed for measurement of the radioactivity in an autogamma counter (Beckman, Irvine, CA). Spontaneous target cell release was determined in the supernatant obtained from the culture of target cells only. The maximum releasable ^{51}Cr value was obtained after five cycles of freezing in dry-ice alcohol and thawing in hot water. The percentage of cytolysis was calculated by means of the following formula;

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100.$$

Each assay was performed in triplicate and data were reported as the mean±standard error.

Lymphocyte Surface Phenotype Analysis The lymphocyte surface markers were determined using a Spectrum III analyzer (Ortho Diagnostics, Raritan, NJ) at the same time as the cell-mediated cytotoxicity assay in all patients. A series of reagents, including Leu 2a (suppresser/cytotoxic T cells), Leu 3a+3b (inducer/helper T cells), Leu 4 (pan T reagent), Leu 7 (NK/killer cells), Leu 11 (NK cells), Leu HLA-DR (activated T, B cells and monocytes) and IL-2 receptor were obtained from Becton Dickinson (Mountain View, CA). OKT 3 (pan T reagent), OKT 4A (inducer/helper T cells), OKT 8 (suppresser/cytotoxic T cells), OKT 9 (activated or proliferative T cells), OKT 10 (precursor cells/activated T cells), and OKIa1 (activated T, B cells and monocytes) were from Ortho Diagnostics.

Statistical Analysis All data were analyzed to determine the significance of differences between means of paired variates by applying the two-tailed Student's *t*-test or Mann-Whitney's U test.

Results

Toxicity of RIL-2 A total of 34 patients with advanced cancer were treated with rIL-2 by one of the three administration schedules. No mortality related to the treatment was observed during the trial even with the high dose of rIL-2, although in seven patients administration of rIL-2 was stopped because of severe toxicities such as hypotension, fluid retention, renal dysfunction and diarrhea three to ten days after the beginning of rIL-2 administration (group A_2, 1 case; group B_2, 1 case; group B_3, 3 cases; and group C, 2 cases). The toxic effects observed in this study are summarized in Table II. Severe renal toxicity was observed in two patients of groups B_3 and C (one in each group). Hepatic toxicity occurred in two and one patients in groups B_2 and C, respectively. Hematological toxicities were not experienced except for one patient in group C who showed thrombocytopenia ($4.2\times10^4/mm^3$). Marked eosinophilia (>20%), but reversible, was observed in 11 patients. In one patient of group B_2, white blood cell count and differential count of eosinophils reached $36,000/mm^3$ and 84%, respectively.

The dose-limiting toxicity of systemic rIL-2 administration was hypotension in group A_2, and fluid retention and renal failure in groups B_2, B_3 and C. In group A_2, a decrease of 50 mmHg or greater in the base-line systolic pressure was observed in three patients, and the treatment of one was discontinued due to severe hypotension. In group B_2, three patients showed weight gain (>108% of the base-line weight) due to fluid retention. Three patients in group B_3 could not complete the schedule of treatment because of severe toxicity such as fluid retention, elevation of serum creatinine (>4.0 mg/dl), or oliguria due to uncontrollable diarrhea. In group C, one patient suffered from rapidly increasing pleural

