*Table 3. Side-effects*

| | Regimen A | Regimen B |
|---|---|---|
| No. of evaluable patients | 57 | 62 |
| Slight sedation | 30 (53%) | 26 (42%) |
| Diarrhoea | 3 (5%) | 1 (2%) |
| Extrapyramidal reactions | 3 (5%) | 4 (6%) |
| Excitability | 4 (7%) | 2 (3%) |
| Epigastric and abdominal pain | 2 (4%) | 0 |
| Other (headache, dry mouth) | 9 (16%) | 8 (13%) |

emetic treatment. This effect was probably due to a slight imbalance between the two groups of patients of some important prognostic factors favouring emesis, such as age and cisplatin dose. Indeed, the group of patients treated first with regimen A had a slightly lower complete protection from vomiting with both antiemetic treatments and were also younger and treated with doses of cisplatin over 90 mg/m$^2$. We cannot explain the period effect on treatment preference since patients favoured the last antiemetic treatment given irrespective of the regimen they were receiving.

The single-dose regimen seems to be preferable on the basis of its therapeutic equivalence, simpler schedule of administration and lower cost.

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# Effect of Intraperitoneal Recombinant Human Tumour Necrosis Factor Alpha on Malignant Ascites

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**29 patients with refractory malignant ascites due to metastatic peritoneal spread of adenocarcinomas originating from the ovary, gastrointestinal tract, liver, breast and uterus were treated in a phase I trial of intraperitoneal infusions of recombinant human tumour necrosis factor alpha (rhTNF-α). Patients received 40–350 μg/m$^2$ rhTNF-α intraperitoneally once weekly for 2 months or for a shorter period in case of early resolution of ascites. Systemic side-effects resembled those reported for rhTNF-α given intravenously. No dose-limiting toxicities were found and thus a maximum tolerated dose of intraperitoneal rhTNF-α was not established. Out of 29 patients, 22 responded with a complete (16) or partial (6) resolution of their ascites. There was a less than 50% reduction in 4, and no increase in ascites in 1. 1 patient showed progressive ascites formation, and another patient was not eligible because of early death unrelated to treatment. Trials in patients with smaller tumour burden are warranted.**
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## INTRODUCTION

IN PRECLINICAL studies recombinant human tumour necrosis factor alpha (rhTNF-α) induced *in vivo* tumour regression in various models [1–3]. Phase I trials with intravenous bolus or long-term infusion (up to 5 days) or intratumoral injection gave dose recommendations for phase II studies. However, except after direct intratumoral injection, objective tumour response was minimal [4–6]. Since intraperitoneal administration of rhTNF-α in patients with malignant ascites in many aspects resembles direct intratumoral application, we started a phase I trial of short-term intraperitoneal infusion. All patients had

*Table 1. Patients' characteristics*

| | |
|---|---|
| F/M | 15/14 |
| Median age (yrs, range) | 58 (27–80) |
| Karnofsky index (%) | |
| 80 | 6 |
| 70 | 10 |
| 60 | 9 |
| 50 | 4 |
| Previous therapy | |
| Surgery, chemotherapy and radiotherapy | 4 |
| Surgery, chemotherapy and intraperitoneal chemotherapy | 5 |
| Surgery and chemotherapy | 15 |
| Chemotherapy alone | 5 |

ascites due to peritoneal spread of metastatic ovarian, breast, endometrial or various gastrointestinal tract adenocarcinomas.

The aims of the study were to establish the maximum tolerated dose (MTD) of intraperitoneally infused rhTNF-α, its dose-limiting toxicities, the recommended dose for phase II trials, the pharmacokinetic properties of TNF-α and its anti-ascites and possible antineoplastic activities.

## PATIENTS AND METHODS

*Patients*

29 patients (10 ovarian, 1 endometrial, 2 breast, 5 colorectal, 6 gastric, 1 hepatic and 4 pancreatic adenocarcinomas) with cytologically confirmed malignant ascites entered the study (Table 1). All patients had measurable and histologically proven disease and fulfiled the following eligibility criteria: (1) Karnosky index over 50%; (2) life expectancy over 2 months; (3) malignant ascites refractory to standard antineoplastic and diuretic treatment with 4 weeks since previous antineoplastic treatment; (4) normal or grade I impairment (WHO criteria) of haematological, hepatic and renal functions at entry; (5) no evidence of other severe non-malignant diseases; and (6) written informed consent. The study was done under the guidelines of the Helsinki declaration.

