this into consideration, it would appear that fotemustine, with a short and original treatment plan and an encouraging rate of objective response and stabilisation (74%) of lesions, may be proposed as safe chemotherapy in the outpatient management of recurrent malignant brain tumour.


1. Levin VA. Chemotherapy of primary brain tumors. *Neurol Clin* 1985, **3**, 855–866.
2. Kornblith PL, Walker M. Chemotherapy for malignant gliomas. *J Neurosurg* 1988, **68**, 1–17.
3. Walker MD, Green SB, Byar DP, *et al.* Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med* 1980, **303**, 1323–1329.
4. Levin VA, Hoffman WF, *et al.* BCNU–5-fluorouracil combination therapy for recurrent malignant brain tumors. *Cancer Treat Rep* 1978, **12**, 2071–2076.
5. Edwards MS, Levin VA, Wilson CB. Brain tumor chemotherapy: an evaluation of agents in current use for phase II and III trials. *Cancer Treat Rep* 1980, **12**, 1179–1205.
6. Montgomery JA. Chemistry and structure activity studies of the nitrosoureas. *Cancer Treat Rep* 1976, **60**, 651–664.
7. Filippesoai S, Colombo T, Bassani O, *et al.* Antitumor activity of the novel nitrosourea S 10036 in rodent tumors. *Anticancer Res* 1988, **8**, 1351–1354.
8. Jacrot M, Giroux B, Deloffre P. Activity of the nitrosourea fotemustine against a human glioma xenograft in the nude mouse. *Proc ECCO* 5, 1989, London, P-0492.
9. Khayat D, Lokiec F, Bizzari JP, *et al.* Phase I clinical study of the new amino acid linked nitrosourea, S 10036, administered on a weekly schedule. *Cancer Res* 1987, **47**, 6782–6785.
10. Zülca KJ. *Types Histologiques des Tumeurs du Système Nerveux Central.* Genève, OMS, Vol. 1, 1979.
11. Order SE, Hellman S, Von Essen CF, *et al.* Improvement in quality of survival following whole brain irradiation for brain metastasis. *Radiology* 1968, **91**, 149–153.
12. Maire JP, Guerin J, Dautheribes M, *et al.* Quality of treatment reponse and survival in patients with supra-tentorial malignant gliomas. In: Chatel M, Darcel F, Pecker J, eds. *Brain Oncology*. 's Gravenhage, Martinus Nijhoff, 1987, 323–328.
13. Kaplan EL, Meier P. Non-parametric estimations from incomplete observations. *J Am Statist Assoc* 1958, **54**, 457–481.
14. Wilson CB, Boldrey EB, Enot KJ. 1,3-bis(2-chloro-éthyl)-1-nitrosourea (NSC-409962) in the treatment of brain tumors. *Cancer Chemother Rep* 1970, **54**, 273–281.
15. Hansen HH, Muggia PM. Treatment of malignant brain tumors with nitrosoureas. *Cancer Chemother Rep* 1971, **55**, 99–100.
16. Georges P, Przedborski S, Brotchi J, *et al.* Effect of HECNU in malignant supratentorial gliomas: a phase II study. *J Neurol Oncol* 1988, **6**, 211–219.
17. Levin VA, Crafts DC, Norman DM, *et al.* Criteria for evaluating patients undergoing chemotherapy for malignant brain tumors. *J Neurosurg* 1977, **47**, 329–335.
18. Maire JP, Caudry M, Demeaux H, *et al.* Le traitement des gliomes malins sustentoriels. Analyse d'une série de 173 patients traités de 1975 à 1985 au CHU de Bordeaux. *J Eur Radiother* 1987, **8**, 1–13.


**Acknowledgements**—We thank Theresa Dunn for reviewing the manuscript, and Isabelle Fillonneau for her secretarial assistance.

Fotemustine was provided by Laboratoires Servier, GIDY, 45400 Fleury Les Aubrais, France.

*Eur J Cancer, Vol. 27, No. 7, pp. 856–863, 1991.*
*Printed in Great Britain*

*0277–5379/91 $3.00 + 0.00*


# Phase I Trial of Recombinant Human Tumour Necrosis Factor α in Patients with Advanced Malignancy

**Heinold Gamm, Albrecht Lindemann, Roland Mertelsmann and Friedhelm Herrmann**

**A phase I clinical trial was conducted with recombinant human tumour necrosis factor alpha (rhTNF-α) in 62 patients with advanced malignancy refractory to previous standard therapy. rhTNF-α was given as a 30 min infusion twice a day at 6 h intervals. A total of 10 different dose levels was escalated in cohorts of 6 patients ranging from 2.5 to 200 μg/m² twice a day for 5 days every second week for a total of 8 weeks followed by a 4-week observation period. Major side-effects of TNF-α therapy, seen in almost all patients studied, were fever and chills. As dose-limiting side-effects hypotension and liver toxicity were recorded in 4 of 5 patients treated with 200 μg/m² twice a day. Pharmacokinetic studies revealed a TNF-α serum half-life of 13 to 25 min, a dose-dependent decrease in TNF clearance, and a dose-dependent increase in the area under the time/concentration curve. No anti-TNF-α antibodies could be detected, except in 1 patient. Tumour response to TNF treatment was poor. Only in 3 of 57 evaluable patients was partial tumour regression observed.**
*Eur J Cancer*, Vol. 27, No. 7, pp. 856–863, 1991

## INTRODUCTION

TUMOUR NECROSIS FACTOR (TNF) was first identified by Carswell and coworkers [1] in 1975 as an antitumour activity found in the serum of BCG or *Clostridium parvum* primed animals upon treatment with endotoxin. In the first preclinical studies partly purified TNF caused not only a haemorrhagic tumour necrosis in the transplantable Meth A sarcoma model of mice, but also had *in vitro* antitumour activity against a broad spectrum of human tumour cell lines including human leukaemia lines [2–5], but not against diploid fibroblasts.

Tumour necrosis factor comes in two forms: TNF-α—a product of a variety of different cells including T-cells, B-

cells, natural killer cells, mononuclear and polymorphonuclear phagocytes; and TNF-β, also referred to as lymphotoxin, which is produced by cells of lymphoid origin only [6–9]. In addition to their direct effects on tumour cells, TNFs display antiviral properties [10, 11] and have been implicated as important mediators of immune and inflammatory responses [12]. In this regard TNF-α has been shown to provide a key signal for phagocyte activation by inducing release of secondary cytokines [12–15], by modulating expression of certain cell surface structures and by enhancing the functional repertoire of these cells [16].

In addition, a marked cytotoxic synergism of TNF-α and interferon-gamma (IFN-γ) has been demonstrated against a variety of murine and human tumour tissues *in vitro* [17–19]. Recently, synergism of interleukin-2 and TNF-α has also been reported [20].