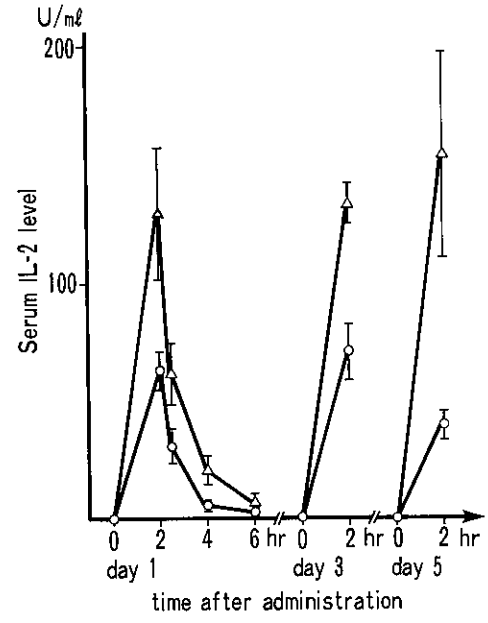

Fig. 1. Kinetics of serum IL-2 level determined by immunoradiometric assay after 2-hr infusion of rIL-2 in group A (2-hr iv infusion for 5 consecutive days): A_1, 6.7×10^5 U/m²/day (○), A_2, 2.2×10^6 U/m²/day (△). Bars represent mean±SE.

effusion requiring tube drainage without evidence of malignant pleuritis, and another one patient fell into non-oliguric renal failure with elevation of serum creatinine and potassium to 4.3 mg/dl and 6.3 mEq/liter respectively. Fever and chills were well controlled by the treatment with acetaminophen and indomethacin. Seventeen patients received an antihistamine, because of a generalized erythematous rash or pruritus. Weight gain and renal dysfunction disappeared promptly in all cases after rIL-2 administration was discontinued, while hypotension and eosinophilia continued for approximately three days and two weeks after the cessation of rIL-2 administration, respectively.

Clinical Response No response was observed in the patients treated with rIL-2 in this study. However, in one patient with pulmonary and subcutaneous metastases from colon cancer,

