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A phase I clinical trial of recombinant human tumor necrosis factor given daily for five days*

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Summary. A phase I trial of human recombinant tumor necrosis factor (rH-TNF) has been carried out in patients with advanced solid tumors. Sixty-six courses of the drug were given by 1 h IV infusion, daily for 5 days to 33 patients at doses of 5, 10, 20, 30, 45, 60, and 80×10^4 U/m^2/day. All patients received isotonic saline (up to 2 l/day) and either indomethacin or ketoprofen. Acute toxicity resembled that seen with the phase I study of a single dose (5). Dose limiting toxicity was acute, rapidly reversible, hepatic dysfunction and hypotension. Hypertension during drug infusion and dyspnea were marked in some patients. There was one complete and one minor response, both in patients with renal cell carcinoma. The dose of 80×10^4 U/m^2/day $\times 5$ was poorly tolerated and the recommended starting dose for phase II studies is 60×10^4 U/m^2/ day $\times 5$. Caution is recommended in treating patients with pre-existing hepatic function abnormalities, hypertension, hypotension or significant obstructive airway disease.

Introduction

Tumor necrosis factor (TNF) was first demonstrated in the serum of mice which had been treated with BCG (Bacillus Calmette Guerin) and then challenged with endotoxin [2]. The serum from these mice, when injected into tumor bearing mice, produced tumor necrosis. The gene for human TNF has recently been cloned and recombinant human TNF (rH-TNF) has been produced in *E. coli* [16].

rH-TNF, a nonglycosylated protein with a molecular weight of 17,000 daltons, has been evaluated for antitumor activity in vitro and in vivo. Of 26 human tumor cell lines tested, 5 showed a high degree of sensitivity, with an IC_{50} of <1 unit/ml [9]. When given i.v., it produced cures in vivo against the meth-A sarcoma and the colon 26 carcinoma but not against the B16 melanoma [9]. The recombinant TNF used in this study (rH-TNF Asahi) was clinically introduced in Japan in 1985 [12]; shortly thereafter, studies were initiated in the United States and Europe. To date, the material has been extensively evaluated on a number of different schedules, including single-dose, 24-h continuous infusion, and 5-day continuous infusion [4, 12, 15, 17, 18, 20]. Studies have also been reported of early clinical trials with TNF from other sources [1, 6, 11, 13, 19, 21]. Major dose-limiting toxicities have included hypotension, abnormalities of liver function, and thrombocytopenia. The 5-day continuous infusion demonstrated thrombocytopenia as a major dose-limiting toxicity [15].

In our initial clinical investigation of rH-TNF we studied the tolerance to single doses given every 3 weeks, with intra- and interpatient escalation. Toxicity was largely acute, with systemic toxicity resolving in 24 h. Hypotension was the dose-limiting toxicity and the maximum tolerated dose was 48×10^4 units/m^2 [4]. The present study was initiated with the following objectives: (1) to determine whether higher total doses could be given if the drug was subdivided and given daily over 5 days; (2) to determine whether dose-limiting, systemic, acute toxicity could be circumvented by giving the drug in this way; (3) to characterize the dose-limiting toxicity of daily administration; (4) to attempt to evaluate the effect, if any, of ketoprofen on the acute hypotensive effect of the drug; and (5) to explore any tachyphylaxis in toxic manifestations with daily dosage. The starting total dose chosen (25×10^4 units/m^2) was approximately 50% of the highest single dose given in the previous study. This was subdivided and given as 5×10^4 units/m^2 per day for 5 days.

Materials and methods

Drug. The drug was supplied by the Asahi Chemical Industry Company Limited (Tokyo, Japan) in vials containing 5×10^4 or 5×10^5 units. The material was purified to homogeneity by extraction and pretreatment followed by anion exchange chromatography, affinity chromatography, and gel filtration [8]. It was assayed for cytotoxicity against L-M cells. The activity (in units/ml) is defined as the reciprocal of the dilution resulting in 50% cell survival [10]; the specific activity of the material was 2.2×10^6 units/mg [8].