The advent of recombinant DNA technology has allowed the production of large quantities of homogenously purified recombinant human TNF-α (rhTNF-α) [21] and this material has recently become available for clinical evaluation in patients with cancer [22–27].

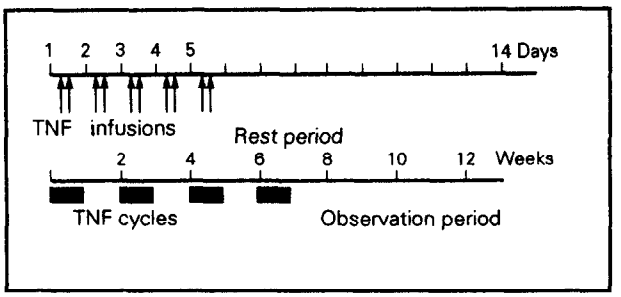
In this article we report on a dose-escalating phase I clinical trial of rhTNF-α in patients with advanced malignancy, aimed at determining the maximum tolerated dose (MTD) of TNF-α using a schedule of 30 min infusions twice a day, with 5 days of therapy and 9 days of rest (1 cycle). A total of four cycles was administered since *in vitro* experiments have shown that prolonged exposure to TNF is required for optimal cytotoxicity. To also obtain information on the *in vivo* antitumour efficacy in "idiosyncratic" tumours a broad spectrum of different neoplastic disorders was included in this study.

## MATERIAL AND METHODS

*Preparation of rhTNF-α*

rhTNF-α was produced by Genentech (San Francisco) and supplied by Boehringer Ingelheim International (Vienna). TNF-α is synthesised in *Escherchia coli* containing complementary DNA encoding TNF-α. Purity was assessed by high performance liquid chromatography HPLC and isoelectric focusing and found to be more than 99% pure. The specific activity was $5 \times 10^7$ U/mg of protein as determined by the lysis of actinomycin D treated mouse L 929 cells [28]. Endotoxin was less than 1 ng/mg of protein (by the limulus assay). Molecular cloning and further biochemical characterisation of the TNF material used were performed by Genentech [21]. This product is not glycosylated and has a molecular weight of approximately 17 000 daltons. Sterility, purity and safety met the standards of the Office of Biologics, Food and Drug Administration, USA and of the Bundesgesundheitsamt, Germany. The solutions for intravenous infusions of TNF-α were prepared by dissolving TNF-α in physiologic saline containing human serum albumin (2 mg/ml).

Correspondence to F. Herrmann, Department of Hematology and Oncology, University of Freiburg Medical Center, Hugstetter Strasse 55, D-7800 Freiburg i.B, Germany.
H. Gamm is at the Department of Hematology, Johannes Gutenberg-University, Mainz; and A. Lindemann, R. Mertelsmann and F. Herrmann are at the Department of Hematology and Oncology, Albert-Ludwigs-University, Freiburg, Germany.
Revised 26 Mar. 1991; accepted 9 Apr. 1991.




| Dose level | Daily dose (μg / m²) | No. of patients investigated |
|---|---|---|
| I | 5 | 6 |
| II | 10 | 6 |
| III | 25 | 7 |
| IV | 50 | 6 |
| V | 75 | 8 |
| VI | 100 | 6 |
| VII | 150 | 6 |
| VIII | 200 | 6 |
| IX | 300 | 6 |
| X | 400 | 5 |

**Fig. 1. Study design.**

*Patient selection and study design*

Only patients with histopathologically proven advanced and otherwise untreatable malignancies who had not received previous antitumour therapy for a minimum of 4 weeks were included in the study. Additional inclusion criteria for patients to be entered into the study were: a Karnofsky performance status of > 60, preserved renal function (creatinine < 2 mg/dl), preserved hepatic function [bilirubin < 1.5 mg/dl, prothrombin time (PT) < 1.3 × control], preserved haematological function [white blood cells (WBC) > 3000/mm³, granulocytes > 1500/μl, platelets > 100000/μl], preserved coagulation (PT < 14 sec, partial thromboplastin time < 17 sec, thrombin time > 90%, fibrinogen 150–400 mg/dl) and a minimum life expectancy of at least 3 months.

Written informed consent from each patient was obtained in accordance with institutional policy and the German Drug Law. Exclusion criteria included: the presence of CNS metastases, patients with significant cardiac disease (NYHA class II–IV), patients with serious active infections, patients with a history of bleeding disorders, hepatitis B surface antigen positive patients as well as pregnant and/or lactating women.

The treatment schedule is depicted in Fig. 1. At least 6 patients were entered sequentially at dose levels I–X. The daily dose was given by 30 min infusions twice a day (6 h between infusions) from day 1 to day 5. During this time the patients received daily 2000 ml NaCl (0.9%) over 24 h. After a rest of 9 days the second cycle was started at the same dose level. Patients who completed the fourth cycle were evaluated at monthly intervals thereafter for at least 6 months. Patients were monitored daily during the 5 days of TNF-α administration. All constitutional symptoms were recorded and classified as minimal (grade I), moderate (II), severe (III), and intolerable or life-threatening (IV). Vital signs were recorded before and 30 min, 1, 2, 3, 4, 6, 8, 12 h after the first TFN-α infusion (day 1). A medical history and physical examination were made before the initial dose of each cycle. Electrocardiogram and chest X-ray

were performed prior to initiation of treatment, during treatment and, if necessary, at the end of study. Complete blood count, coagulation profile, serum electrolytes, serum chemistry profile including renal and liver function tests, triglycerides, cholesterol and low-density lipoproteins, as well as urine analysis were obtained before treatment and at days 2, 5, 8, 15, 22, 50, 57, 71 and 85.

Tumour size was evaluated by physical examination or other appropriate methods, i.e. ultrasound and computed tomography of chest and abdomen as well as by assessment of tumour markers including carcinoembryonic antigen (CEA), alpha fetoprotein or beta human chorionic gonadotropin. Response criteria were classified according to UICC guidelines, i.e. complete remission (CR, disappearance of all clinical and laboratory signs for at least 4 weeks); partial response (PR, with a minimum of 50% reduction of tumour size, as measured by the sum of the products of the longest perpendicular diameters of indicator lesions without appearance of new lesions or progression of any lesions); minor response (MR, reduction of 25–50% of tumour size); no change (NC, unchanged disease) and progressive disease (PD, increase of 25% or more of tumour size or appearance of any new lesion). Patients receiving at least one complete cycle of therapy were considered evaluable for response.

*Pharmacokinetic studies*

In order to evaluate the pharmacokinetic properties of TNF-α, venous blood samples were collected from selected patients on the day of first application of both first and second cycle, at the following times: preinfusion and at 10, 20, 25, 29 (end of infusion), 30, 33, 37, 45, 60, 75, 90, 120, 150 min and 4 h after initiation of infusion. Blood samples were centrifuged. The serum was separated, frozen and stored at −20° until analysed.