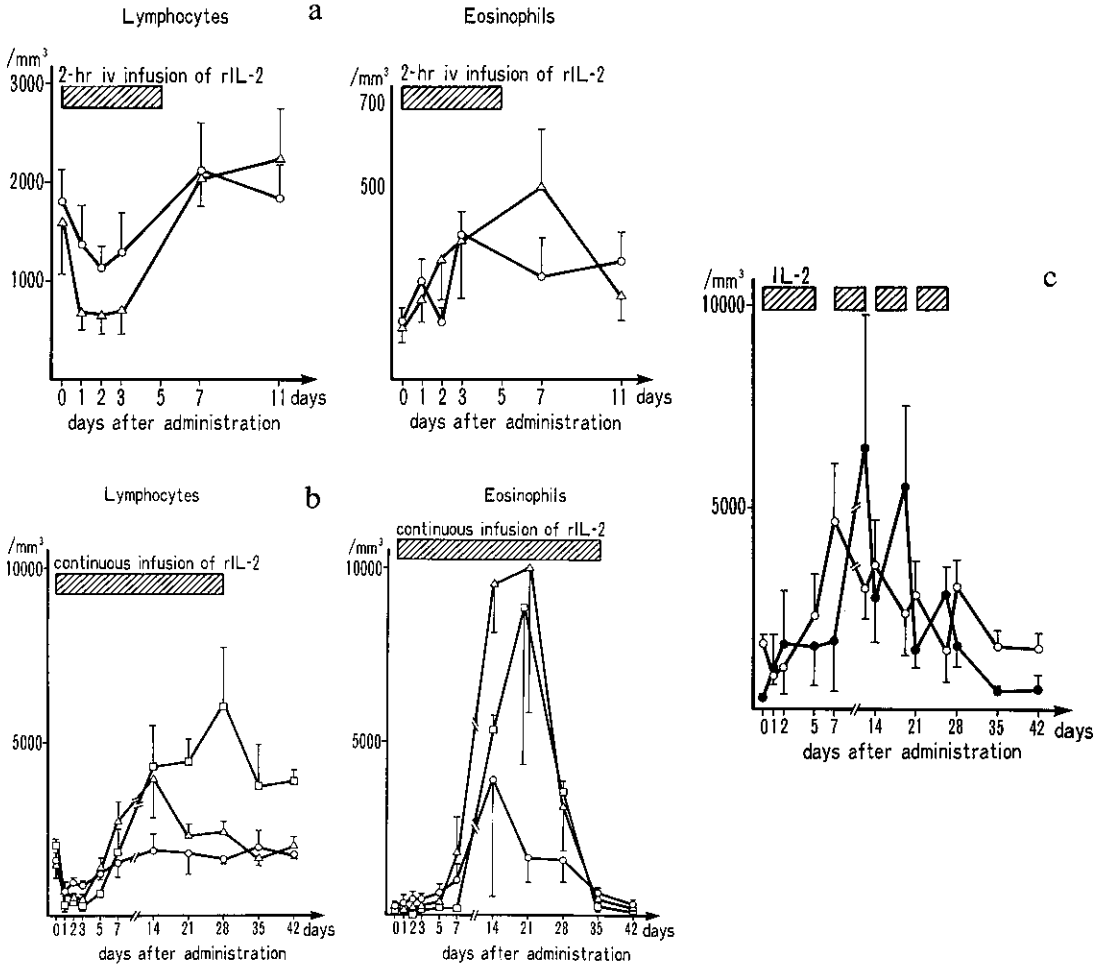

Fig. 2. a. Changes in lymphocyte and eosinophil counts after rIL-2 administration in group A (2-hr iv infusion for 5 consecutive days): A_1, 6.7×10^5 U/m²/day (○), A_2, 2.2×10^6 U/m²/day (△). Bars represent mean±SE. b. Changes in lymphocyte and eosinophil counts after rIL-2 administration in group B (24-hr continuous iv infusion for 28 consecutive days): B_1, 3.3×10^5 U/m²/day (○); B_2, 6.7×10^5 U/m²/day (△); B_3, 1.1×10^6 U/m²/day (□). Bars represent mean±SE. c. Changes in lymphocyte (○) and eosinophil (●) counts after rIL-2 administration in group C (24-hr continuous iv infusion of 6.7×10^5 U/m²/day for 5 consecutive days weekly, 4 cycles). Bars represent mean±SE.

resected previously, in group B_3, more than 50% decrease in the diameter product lasting more than one month was observed in the subcutaneous lesions during rIL-2 administration. In two patients of group B_2, serum carcinoembryonic antigen was significantly decreased (from 70 to 25.9 and from 39.9 to 22.7 ng/ml). Of 24 evaluable patients, four showed progressive disease, and 20 showed no change.

Serum IL-2 Level Serum IL-2 activity was rapidly cleared in patients of group A (half life: approximately 30 min) (Fig. 1). No IL-2 activity was detected just before the rIL-2 administration on day 3 and day 5, suggesting that there was no accumulation of rIL-2. On the other hand, serum IL-2 level was maintained between 0.7 to 15.2 U/ml in ten patients of groups B_1 and B_2, and between 1.5 to 17.6 U/ml in five of group B_3.

Serum INF-γ and Anti-IL-2 Antibody Level One to two U/ml of IFN-γ was detected in the serum of patients receiving 2-hr infusion of rIL-2 (groups A_1 and A_2). However, IFN-γ was undetectable in the serum of patients treated with 24-hr continuous infusion of rIL-2 (groups B_1, B_2, B_3 and C). Anti-IL-2 antibody was not detected in any patient receiving rIL-2 throughout the trial.

Change in Lymphocyte and Eosinophil Counts A significant decrease in lymphocytes was seen after the administration of rIL-2 (days 1 to 3) in all patients of groups A and B (A_1, $P<0.02$; A_2, $P<0.05$; B_1, $P=0.05$; B_2, $P<0.02$; B_3, $P<0.01$) (Fig. 2a and 2b). In group B_2, the total number of lymphocytes was reduced to <50% on days 1 to 3 and returned to the pretreatment level on day 5. In all patients of group B_3, the lymphocyte count was depressed for three to five days after the start of rIL-2 infusion and thereafter increased progressively to three times the pretreatment level on day 28 (Fig. 2b). The degrees of lymphocytosis in group B were related to the dose of rIL-2 administered. In group C, an increase in lymphocytes was reproducibly found during the intermission of rIL-2 administration.