Patients. Patients with advanced cancer not amenable to other treatment or for whom other treatment had proven ineffective were entered in the study after their written, informed consent was obtained. The study protocol was reviewed and approved by the Institutional Review Board of Roswell Park Memorial Institute. The requirements for entry were an age of 18–70 years, an expected survival of

* Preliminary reports of some of these data have previously been published [3, 5]

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at least 2 months, at least a 3-week interval since the last dose of potentially myelosuppressive therapy (6 weeks for nitrosourea and mitomycin C) and recovery from reversible toxicity, a 2-week interval since radiation therapy or surgery (except minor procedures), and the absence of acute intercurrent complications, pregnancy, or a history of asthma. The minimal hematologic parameters required were a WBC count of $3.5 \times 10^3/mm^3$ and platelet count of $10^5/mm^3$. The minimal biochemical parameters required were an SGOT level of ≤ 100 IU/l and serum bilirubin and serum creatinine levels of ≤ 2 mg/dl.

Patients were monitored as follows: temperature, pulse, and blood pressure (BP) every 2 h for 12 h and then every 4 h until they returned to baseline after each dose; complete blood count, including differential count and platelet count, daily during treatment and weekly between courses; serum chemistry (Na^+, K^+, CA^{2+}, PO_4^{3-}, creatinine, uric acid, total protein, albumin, bilirubin, alkaline phosphatase, lactate dehydrogenase, SGOT, and blood urea nitrogen) was recorded pretreatment, on day 5, and then weekly (at higher drug doses measurements were done daily). Prothrombin time, partial thromboplastin time, serum fibrinogen, fibrin degradation products, and fibrin monomer were measured at 48 h and then weekly for 3 weeks. An electrocardiogram was recorded at 24 h after treatment.

All patients were hospitalized for the duration of the treatment. On the day before treatment blood was drawn for antibodies to TNF, and 1 h prior to infusion of the first daily drug dose, each patient was skin-tested by the i.d. injection of 0.025 ml infusion solution.

At doses of $5-45 \times 10^4$ units/m^2 per day, patients were begun on indomethacin (50 mg orally 3 times daily) on the day before the start of therapy, and indomethacin was continued throughout the 5 days of treatment. At doses of 60 and 80×10^4 units/m^2 per day, the patients were pretreated with ketoprofen (75 mg orally 3 times daily) instead of indomethacin, and this drug was also continued throughout the 5 days of rH-TNF treatment. In addition, 12 h before the initiation of drug therapy, patients were started on an i.v. infusion of normal saline (2 l/24 h). Hydration was continued throughout the 5 days of treatment except when an undue increase in body weight indicated that it should be discontinued.

Results

Treatment

A total of 33 patients were entered in the study; patient characteristics are listed in Table 1. In all, 85% of the patients were fully ambulatory, with a performance status (PS) of 0 or 1. A total of 66 courses were given (Table 2); 7 courses were terminated prematurely for drug-related reasons, 4 of which involved respiratory distress, 2, hepatotoxicity, and 1, hypotension. The respective doses (units/m^2 per day) were 20, 30, 60 (3 patients), and 80 (2 patients). The two patients who had incomplete courses at 80×10^4 units/m^2 per day subsequently received complete courses at 60×10^4 units/m^2 per day.

Table 1. Patient characteristics

			N	(%)
Entered			33	
Sex:	Male		23	70
	Female		10	30
Age:	(mean)		53.6 years	
	(range)		27–70 years	
Diagnoses:	Renal cell carcinoma		17	52
	Non-small-cell carcinoma, lung		6	18
	Squamous cell carcinoma, head and neck		3	9
	Adenocarcinoma pancreas		2	6
	Miscellaneous		5	15
Prior therapy:	Radiotherapy		8	24
	Chemotherapy		28	85
	Immunotherapy		2	6
Performance status (ECOG):	0	13		39
	1	15		45
	2	4		12
	3	1		3

Toxicity

Acute systemic toxicity was seen in all patients and did not differ markedly from that previously described [4]; the major systemic toxicities are shown in Table 3. Fever and rigors were not dose-limiting. Hypertension occurred during infusion, resolving rapidly after infusion was stopped. Pharmacologic intervention was required in 13 patients at doses of 20 (1 patient), 30 (2 patients), 60 (9 patients), and 80 (1 patient) $\times 10^4$ units/m^2 per day (pharmacologic intervention was generally considered when systolic BP >200 mm Hg or diastolic BP >110 mm Hg).