TNF-α in the serum was measured by means of a sensitive and specific ELISA. The standard curve was performed with an in-house standard of Genentech, using the reference preparation TH 151A with a specific activity of $5 \times 10^7$ U/mg. The lower detection limits of the ELISA are 15.6–2000 pg/ml. The interassay variation coefficient is 10.4% at 1139 pg/ml, 13.3% at 223 pg/ml and 21.3% at 41.2 pg/ml. For the interassay variation the corresponding values are 4.3%, 6.1% and 10.4%, respectively. Serum concentration at each time were calculated as means of at least two separate tests (performed as duplicates). For the individual patients, serum/TNF-α curves were fitted to the observed date and evaluated using the peeling algorithm of the program PHARM [29]. In 13 cases a two-compartment model was necessary and in 5 cases a one-compartment model afforded the best fit to the data. Serum half-life, clearance and area under the curve (AUC) were calculated for individual patients.

*Anti-TNF-α antibodies*

For determination of anti-TNF-α antibodies, blood samples were collected before onset of therapy and on days 15, 29, 43, 51 or when the patients were removed from study, and 1 month after the last infusion. Serum samples were assessed for development of anti-TNF-α antibodies by a neutralising antibody bioassay that is based on a serum-free *in vitro* bioassay for the detection of tumour necrosis factor [28]. In this modification the "LM-cell" (murine connective tissue clone of L929 cells) sheet is checked in a double setting for lysis caused by untreated serum and that caused by serum that has been previously incubated with a known amount of TNF-α. In the absence of anti TNF-α antibodies the cell-sheet should be intact with serum alone, but destroyed with serum plus TNF-α. In the presence of neutralising anti-TNF-α antibodies the cell-sheet should not undergo lysis even with TNF-α added. Cell viability is visualised by staining with crystal violet and determining optical density at 540 nm.

*Table 1. Patients' characteristics*

| | |
|---|---|
| No. of patients | 62 |
| Median age (range) | 50 (16–74 years) |
| Sex (male : female) | 36 : 26 |
| Performance status (Karnofsky; range) | 80–90% (60–100) |
| Previous anticancer treatment | |
| Radiotherapy | 21 |
| Chemotherapy | 38 |
| Diagnostic groups | |
| Solid tumours | |
| Colorectal cancer | 18 |
| Renal cell cancer | 5 |
| Soft tissue sarcoma | 3 |
| Thyroid cancer | 2 |
| Melanoma | 2 |
| Pancreatic carcinoma | 2 |
| Squamous cell cancer | 2 |
| Ovarian cancer | 2 |
| Lung cancer | 2 |
| Others | 8 |
| Haematological malignancies | |
| Multiple myeloma | 4 |
| Acute non-lymphocytic leukaemia | 4 |
| Non-Hodgkin lymphoma | 3 |
| Acute lymphocytic leukaemia | 2 |
| Chronic myelogenous leukaemia | 2 |
| Chronic lymphocytic leukaemia | 1 |

## RESULTS

*Patients' characteristics*

62 patients with a broad spectrum of haematological and non-haematological malignancies entered the study. Patients' characteristics and diagnostic categories are summarised in Table 1. A total of 57 patients were considered evaluable for tumour response and 60 patients were evaluable in detail for tolerance of the therapy scheme.

*Pharmacokinetics*

18 patients were analysed for pharmacokinetics of TNF-α in the serum. TNF-α serum levels were detectable from doses of 25 μg/m² onwards. Serum half-life increased from 13 min (at doses of 25 and 37.5 μg/m²) to up to 26 min at the higher doses (Table 2). The maximal peak serum concentration reached 26.4 ng/ml at a dose-level of 200 μg/m² per day. Clearance decreased with increasing dose, while the AUC also increased. However, the AUC data did not support dose linearity. There was no evidence for accumulation of the drug in the blood with the schedule applied.

*Anti-TNF-α antibodies*

83 serum samples (baseline samples not included) from 49 patients were tested for antibody development. 1 patient whose pretreatment serum samples was negative showed a low positive antibody titre of 24 on day 29. A titre of 6 describes the lower detection limit for antibodies while titres under 6 already indicate a negative result. 9 days after discontinuation of therapy the

*Table 2. Pharmacokinetics*

| Dose (μg/m²) | Patients | Half-life beta phase (min) | Total clearance (l/min) | AUC (ng min/ml) |
|---|---|---|---|---|
| 25 | 2 | 13.3 (1.32) | 1.932 (0.07) | 24.73 (2.4) |
| 37.5 | 1 | 12.58 | 1.425 | 52.64 |
| 50 | 6* | 22.77 (3.6) | 0.731 (0.19) | 132.33 (48.8) |
| 75 | 2 | 19.42 (0.9) | 0.638 (0.08) | 259.37 (8.4) |
| 100 | 4 | 16.61 (3.1) | 0.456 (0.05) | 397.80 (49.7) |
| 150 | 1 | 26.81 | 0.498 | 542.45 |

Mean (S.D.).
Evaluation was done under the assumption that renal clearance was equal to total clearance. Creatinine values of the evaluable patients were all within the normal range. Clearances are computed assuming complete availability.
* From patients 2 separate series of samples, each eligible for pharmacokinetic evaluation, were made available, thus allowing 8 determinations.

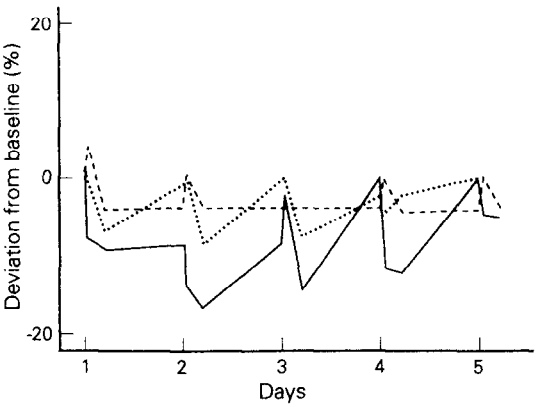



**Fig. 2. Variation of systolic blood pressure medians at different dose-levels upon intravenous administration of TNF-α. ----- 5–25 μ/m² per day ($n$ = 19), ····· 50–150 μg/m² per day ($n$ = 26), —— 200–400 μg/m² per day ($n$ = 14).**

anti-TNF-α antibody titre of this patient had returned to starting levels of below 6 (data not shown).

*Toxicity*

For evaluation of toxicities dose levels were clustered into three groups (5–25 μg/m², 50–150 μg/m² and 200–400 μg/m²).