An increase in peripheral blood eosinophils (>10%) was observed during the administration in 22 patients. In groups B_1, B_2, B_3 and C, eosinophilia (>20%) was observed in one, five, two and four patients, respectively. Eosinophils increased with rIL-2 administration, and began to decrease 14, 21 and 21 days later in groups B_1, B_2 and B_3 in spite of continuing infusion of rIL-2, respectively (Fig. 2b). In group C, eosinophils increased during rIL-2 administration and decreased during intermission.

Cell-mediated Cytotoxicity A reproducible increase in both NK and LAK activities was seen after rIL-2 administration in group A ($P<0.05$). Figure 3 shows the NK and LAK

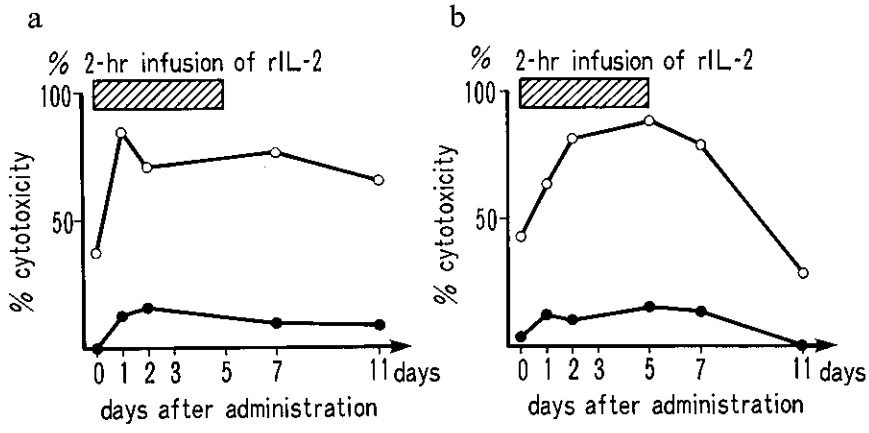

Fig. 3. Effects of rIL-2 on NK and LAK activities in group A (2-hr iv infusion for 5 consecutive days): A_1, 6.7×10^5 U/m^2/day (a); A_2, 2.2×10^6 U/m^2/day (b). NK and LAK activities were determined by a 5-hr ^{51}Cr release assay at an E/T ratio of 25:1 against K562 (○) and PC-9 (●), respectively.