Hypotension occurred 2–15 h after drug administration; it was dose-related and appeared to display tachyphylaxis with repeated doses (Fig. 1). This effect was less marked at higher doses: the difference between the median nadir of systolic BP after days 1 (88 mm Hg) and 5 (97 mm Hg)

Table 2. Courses given at each dose level[a]

Patients	Dose (units/$m^2 \times 10^4$)							
	5	10	20	30	45	60	80	Total
New	6/5	5/3	7/6	5/5	6/3	13/9	2/2	44/33
Escalated from lower dose	–	3/3	8/5	3/3	2/1	4/4[b]	2/2	22/18
Total	6/5	8/6	15/11	8/8	8/4	17/13	4/4	66

[a] Number of courses/number of patients

[b] Includes two patients entered at 80 and deescalated to 60×10^4 units/m^2 per day

Table 3. Systemic toxicity

Toxicity	Dose (units/m² per day × 10⁴)							
	5	10	20	30	45	60	80	Overall
	Percentage of evaluable courses showing the toxic effect							
Rigors	100	75	75	100	63	88	100	84
Fever (T > 38°C)	17	50	25	86	75	94	75	63
Hypertension (Systolic BP > 160 mm Hg)	33	38	67	57	50	76	75	60
Back pain	50	38	17	29	88	82	75	55
Hypotension (Systolic BP < 90 mm Hg)	0	38	8	14	75	88	100	48
Nausea and vomiting	17	38	17	57	63	71	25	45
Headache	33	50	42	57	25	53	25	44
Peripheral cyanosis	67	38	25	57	0	59	25	40
Dyspnea	17	13	8	14	0	47	50	23
Neck pain/stiffness	–	–	–	–	–	53	–	15
Chest tightness	–	–	17	–	–	18	25	10
Erythema	–	–	–	–	13	6	25	5
Diarrhea	–	–	8	–	13	–	–	3

at a dose of 60×10^4 units/m² per day is, however, significant ($P < 0.02$, Mann-Whitney rank-sum test). One patient receiving 20×10^4 units/m² per day and six patients given 60×10^4 units/m² per day required extra fluid for BP support; in addition, one patient receiving 80×10^4 units/m² per day required an infusion of dopamine.

Laboratory studies

Hematologic changes. Leukopenia occurred sporadically in a non-dose-related fashion throughout the study, being observed in 18 courses in 11 patients. The median nadir of WBC in these 18 courses was 2.9×10^3/mm³, generally occurring on days 3–5. Thrombocytopenia did not occur. Myelosuppression was not a clinically significant toxicity. The most striking hematologic change was a variable but, in some patients, marked leukocytosis and left shift seen on day 1 at the highest doses (Fig. 2). (The day of treatment is day 0). This resolved rapidly, even with continued drug administration. As with the single dose, sporadic eosinophilia (eosinophil count > 700/m³) and monocytosis (monocyte count > 1000/mm³) were seen, tending to recur in subsequent courses in the same patient: monocytosis was observed in 22 courses in 15 patients and eosinophilia, in 4 courses in 2 patients.

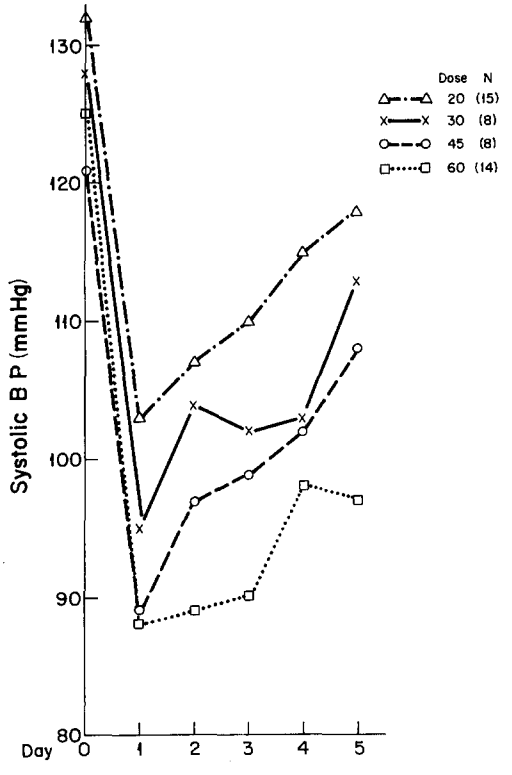

Fig. 1. Nadir of systolic BP following treatment with rH-TNF at doses of $20–60 \times 10^4$ units/m² per day. Each point represents the median of the lowest values recorded in the 24-h period for the courses given at that dose