*Constitutional symptoms*

The most common side-effects included chills and fever and were seen in almost all patients at all dose levels. Chills were not dose-dependent and not dose-limiting and generally appeared within 15 min after initiation of TNF-α infusion and resolved within 5–30 min. Fever was also a common side-effect, but seemed to be dose-dependent (Table 3). The highest fever peak occurred about 1 h after the start of infusion and returned to normal after 4–6 h. Both chills and fever were better tolerated with paracetamol—with or without the addition of pethidine—that was administered to the patients before start of infusion. There was no evidence for attenuation of fever or chills during subsequent infusions or cycles. Other common subjective side-effects included fatigue, headache, nausea, anorexia, myalgia and in some patients pain at the primary tumour site or site of metastatic lesion, which mostly occurred during TNF infusion. In general, constitutional side-effects were self-limiting and rarely exceeded grade II.

*Table 3. Summary of all systemic side-effects in various dose groups encountered with TNF-α therapy*

| Total treatment | Dose (μg/m² per day) 5–25 | 50–150 | 200–400 | Total |
|---|---|---|---|---|
| Days* | 272 | 353 | 211 | 836 |
| Chills | 82† (0.4)‡ | 82 (20.7) | 87 (22.3) | 84 (14.4) |
| Fatigue | 29 (0) | 74 (0.8) | 74 (0.9) | 59 (0.6) |
| Fever | 26 (0) | 44 (0.6) | 79 (13.7) | 47 (3.7) |
| Headache | 27 (1.1) | 58 (9.3) | 53 (12.3) | 46 (7.4) |
| Nausea | 16 (0.4) | 36 (4.2) | 49 (1.9) | 33 (2.4) |
| Anorexia | 17 (0) | 30 (2.8) | 54 (1.4) | 32 (1.6) |
| Myalgia | 24 (1.1) | 28 (3.1) | 27 (3.3) | 26 (2.5) |
| Vomiting | 13 (0) | 23 (1.1) | 24 (0.9) | 20 (0.7) |
| Diarrhoea | 2 (0) | 5 (0) | 4 (0.5) | 3 (0.1) |

* All days of treatment at the indicated dose level were included in this analysis.
† Values are expressed as percentage of applied days.
‡ Values in parentheses represent percent of application days of grade III and IV side-effects.

*Cardiovascular toxicity*

A dose-dependent reduction in blood pressure was seen in all patients. As the variation of systolic and diastolic blood pressure always changed in parallel, only the systolic values are shown in Fig. 2. The most pronounced effect was seen 5 h after the third infusion (first infusion of day 2). The mean difference for the highest dose group was 19% below base line value and was significant ($P < 0.01$) (Wilcoxon signed-rank test).

In the highest dose group (100–200 μg/m² per infusion) 3 patients experienced grade IV toxicity with a drop of systolic and diastolic blood pressure below 50% of baseline, requiring discontinuation of TNF. 1 patient also developed respiratory distress syndrome with pulmonary oedema. Another patient receiving 200 μg/m² per infusion developed milder hypotension (grade II).

*Metabolic changes*

A slight dose dependent increase of aspartate aminotransferase (AST) was seen in most of our patients (Table 4). 1 patient receiving 200 μg/m² twice a day of TNF-α experienced, however, liver toxicity grade IV with increase of AST from 7 to 301 U/l and alanine aminotransferase from 9 to 348 U/l on the second day of therapy. In the highest dose group serum alkaline phosphatase showed a statistically significant increase from 189 U/l to 358 U/l at day 2. Coagulation variables showed no significant changes. Only fibrinogen decreased slightly during the first 5 days of treatment to approximately 20% below baseline ($P < 0.01$), but this effect was not dose-dependent and no significant differences were found between day 0 and 15. All other parameters (renal function, electrolytes) remained unchanged during the treatment. Only triglycerides showed a dose dependent increase found on day 5 of the first cycle. This effect was, however, not statistically significant due to large variations between patients. Concomitantly, the total serum cholesterol (Table 4) and high-density lipoproteins (HDL) tended to decrease in these patients. Changes of immunoglobulin

*Table 4. Metabolic changes during first cycle of treatment with TNF-α*

| Parameter | Dose group* | Time points (1st cycle) 20/day 2 | 20/day 5 | 20/day 15 |
|---|---|---|---|---|
| AST (U/l) | I | 13/15 | 14/19‡ | 13/15 |
| | II | 25/16§ | 11/18‡ | 11/13 |
| | III | 14/33§ | 12/22 | 10/13 |
| Alkaline phosphatase (U/l) | I | 176/179 | 172/149 | 202/205 |
| | II | 159/170 | 159/195 | 164/190 |
| | III | 189/358‡ | 216/432 | 185/308§ |
| Bilirubin (mg) % | I | 0.4/0.30 | 0.3/0.4 | 0.4/0.5 |
| | II | 0.4/0.35 | 0.4/0.4 | 0.4/0.4 |
| | III | 0.3/0.30 | 0.3/0.6 | 0.3/0.4 |
| LDH (U/l) | I | 220/300 | 219/278 | 219/196 |
| | II | 204/235 | 206/227 | 221/234 |
| | III | 182/264‡ | 248/279 | 205/210 |
| PT (%) | I | 87/80 | 86/85 | 77/78 |
| | II | 100/85‡ | 100/98 | 100/100 |
| | III | 83/72‡ | 85/90 | 85/88 |
| PTT (s) | I | 35/33 | 32/34 | 34/34 |
| | II | 33/34 | 33/32 | 33/32 |
| | III | 35/39‡ | 35/36 | 35/34 |
| TT (s) | I | 17/17 | 17/17 | 17/16 |
| | II | 16/16 | 16/16 | 16/15 |
| | III | 17/16 | 17/17 | 17/16 |
| Fibrinogen (mg) % | I | 475/397 | 422/355‡ | 444/489 |
| | II | 409/352 | 409/350‡ | 432/378 |
| | III | 502/492 | 488/452 | 520/500 |
| Creatinine (mg) % | I | 1.0/0.9 | 0.9/0.9 | 0.9/0.9 |
| | II | 0.8/0.8 | 0.8/0.8 | 0.9/0.8 |
| | III | 0.9/0.9 | 1.0/0.8 | 1.0/0.8 |
| Uric acid (mg) % | I | 5.1/4.7 | 5.1/4.5 | 5.1/5.3 |
| | II | 5.4/4.5‡ | 5.4/4.5 | 5.4/4.6 |
| | III | 5.0/5.4 | 4.5/4.7 | 5.7/5.0 |
| BUN (mg) % | I | 14.5/11.0‡ | 14.0/9.0§ | 14.0/11.0 |
| | II | 12.0/10.0 | 12.0/7.0§ | 13.5/12.5 |
| | III | 13.0/14.0 | 13.0/9.0‡ | 13.5/14.0 |
| Total serum protein (g/100 g) | I | 6.60/6.65 | 7.00/6.90 | 7.00/7.25 |
| | II | 7.10/6.60 | 7.05/6.10§ | 7.10/6.70 |
| | III | 7.00/6.00 | 7.90/5.80‡ | 7.20/6.60 |
| Glucose (mg) % | I | 91/94 | 93/95 | 93/112 |
| | II | 92/95 | 92/96 | 90/90 |
| | III | 90/99 | 90/95 | 90/92 |
| Sodium (mmol/l) | I | 139.0/143.0 | 138.5/140.5 | 140.0/139.0 |
| | II | 139.0/138.0 | 140.0/138.0 | 140.0/140.0 |
| | III | 137.0/139.0 | 140.0/139.0 | 140.0/138.5 |
| Potassium (mmol/l) | I | 3.95/4.15 | 4.00/4.00 | 3.90/4.20 |
| | II | 4.00/3.90 | 4.05/3.80 | 4.10/4.30 |
| | III | 4.50/4.20‡ | 4.50/4.90‡ | 4.50/4.15 |
| Calcium (mmol/l) | I | 2.35/2.30 | 2.35/2.33 | 2.40/2.40 |
| | II | 2.35/2.28§ | 2.35/2.25§ | 2.35/2.40 |
| | III | 2.40/2.20‡ | 2.35/2.18 | 2.35/2.30 |
| Phosphorus (mg) % | I | 3.55/3.35‡ | 3.50/3.20 | 3.50/4.00 |
| | II | 3.60/3.00§ | 3.40/2.60§ | 3.35/3.35 |
| | III | 3.90/3.70 | 3.70/2.70‡ | 3.65/3.90 |
| Total cholesterol (mg) % | I | 214/158 | 217/162§ | 192/168 |
| | II | 181/195 | 206/161§ | 211/199 |
| | III | 152/142 | 207/114 | 154/214 |
| Triglyceride (mg) % | I | 119/112 | 122/156 | 115/109 |
| | II | 148/116 | 149/262 | 158/149 |
| | III | 136/109 | 120/258 | 150/213 |
| HDL (%) | I | 39/37 | 42/33 | 40/40 |
| | II | 42/37 | 49/23‡ | 49/42 |
| | III | 34/28 | 24/13 | 20/36 |