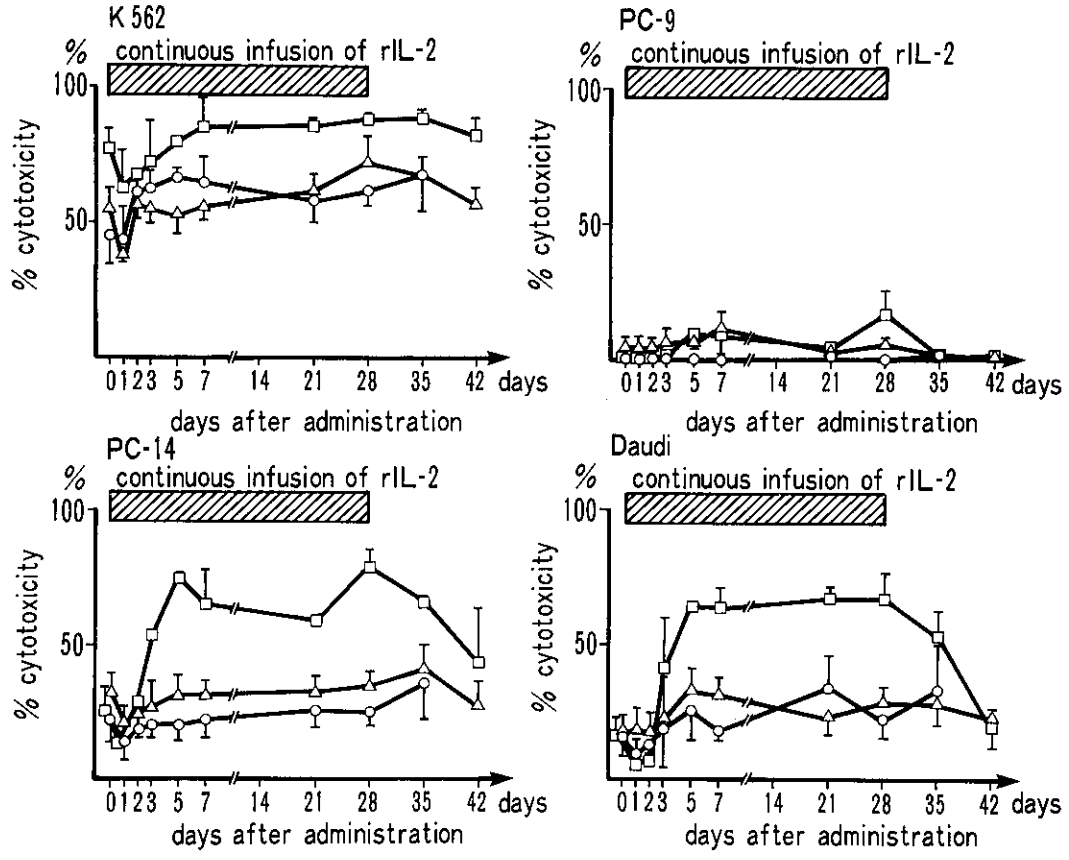

Fig. 4. Effects of rIL-2 on NK and LAK activities in group B (24-hr continuous iv infusion for 28 consecutive days): B_1, 3.3×10^5 U/m^2/day (○); B_2, 6.7×10^5 U/m^2/day (△); B_3, 1.1×10^6 U/m^2/day (□). Cytotoxicity against established tumor cell lines (K562, PC-9, PC-14 and Daudi) were measured by a 5-hr ^{51}Cr release assay at an E/T ratio of 50:1. Bars represent mean±SE.

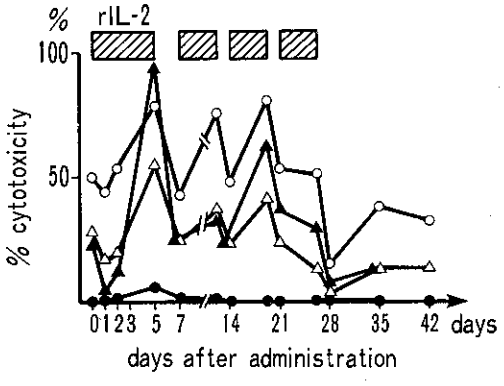

Fig. 5. Effects of rIL-2 on NK and LAK activities in group C (24-hr continuous iv infusion of 6.7×10^5 U/m^2/day for 5 consecutive days weekly, 4 cycles). NK and LAK activities were determined by a 5-hr ^{51}Cr release assay at an E/T ratio of 50:1 against K562 (○) for NK activity, and PC-9 (●), PC-14 (△) and Daudi (▲) for LAK activity.

activities in representative cases of groups A_1 and A_2. The cell-mediated cytotoxicities against four different cell lines in group B are shown in Fig. 4. NK activity against K562 and LAK activities against PC-14 and Daudi decreased transiently on day 1 in group B_3. In group B_2, the decrease in NK and LAK activities against PC-14 was observed on day 1. NK activity significantly increased five to seven days after the beginning of rIL-2 admin-