Biochemical changes. The most important biochemical change was noted in liver function tests (Fig. 3). A rise in SGOT, generally maximal on day 1, was seen; however, it rapidly returned to normal in most patients during drug administration (Fig. 4). The increases in alkaline phosphatase and bilirubin did not show this pattern: at a dose of 80×10^4 units/m² per day, the two patients who completed the 5-day course had serum bilirubin levels of 3.1 and 4.5 mg/dl at the completion of the course; a third patient showed a rise in bilirubin from 0.3 to 2.1 mg/dl by day 2 (and a rise in SGOT from 8 to 370 IU/l), resulting in the discontinuation of treatment. Minor and clinically insignificant decreases in serum calcium and changes in coagulation parameters were seen.

Response

One patient with renal cell carcinoma with pulmonary metastases had a complete response, and a second patient with renal cell carcinoma with bony metastases had a minor response. The complete response lasted 3 months; the patient had multiple pulmonary metastases that diminished in size after one course of rH-TNF, diminished further in size and in number after two courses, and completely disappeared after a third course. A computerized axial tomographic (CAT) scan then revealed a brain lesion; however, biopsy of the lesion showed only blood clots and necrotic brain tissue. A follow-up CAT scan showed a second intracranial lesion, which was biopsied and tested positive

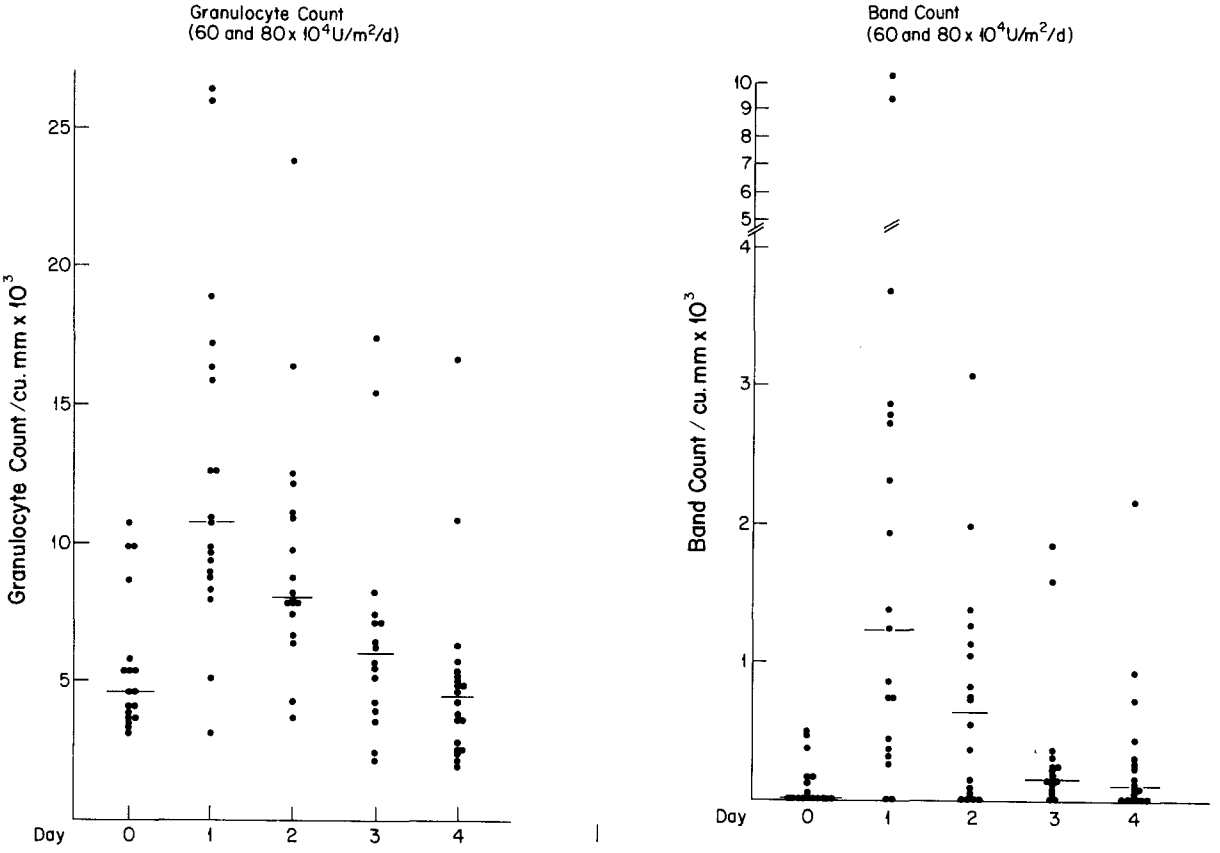

Fig. 2. Absolute granulocyte count (*left panel*) and absolute band count (*right panel*) during the 5 days of treatment with rH-TNF. The bars show the median values

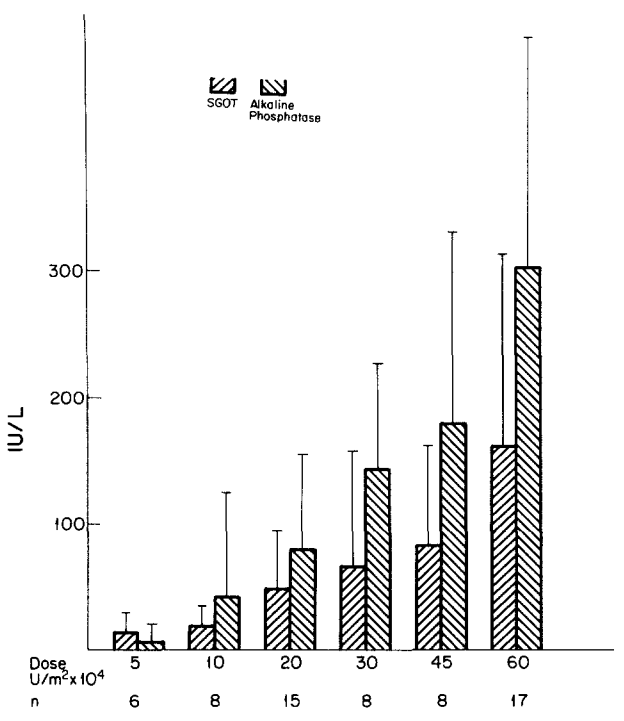

Fig. 3. Increase in SGOT and alkaline phosphatase following the administration of rH-TNF. Each value is a mean maximal increase over the baseline. The bars show the SD

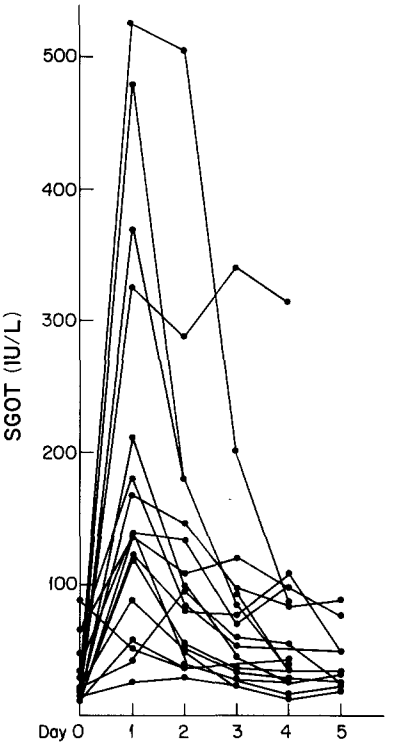

Fig. 4. SGOT during the 5-day administration of rH-TNF at a dose of 60×10^4 units/m^2 per day

for renal cell carcinoma. Because the patient was in systemic remission, this lesion was removed; the patient remained free of disease for 1 year after completion of treatment with rH-TNF, having received a total of four courses at a dose of 45×10^4 units/m^2 per day. The second patient, who had received treatment in the single-dose study and was subsequently entered in the present 5-day study, showed a >50% decrease in the size of a bony lesion by direct measurement. Although this partial response lasted for 3 months, other lesions remained unchanged.