Pretreatment/day of treatment values. All values are medians of data available at time points compared.
LDH = lactate dehydrogenase, BUN = blood urea nitrogen.
* Group I = 5–25, II = 50–150 and III = 200–400 mg/m² per day.
The pretherapy values vary due to the fact that values missing at one or the other day for comparison led to a change in the number of patients being compared for each time point.
Comparison between pretherapy and day listed: ‡ $P \leq 0.01$, § $P \leq 0.001$.

levels (IgG, IgA and IgM), C reactive protein, beta-2-microglobulin and lysozyme were detectable (not shown) but were not statistically significant.

*Table 5. Changes in haematological parameters*

| Parameter | Dose group | Time points (first cycle) Day 0/day 2 | Day 2/day 5 | Day 0/day 5 | Day 0/day15 |
|---|---|---|---|---|---|
| Platelets | I | 333/303* | 303/286† | 296/216‡ | 269/340† |
| | II | 249/225† | 215/164‡ | 247/164* | 270/353* |
| | III | 352/279* | 289/140†* | 310/146‡‖ | 321/450 |
| Leucocytes | I | 6300/4700 | 4600/4800 | 5800/4600† | 5700/7800* |
| | II | 5200/5300 | 5100/2900‡ | 5000/2900‡ | 5100/6000‡ |
| | III | 9250/12350†‖ | 11800/5100‖ | 7750/5400 | 8100/8100 |
| Polymorphs | I | 3834/3560 | 3468/2830 | 3654/2508† | 3705/6084† |
| | II | 3215/2717 | 2730/1419‡ | 3215/1431‡ | 3644/4757‡ |
| | III | 5271/7395†‖ | 6322/1917§ | 4818/2183 | 4752/5150† |
| Lymphocytes | I | 756/752 | 699/746 | 756/507 | 1026/900 |
| | II | 825/696 | 672/598 | 769/624 | 769/998 |
| | III | 1512/1260 | 603/1075 | 1052/1282 | 1573/1508 |
| Monocytes | I | 360/265 | 253/372* | 400/476 | 509/362 |
| | II | 343/140† | 156/288† | 300/287 | 384/303 |
| | III | 426/201 | 184/407 | 389/329 | 387/411 |
| Erythrocytes | I | 4.42/4.26 | 4.18/4.03 | 4.42/4.04† | 4.57/4.46 |
| | II | 4.01/3.53‡ | 3.46/3.68 | 3.99/3.68* | 4.06/3.81 |
| | III | 4.24/3.78‡§ | 3.77/3.76 | 4.24/3.86 | 4.25/3.81* |
| Haemoglobin | I | 13.0/12.0* | 11.5/11.0 | 13.0/11.0‡ | 13.0/12.0* |
| | II | 12.0/11.0‡ | 10.5/11.0 | 12.0/11.0† | 12.0/11.0 |
| | III | 12.0/10.0† | 10.0/10.0 | 12.0/10.5 | 12.0/11.0* |

Pretreatment/day of treatment values. All values are medians of data available at time points compared.
Comparison between pretherapy and day listed: * $P \leq 0.05$, † $P \leq 0.01$, ‡ $P \leq 0.001$.
Comparison between the various dose groups: § $P \leq 0.05$, ‖ $P \leq 0.01$, * $P \leq 0.001$.