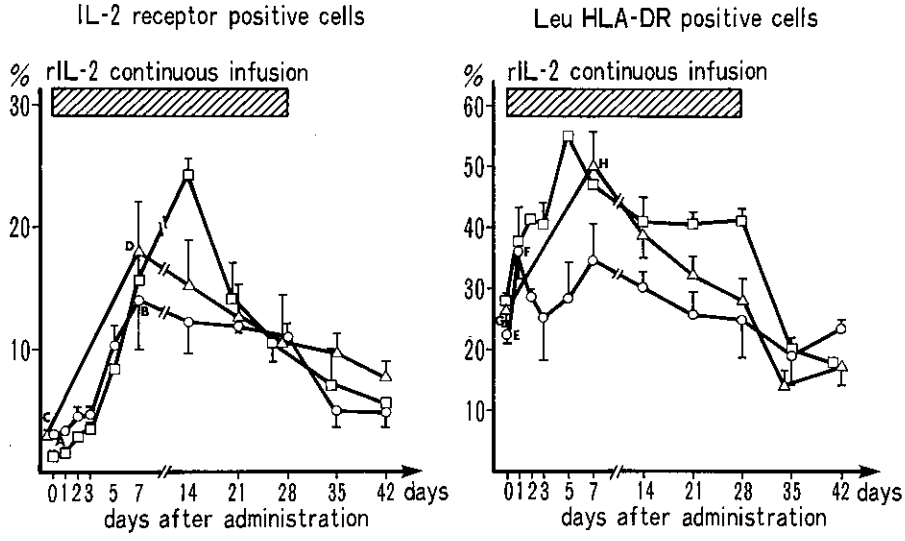

Fig. 6. Changes in IL-2 receptor positive and Leu HLA-DR positive cells in group B (24-hr continuous iv infusion for 28 consecutive days): B_1, 3.3×10^5 U/m^2/day (○); B_2, 6.7×10^5 U/m^2/day (△); B_3, 1.1×10^6 U/m^2/day (□). Bars represent mean±SE. *P* values: A vs B, =0.05; C vs D, <0.05; E vs F, =0.05; G vs H, <0.05.

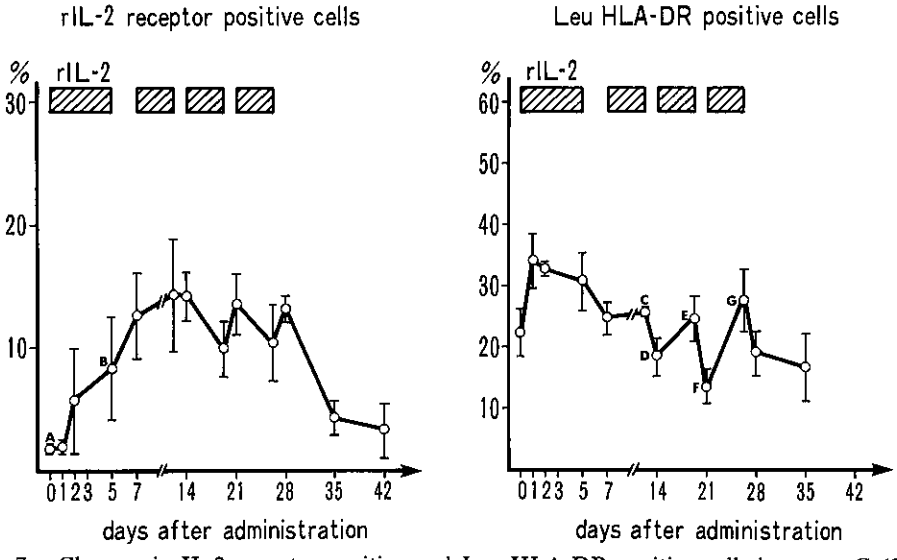

Fig. 7. Changes in IL-2 receptor positive and Leu HLA-DR positive cells in group C (24-hr continuous iv infusion of 6.7×10^5 U/m^2/day for consecutive 5 days weekly, 4 cycles). Bars represent mean±SE. *P* values: A vs B, =0.05; C vs D, <0.05; E vs F, =0.075; F vs G, =0.05.

istration in group B_3. In this group, LAK activities against PC-14 and Daudi cells were significantly augmented. LAK activity against Daudi cells was also increased in groups B_1 and B_2. Figure 5 shows representative chages in NK and LAK activities of lymphocytes in group C. The NK and LAK activities increased during rIL-2 administration, and decreased during intermission for two days.