Discussion

In the previously reported study of rH-TNF using single-dose administration, the highest dose reached was 48×10^4 units/m^2 per day and hypotension was dose-limiting [4]. Kimura et al. [12] reported a dose of 50×10^4 units/m^2 per day as the highest safe dose when hypotension was taken into account, although doses up to 160×10^4 units/m^2 per day were given when hypotension was circumvented. The maximum tolerated dose (MTD) was considered to be 120×10^4 units/m^2 per day, and hepatotoxicity and thrombocytopenia were considered to be dose-limiting in that study. In the present study, four patients were entered at a dose of 80×10^4 units/m^2 per day; three developed hepatotoxicity, and one developed hypotension requiring dopamine. We consider this dose to be too high and believe that 60×10^4 units/m^2 per day is the MTD. The latter dose caused significant hypertension during infusion, hypotension following drug administration, and abnormalities in liver function tests, which were generally the dose-limiting toxicity.

The dose-limiting toxicity of this material appears to be schedule-dependent. Thus, the single-dose administration and the 24-h continuous infusion showed hypotension to be the dose-limiting toxicity [5, 17] and the 5-day continuous infusion was limited by thrombocytopenia [15], whereas in the present study, in which the drug was given daily for 5 days, hepatotoxicity was dose-limiting and no thrombocytopenia occurred.

A feature of the doses of 60 and 80×10^4 units/m^2 per day was marked leukocytosis with a left shift, occurring in approximately half of the patients treated. Leukocytosis at 24 h has been observed by Gabrilove et al. [6], who also noted an increase in serum cortisol. It is of interest that the blood counts returned to normal during drug administration. This tendency for a side effect to reverse under continued drug administration was also seen with the increase in SGOT, but not with that of alkaline phosphatase or bilirubin. Tachyphylaxis of the hypotensive effect of rH-TNF was also observed in some patients in this study.

Of interest in the present study was the occurrence of one complete and one minor response in two patients with renal cell carcinoma. Tumor shrinkage with TNF therapy has been reported in renal cell carcinoma by Blick et al. [1], and tumor shrinkage and/or patient benefit has been reported in a variety of other tumors, including gastric carcinoma [19], lymphoma [14, 19], colorectal carcinoma [1, 11, 13], multiple myeloma [7], and fibrous histiocytoma [20].

In the present trial, escalation of the daily dose beyond that which had previously been shown to be the tolerated single dose was carried out following reports that ketoprofen could prevent the hypotensive effect of TNF [12]. Since our studies were not carried out at the same doses with and without ketoprofen, no definitive statement about its effect can be made. Our experience, however, suggests that this drug possibly ameliorates the hypotensive effect of TNF.

The recommended dose for phase II studies is 60×10^4 units/m^2 per day $\times 5$. Treatment with saline and ketoprofen is recommended. Caution should be observed in treating patients with preexisting hepatic function abnormalities, hypertension, hypotension, or significant obstructive airway disease.