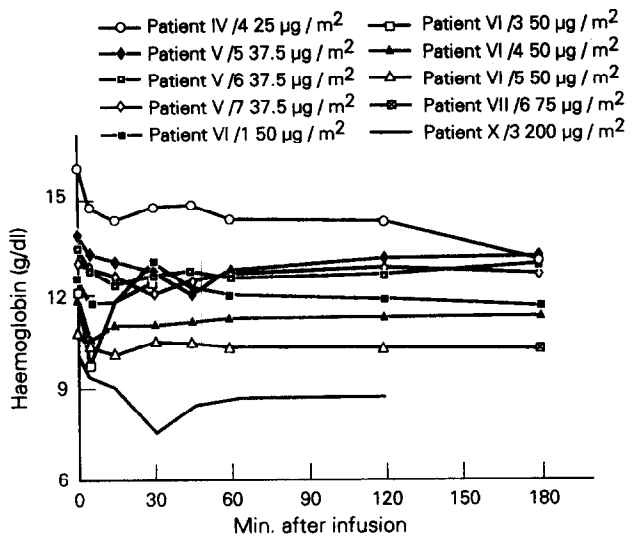



**Fig. 3. Changes in haemoglobin levels during the first 180 min after TNF-α injection.**

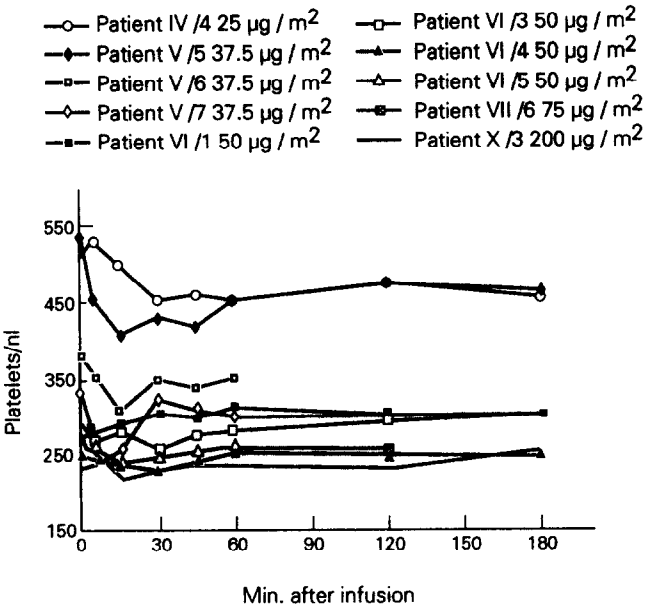



**Fig. 5. Changes in platelet counts during the first 180 min after TNF-α injection.**

*Changes in haematological variables*

*Haemoglobin.* A comparison of the medians of haemoglobin values for the three dose groups revealed a drop of approximately 10% on the second day of treatment (Table 5). For the lower dose range the values returned to normal in most cases but remained below baseline values for the highest dose group. In 10 randomly selected patients receiving doses from 25 to 200 µg/m² per infusion, the changes in haemoglobin were followed from the start of infusion up to 180 min thereafter (Fig. 3). The decrease of haemoglobin values generally occurred during the first minutes of TNF infusion and were most pronounced by the end of infusion. This effect did not appear to be dependent on either the dose applied or the pretherapy level of haemoglobin.

*Platelets.* As shown in Fig. 4 there was a significant decrease of platelet counts in all three dose groups during the 5 days of TNF infusion ($P < 0.004$ for dose group III, days 0 and 5). Interestingly, platelet counts normalised in the therapy-free interval in the dose group II and III but increased significantly above baseline levels in the lowest dose group. During infusion of TNF there was no variation of platelet counts (Fig. 5), as seen for haemoglobin values (Fig. 3) and leucocyte counts (Fig. 6).

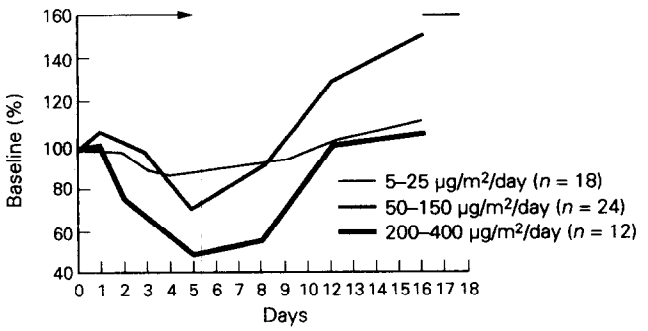



**Fig. 4. Changes of platelet counts at different dose levels upon intravenous administration of TNF-α.**

*Leucocytes.* The most prominent effects of TNF-α on leucocyte number were: a dose independent decrease during the 5 days of treatment, an increase above baseline for lower dose groups during the therapy-free period and an increase during treatment at higher doses on day 2 (Fig. 7).

In 8 patients at different dose levels differential blood counts were analysed every 10 min for up to 180 min after start of infusion (Fig. 6). During the 30 min TNF-α infusion there was a dramatic drop of granulocytes, monocytes and lymphocytes, but in contrast to the granulocytes that began to recover shortly after the end of infusion, recovery of monocytes and lymphocytes was more prolonged.

*Tumour response*

Among the 57 evaluable patients 3 showed a partial remission. 1 patient with metastatic colon cancer received 4 cycles of TNF-α at a dose of 5 µg/m² per infusion. Although during the treatment phase no reduction of tumour mass could be detected, CEA decreased from 146 ng/ml before treatment to 86 ng/ml on day 81. 5 months after discontinuation of therapy the metastasis involving the duodenum showed a decrease in size of less than 50% before therapy, and CEA had dropped to 16 ng/ml. Another patient (no. 32) with pancreatic carcinoma and liver metastasis of 4.4 × 3.4 cm showed a decrease in the size of the metastasis to 2 × 2 cm 8 weeks after start of therapy. The patient had no further treatment until he developed brain metastasis 8 months later. The third patient with chronic lymphocytic leukaemia received 4 cycles of TNF-α at a dose of 150 µg/m² infusion. Leucocyte counts dropped from 20 000/µl before onset of treatment to 15 200/µl at the beginning of the second cycle (day 15) and to 4600µl at the end of the fourth cycle (day 50). 4 weeks later, the patient still had a leucocyte count of 6900/mm³. No changes in platelet counts or haemoglobin values were detectable in this patient. 4 other patients had no tumour progression or minimal response during TNF therapy. Among them 2 patients had colon cancer: 1 patient received 5 cycles of rhTNF-α at a dose of 50 µg/m²/infusion. 7 weeks after the end of therapy one large liver metastasis showed