Lymphocyte Surface Phenotype A reproducible relative increase in IL-2 receptor positive cells and Leu HLA-DR positive cells was seen on day 5 in peripheral blood in group A (data are not shown). The percentages of lymphocytes with the other surface phenotypes were unchanged.

In group B, IL-2 receptor positive cells markedly increased up to $14.4\pm4.0\%$ ($P=0.05$), $18.2\pm3.8\%$ ($P<0.05$), $24.2\pm1.4\%$ (NS: not statistically significant) in groups B_1, B_2 and B_3, respectively, and Leu HLA-DR positive cells were also increased up to $36.1\pm4.4\%$ ($P=0.05$), $49.9\pm5.7\%$ ($P<0.01$) and $46.9\pm0.4\%$ (NS) in groups B_1, B_2 and B_3, respectively. However, the percentages of Leu HLA-DR and IL-2 receptor positive cells decreased gradually in spite of continuing infusion of rIL-2 (Fig. 6). The percentage of IL-2 receptor positive cells on day 42 still remained higher than that before rIL-2 administration. Other lymphocyte surface phenotypes such as Leu 2a, Leu 3a+3b, Leu 4, Leu 7, Leu 11, OKIa1, OKT 3, OKT 4 and OKT 8 showed no significant change (data are not shown).

A similar increase in the percentage of IL-2 receptor positive cells was observed during administration of rIL-2 in group C (Fig. 7). The percentage of Leu HLA-DR positive cells in group C increased during continuous infusion of rIL-2, and decreased upon discontinuation of rIL-2.

Discussion

There are three strategies for treatment with IL-2. In the first, LAK cells are administered to support the host cellular immunity in the hope that administration of large quantities of the effector cells induced by IL-2 *in vitro* may be effective therapeutically.[23, 24] The second is to induce LAK cells *in vivo* by administration of IL-2. In 1985, as the third approach, Rosenberg *et al.*[25, 26] obtained exciting results by the administration of rIL-2 and LAK cells, registering a 44% response rate in 25 advanced tumors, including one complete response. However, the most effective dose and method of rIL-2 administration have not been identified. This study was conducted to find the most appropriate dose and method of rIL-2 administration for the maximum antitumor effect by using three schedules.

Toxicities observed were similar to those described in the previous report,[21] including fever, chills, anorexia, general malaise, mild gastrointestinal symptoms, rash and pruritus, with dose-limiting toxicities of hypotension, ascites and marked fluid retention. Weight gain was thought to be secondary to extravascular fluid accumulation, named "capillary leak syndrome."[26] The mechanism of hypotension and renal dysfunction is still unknown. Pleural effusion seems to be due to an aseptic inflammation associated with rIL-2 administration.

Although the mechanism of IL-2-induced fever is unclear, fever may be caused by rIL-2 itself or contaminating endotoxins or other substances. Since IFN-γ was detected only in group A and high fever was experienced in patients of groups B and C it is possible that inducible cytokines other than IFN-γ may contribute to the fever associated with IL-2.

The maximum tolerable dose of rIL-2 was considered to be 6.7×10^5 U/m^2/day in all the schedules of administration.