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References

1. Blick M, Sherwin SA, Rosenblum M, Gutterman J (1987) Phase I study of recombinant tumor necrosis factor in cancer patients. Cancer Res 47: 2986–2989
2. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72: 3666–3670
3. Creaven PJ, Huben RP, Takita H, Brenner DE, Cowens JW, Zera R, Proefrock A (1987) Recombinant human tumor necrosis factor (rH-TNF) daily $\times 5$ with ketoprofen: a phase I study. Immunobiology 175: 92
4. Creaven PJ, Plager JE, Dupere S, Huben RP, Takita H, Mittelman A, Proefrock A (1987) Phase I clinical trial of recombinant human tumor necrosis factor. Cancer Chemother Pharmacol 20: 137–144
5. Creaven PJ, Brenner DE, Cowens JW, Huben RP, Takita H, Arbuck S, Razack MS, Parsons J, Solomon JK (1988) Tumor necrosis factor: phase I trial of a daily $\times 5$ schedule. Proc Am Soc Clin Oncol 7: 66
6. Gabrilove JL, Starnes HF, Begas A, Casper E, Chapman P, Engert A, Jakubowski A, Templeton MA, Sherwin S, Brennan M, Oettgen HF (1987) Phase I studies of rTNF-α in a patients with advanced cancer. Immunobiology 175: 12–13
7. Gamm H, Meyenburg W, Herrmann F, Mertelsmann R (1987) Phase I trial of recombinant tumor necrosis factor-α (TNF) in advanced malignancy. Immunobiology 175: 22
8. Hayashi H, Kiyota T, Kajiwara J, Otsuka Y, Inada T, Yoshida N (1985) Physicochemical and biological properties of recombinant human tumor necrosis factor in large scale purification. In: Ishigami J (ed) Recent advances in chemotherapy. Proceedings of the 14th International Congress of Chemotherapy. University of Tokyo Press, Tokyo, pp 816–817
9. Hayashi H, Oishi J, Hori K, Maeda N (1985) Biological activities of recombinant human tumor necrosis factor. In: Ishigami J (ed) Recent advances in chemotherapy. Proceedings of the 14th International Congress of Chemotherapy. University of Tokyo Press, Tokyo, pp 818–819
10. Hayashi H, Kiyota T, Sakamoto H, Seto M (1985) An enzyme-linked immunosorbent assay for recombinant human tumor necrosis factor using monoclonal antibody. In: Ishigami J (ed) Recent advances in chemotherapy. Proceedings of the 14th International Congress of Chemotherapy. University of Tokyo Press, Tokyo, pp 820–821
11. Hersh EM, Schwartz J, Scuderi P (1987) Phase I study of tumor necrosis factor (TNF) administered by continuous intravenous infusion to cancer patients. Immunobiology 175: 94–95
12. Kimura K, Taguchi T, Urshizaki I, Ohno R, Abe O, Furue H, Hattori T (1987) Phase I study of recombinant human tumor necrosis factor. Cancer Chemother Pharmacol 20: 223–229

13. Moritz T, Niederle N, Kurschel E, May D, Schlick E, Schmidt CG (1987) Phase I study of recombinant human tumor necrosis factor in patients with advanced malignancies. Immunobiology 175: 23–24
14. Selby P, Hobbs S, Jackson E, Viner C (1987) Early clinical studies of recombinant human tumor necrosis factor. Immunobiology 175: 100–101
15. Sherman ML, Spriggs DR, Arthur KA, Imamura K, Frei E, Kufe DW (1988) Recombinant human tumor necrosis factor administered as a five-day continuous infusion in cancer patients: phase I toxicity and effects on lipid metabolism. J Clin Oncol 6: 344–350
16. Shirai T, Yamaguchi H, Ito H, Todd CW, Wallace RB (1985) Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor. Nature 313: 803–806
17. Spriggs DR, Sherman ML, Frei E (1987) Clinical studies with tumor necrosis factor. In: Bock G, Marsh J (eds) Tumor necrosis factor and related cytotoxins. Ciba Foundation Symposium 131. Chichester, Wiley, pp 206–227
18. Steinmetz T, Schaadt M, Schenk V, Tuschen R, Pfreundschuh M, Diehl V (1987) Phase I study of intratumoral (i.t.) and intravenous (i.v.) application of recombinant human tumor necrosis factor (rHuTNF) in patients with malignant diseases. Immunobiology 175: 8–9
19. Taguchi T (1987) Clinical studies of recombinant human tumor necrosis factor (rHu-TNF). Immunobiology 175: 37
20. Tanneberger St, Lenk H, Muller U, Shiga T (1987) Human pharmacological investigation of a human recombinant tumour necrosis factor preparation PAC-4D (Asahi) – a phase I trial. Immunobiology 175: 38–39
21. Wiedemann B, Theilmann L, Schule B, Kommerell B, Schlick E, Rath U (1987) Phase I trial of intravenous 24-h-infusion of tumor necrosis factor (TNF) in patients with gastrointestinal metastasing adenocarcinoma. Immunobiology 175: 105–106

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