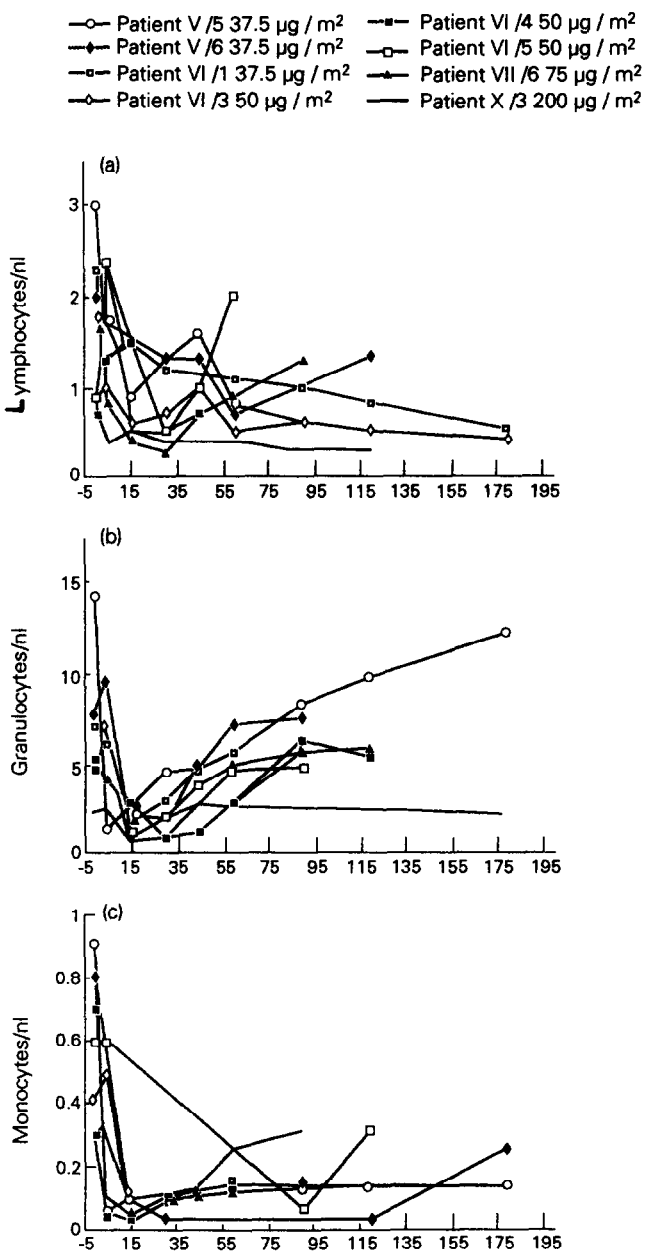



**Fig. 6. Changes in (a) lymphocyte, (b) granulocyte and (c) monocyte counts during the first 180 min after TNF-α injection.**

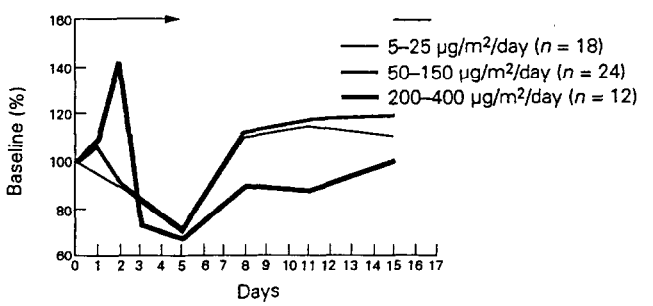



**Fig. 7. Changes in leucocyte counts at different dose levels upon intravenous administration of TNF-α.**

a reduction in size from 10 × 10 cm to 7 × 9.5 cm with increasing intrametastatic calcifications. CEA dropped from 5481 ng/ml before therapy to 3143 ng/ml on day 15, but increased again to 6788 ng/ml 7 weeks after end of treatment.

## DISCUSSION

This report describes the pharmacokinetics, tolerance and toxicity of rhTNF-α in patients with advanced malignancy refractory to standard anticancer therapy. The drug was administered as a 30 min intravenous infusion twice a day at 10 different dose levels (2.5–200 µg/m²) for 5 days every second week for a total of 8 weeks. A total of 62 patients was investigated. The clearance of TNF-α after intravenous infusion followed in most patients investigated a two-compartment model. Serum half-life of TNF-α ranged between 13 min for the lower doses and 26 min when higher doses were administered. Almost identical TNF-α half-life calculations have been described by others using a similar intravenous regimen [22]. Serum concentrations of TNF were detected at a dose of 25 µg/m² or greater, confirming previous findings by others [22]. The maximal peak serum concentration in our study reached 26.4 ng/ml at a dose of 200 µg/m², and was thus comparable to results obtained by others [23]. Continuously infused rhTNF-α (200 µg/m²) resulted only in serum TNF concentrations of 700 pg/ml or less [25]. In contrast to the intravenous route of administration, intramuscular administration of rhTNF-α has been shown to result in sustained serum concentrations [22].

The most frequent systemic toxicity encountered with TNF-α treatment were chills and fever that occurred in almost all patients. The pyrogenic effect of TNF-α may relate to a direct effect on hypothalamic thermoregulatory centres, but may also be mediated indirectly by inducing the release of interleukin-1 by phagocytes [12, 15]. Some patients also complained of pain at the site of primary tumour or metastases during TNF-α infusion. The dose-limiting toxicity was hypotension that was associated with increased fluid retention most likely due to TNF-α-mediated damage of the vascular endothelium administered, if intensive care level support were routinely employed, as it has been reported for treatment with interleukin 2 [27].

At the highest doses TNF-related hepatoxicity required treatment discontinuation in 1 patient. Haematological toxicity was not dose-limiting. Dose-dependent decreases in platelet counts were recorded in most patients most likely due to increased platelet aggregation or platelet consumption, but never requiring platelet transfusion. In this regard it is of note that an inhibitory effect of TNF-α on megakaryocyte progenitor *in vitro* growth has also been reported [18]. Whereas haemoglobin values remained unaffected by TNF-α treatment in most patients, mild to moderate but not dose-related decreases of peripheral leucocyte counts were recorded. Interestingly, a sharp decrease of circulating leucocytes (granulocytes, monocytes and to a lesser extent of lymphocytes) was observed within the first 120 min after TNF infusion. Occurrence of mild dyspnoea in some patients at this time suggests leucocyte trapping within the lung vasculature. Induction of leucocyte adhesion molecules by TNF-α may account for this observation. Slight decreases (up to 20% below baseline levels) of fibrinogen were recorded in one third of our patients during the first 5 days of TNF treatment that appeared, however, not to be dose-related. Prothrombin time or activated partial thromboplastin time was not altered.

As TNF-α is homologous to cachectin, the major inhibitor of lipoprotein lipase in adipocytes, we analysed various lipid parameters upon TNF-α treatment. Increases of serum trigly-

cerides and decreases of serum cholesterol and high-density lipoproteins were recorded, but the magnitude of these alterations was, however, small and statistically not significant.

We conclude that up to a dose of 150 μg/m² twice a day (300 μg/m² per day) TNF-α was well tolerated when the dose was split into two infusions.

It is not yet clear if this increase of MTD compared with MTD doses seen in patients subjected to single daily infusions might be useful to increase tumour response. Up to now there is no evidence to suggest a superior tumour response at higher doses of TNF-α. 2 of our patients with adenocarcinoma experiencing a partial remission had received lower doses of the drug (5 μg/m²). Also, the 4 patients with stable disease or minimal response had received doses of 150 μg/m² or less. One should caution as to the use of the phase I results as a positive or negative basis for antitumour efficacy. This is particularly important as the tumoricidal mechanisms of TNF are still poorly understood. Thus, we suggest the use of our study design in selected indications to assess the efficacy of the MTD while limiting the tumour load by selecting patients with only limited metastatic disease. Also, the synergistic antitumour effect of TNF-α with IFN-γ and IL-2 shown by others may lead to combination cytokine therapy that may result in improved tumour responses. Furthermore, toxicity of TNF-α infusions may be decreased by the use of indomethacin. Cyclooxygenase inhibition has also been reported to decrease toxicity of TNF infusions in animals [30]. Thus, administration of ibuprofen may prevent undesired side-effects of TNF.


1. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 1975, **72**, 3666–3670.
2. Carswell EA, Green S, Everson TC, Nathanson T, Biedler JL. Effect of tumor necrosis factor on cultured human melanoma cells. *Nature* 1975, **258**, 731–732.
3. Oettgen HF, Carswell EA, Kassel RL, *et al.* Endotoxin-induced tumor necrosis factor. *Recent Results Cancer Res* 1980, **75**, 207–212.
4. Haranaka K, Satomi N, Sakurai N. Antitumor activity of murine tumor necrosis factor (TNF) against transplanted murine tumors and heterotransplanted human tumors in nude mice. *Int J Cancer* 1984, **34**, 263–267.
5. Haranaka K, Satomi N. Cytotoxic activity of tumor necrosis factor (TNF) on human cancer cells in vitro. *Jpn J Exp Med* 1981, **51**, 191–194.
6. Männel DN, Moore RN, Mergenhagen SE. Macrophages as a source of tumoricidal activity (tumor-necrotizing factor). *Infect Immun* 1980, **30**, 523–530.
7. Aggarwal BB, Kohr WJ, Hass PE, *et al.* Human tumor necrosis factor. *J Biol Chem* 1985, **260**, 2345–2354.
8. Ganger G, Orr S, Yamamoto R. Lymphotoxins, macrophages cytotoxins and tumor necrosis factor: a interrelated family of antitumors effector molecules. *J Clin Immunol* 1985, **5**, 217–219.
9. Aggarwal BB, Henzel WJ, Moffat B, Kohr WJ, Harkins RN. Primary structure of human lymphotoxin derived from a lymphoblastoid cell line. *J Biol Chem* 1985, **260**, 2334–2344.
10. Mestan J, Digel W, Mittnacht S. Antiviral effects of recombinant tumor necrosis factor in vitro. *Nature* 1986, **323**, 816–819.
11. Wong GHW, Goeddel DV. Tomor necrosis factors alpha and beta inhibit virus replication and synergize with interferons. *Nature* 1986, **323**, 819–822.
12. Dinarello CA, Cannon JG, Wolff SM, *et al.* Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med* 1986, **163**, 1433–1450.
13. Munker R, Gasson J, Ogawa M, Koeffler HP. Recombinant human TNF induces production of granulocyte-monocyte colony-stimulating factor. *Nature* 1986, **323**, 79–82.
14. Oster W, Lindemann A, Horn S, Mertelsmann R, Herrmann F. Tumor necrosis factor (TNF)-alpha but not TNF-beta induces secretion of colony stimulating factor for macrophages (CSF-1) by human monocytes. *Blood* 1987, **70**, 1700–1703.
15. Nawroth PP, Bank I, Handley F, Cassimeris J, Chess L, Stern D. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. *J Exp Med* 1986, **163**, 1363–1375.
16. Larrick JW, Graham D, Tox K, Lin LS, Senyk G, Fendly BM. Recombinant tumor necrosis factor causes activation of human granulocytes. *Blood* 1987, **69**, 640–644.
17. Aggarwal BB. Characterization of receptors for human tumor necrosis factor and their regulation by gamma-interferon. *Nature* 1985, **318**, 665–667.
18. Brox Meyer HE, Williams DE, Lu L. The suppressive influences of human tumour necrosis factor on bone marrow hematopoietic progenitor cells from normal donors and patients with leukemia: synergism of tumour necrosis factor and interferon-γ. *J Immunol* 1986, **136**, 4487–4495.
19. Williamson BD, Carswell EA, Rubin BY, Prendergast JS, Old LY. Human tumor necrosis factor produced by human B-cell lines: synergistic cytotoxic interaction with human interferon. *Proc Natl Acad Sci* 1983, **80**, 5297–5401.
20. Owen-Schaub LB, Guttermann JU, Grimm EA. Synergy of tumor necrosis factor and interleukin 2 in the activation of human cytotoxic lymphocytes: effect of tumor necrosis factor alpha and interleukin 2 in the generation of human lymphokine-activated killer cell cytotoxicity. *Cancer Res* 1988, **48**, 788–792.
21. Gray PW, Aggarwal BB, Benton CV, *et al.* Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumor necrosis activity. *Nature* 1984, **312**, 721–724.
22. Blick M, Sherwin SA, Rosenblum B, Gutterman J. Phase I study of recombinant tumor necrosis factor in cancer patients. *Cancer Res* 1987, **47**, 2986–2989.
23. Feinberg B, Kurzrock R, Talpaz M, Blick M, Saks S, Gutterman J. A phase I clinical trial of intravenously administered recombinant tumor necrosis factor-alpha in cancer patients. *J Clin Oncol* 1988, **6**, 1328–1334.
24. Creaven PJ, Plager YE, Dupere S, *et al.* Phase I clinical trial of recombinant human tumor necrosis factor. *Cancer Chemother Pharmacol* 1987, **20**, 137–144.
25. Spriggs DR, Sherman ML, Michie H, *et al.* Recombinant human tumor necrosis factor administered as a 24-hour intravenous infusion. A phase I and pharmacologic study. *J Natl Cancer Inst* 1988, **80**, 1039–1044.
26. Gutterman JU, Fein S, Quesada J, *et al.* Recombinant leukocyte A interferon: pharmacokinetics, single-dose tolerance, and biologic effects in cancer patients. *Ann Intern Med* 1982, **96**, 549–556.
27. Atkins MB, Gould JA, Allegretta M, *et al.* Phase I evaluation of recombinant Interleukin 2 in patients with advanced malignant disease. *J Clin Oncol* 1986, **4**, 1380–1391.
28. Kramer SM, Carver ME. Serum-free *in vitro* bioassay for the detection of tumor necrosis factor. *J Immunol Meth* 1986, **93**, 201–206.
29. Gomeini R. Pharm: an interactive graphic program for individual and population pharmacokinetic parameter estimation. *Computer Biol Med* 1984, **14**, 25–34.
30. Kettelhut IS, Fiers W, Goldberg AL. The toxic effects of tumor necrosis factor *in vivo* and their prevention by cyclooxygenase inhibitors. *Proc Natl Acad Sci USA* 1987, **84**, 4273–4277.


**Acknowledgement**—This work was supported by grants from the Deutsche Forschungsgemeinschaft and Bochringer Ingelheim International.