We have previously demonstrated that cytotoxicity of lymphocytes to K562 and PC-9 was significantly enhanced by incubating PBL with more than one U/ml of rIL-2 for 72 hr *in vitro*,[27] suggesting that it is desirable to maintain at least one U/ml IL-2 level for more than three days to generate LAK cells *in vivo* effectively. As an initial trial, we administered rIL-2 by 2-hr iv infusion. Although more than one U/ml of serum IL-2 activity was maintained for at least 6 hr with this method, no activity was detected in the serum 24 hr after rIL-2 administration, and a significant decrease in lymphocyte counts was seen from days 1 to 3 after the beginning of the rIL-2 administration. On the other hand, NK and LAK activities remained at high levels during seven days after the beginning of treatment. Then we tried to give rIL-2 by 24-

hr continuous infusion to maintain an adequate serum IL-2 level (>1.0 U/ml) continuously. Five among 15 patients in group B achieved more than one U/ml of IL-2 in the serum constantly for 28 days, and the other ten maintained at least 0.7 U/ml. In these groups the initial decrease in lymphocyte count was similar to that in group A. However, the lymphocyte count thereafter progressively increased. NK and LAK activities were consistently elevated in group B_3, and NK and LAK activity against Daudi cells were augmented in group B_2. These results suggest that treatment schedule B_3 is the best for further clinical trials. However, in three out of five patients in group B_3, treatment had to be stopped because of the severe side effects, suggesting that the dose of group B_3 is not generally tolerable.

Next we planned 24-hr continuous infusion of five consecutive days with two days off weekly, four cycles. The reasons for adopting this administration method were as follows.

1) As mentioned previously, serum IL-2 level was maintained at 0.7 to 15.2 U/ml in patients of group B_2 for 28 days, with subsequent enhancement of NK and LAK activities. 2) The ratio of IL-2 receptor positive lymphocytes to PBL markedly increased to reach the maximum level on day 7 in group B_2, but thereafter gradually decreased while rIL-2 was continuously infused, suggesting that precursors of LAK cells might be depleted. It was also reported that lymphocytes including LAK precursors increased markedly shortly after the discontinuation of rIL-2.[21] 3) Both NK and LAK activities were maintained during four weeks of administration of rIL-2 in group B_2.

With the method of group C, augmented NK and LAK activities and an increased number of IL-2 receptor positive lymphocytes could be maintained during four weeks of rIL-2 infusion. In this group the transient decrease in lymphocyte count as well as in NK and LAK activities was also observed one day after the beginning of treatment. The mechanism of the transient initial decrease in lymphocytes in groups B and C after the administration of rIL-2 still remains unclear.

The relationship between LAK activity and percentage of IL-2 receptor positive cells was not demonstrated. In group A, the percentage of IL-2 receptor positive cells did not increase upon rIL-2 administration, but the NK and LAK activities were augmented. In group B, the percentage of IL-2 receptor positive cells increased for seven or 14 days and later decreased gradually in spite of continuing infusion of rIL-2, without detection of anti-rIL-2 antibody, while NK and LAK activities remained at an increased level for four weeks. These results suggest that it may not be necessary for augmenting NK and LAK activities to increase IL-2 receptor positive cells. In group C, the change in percentage of IL-2 receptor positive cells was opposite to that of Leu HLA-DR positive cells during the intermission period. The percentage of Leu HLA-DR positive cells as well as NK and LAK activities decreased upon discontinuation of rIL-2, while IL-2 receptor positive cells increased. It is possible that lymphocytes with receptors unbound with IL-2 are increased by the intermission. However, a marked increase in IL-2 receptor positive cells during seven days after the beginning of rIL-2 cannot be explained. These results suggest that a rebound increase in IL-2 receptor positive cells and maintenance of IL-2 receptor positive cells can be expected as a result of a short discontinuation of rIL-2 administration.

Although no clinical partial response was found in this study by these three schedules, immunologic studies showed an increase of IL-2 receptor positive cells as well as of NK and LAK activities against tumor cells by rIL-2 administration, especially in 24-hr continuous infusion. The important question, whether or not the maintenance of IL-2 receptor bearing cells as well as NK and LAK activities at a high level could produce the optimal therapeutic effect, still remains unsolved. Attempts to find the best schedule of rIL-2 administration for optimal biological response will be continued.

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