*TNF preparation and application*

Lyophilised rhTNF-α was provided by Knoll AG (Ludwigshafen). The compound was dissolved in distilled water and the required amount diluted in 500–2000 ml of 0.9% saline containing 0.5% human serum albumin. By means of a standard disposable plastic cannula, ascites fluid was completely drained if possible. After drainage, rhTNF-α was infused intraperitoneally over 20 min to 2 h. After the infusion, a sample of ascites was obtained and the cannula removed. At 24 h postinfusion a sample of ascites was obtained by ultrasonically guided fine-needle puncture. In the absence of major side-effects, weekly dose escalations (Table 2) were done until a complete stop of ascites formation or up to the end of the scheduled treatment period of 2 months.

*Ultrasound estimation of ascites volume*

The volume of the ascites was estimated by a standard abdominal ultrasound examination with a Picker LSC 7000 B-mode real-time scanner with a 3.5 mHz curved array scanhead. The ultrasound examination was done with the patient in a supine position, before entry into the study and before each treatment. The amount of ascites was graded semiquantitatively with a modification of the recommendations by Goldberg and Proto [7, 8]: no ascites = no fluid or only occasional thin layers of subdiaphragmatic and perihepatic fluid detectable (estimated intraperitoneal fluid volume 0–500 ml); minor ascites = formation of subdiaphragmatic and perihepatic fluid collections with layers of fluid around bowel loops (estimated volume 500–1000 ml); and major ascites = extensive fluid collections in all parts of the abdominal cavity with floating bowel loops (estimated fluid volume exceeding 1000 ml).

Therapeutic response for the ascites volume was graded thus: complete response (CR) = complete resolution of ascites; partial response (PR) = over 50% reduction of ascites volume; minor response (MR) or stable disease (SD) = less than 50% reduction or no increase in ascites volume; and progressive disease (PD) = increase in ascites volume.

*Clinical and laboratory examinations*

Before and at 2 and 24 h after each rhTNF-α infusion, the patient was clinically examined. The occurrence of headache, prostration, pain, chills, nausea/vomiting, loss of appetite, abdominal discomfort, signs of bleeding and allergic reactions were monitored. Vital signs, including pulse rate, blood pressure and body temperature, were measured before and at 1, 2, 6 and 24 h after the start of the infusion. The following laboratory indices were monitored before and 24 h after each infusion: haemoglobin, haematocrit, erythrocyte, reticulocyte, leucocyte and platelet counts and leucocyte differential count; partial thromboplastin time, thrombin time and fibrinogen; alkaline phosphatase, serum transaminases, gamma-glutamyl transferase, lactate dehydrogenase, total bilirubin, blood sugar, creatinine, urea, uric acid, cholesterol, triglycerides, total proteins, sodium, potassium, calcium and inorganic phosphate. Microscopic and chemical urine analyses was done before and 24 h after each infusion. All clinical and laboratory variables were graded with WHO criteria.

*Pharmacokinetic studies*

Plasma and ascites samples for assay of rhTNF-α levels were obtained from each patient immediately before, immediately after and 24 h after the start of infusion. These samples were frozen at −20°C until assay by ELISA [9] with two monoclonal antibodies that recognise two different epitopes of TNF-α (detection limit 5 pg/ml). The presence of serum antibodies to rhTNF-α was monitored by an enzyme immunoassay [6, 10].

*Cytological and immunological studies*

With the Papanicolaou classification, a cytological examination of the ascites was done before and 24 h after each infusion. Cellular compounds were isolated from the ascites fluid before and 1 and 24 h after completion of the infusion by density gradient centrifugation. From these cells a differential count of monocytes/macrophages, lymphocytes and granulocytes was done.

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*Table 2. Dose escalation and side-effects*

| | Dose (μg/m²) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 20 | 40 | 80 | 120 | 140 | 200 | 250 | 300 | 350 |
| Fever | 1 | 11 (48%) | 7 (47%) | 0 | 2 | 2 (25%) | 1 (14%) | 1 | 1 |
| Chill | 4 | 13 (57%) | 4 (57%) | 0 | 2 | 3 (38%) | 4 (57%) | 2 | 1 |
| Nausea/vomiting | 2 | 1 (4%) | 2 (13%) | 0 | 1 | 1 (13%) | 2 (29%) | 1 | 0 |
| Abdominal pain | 0 | 8 (35%) | 3 (20%) | | 1 | 4 (50%) | 2 (29%) | 2 | 1 |
| Fatigue/malaise | 1 | 14 (61%) | 5 (33%) | 1 | 2 | 3 (38%) | 2 (29%) | 1 | 0 |
| Loss of appetite | 1 | 6 (26%) | 0 | 1 | 1 | 1 (13%) | 1 (14%) | 0 | 0 |
| Pulmonary | 0 | 1 (4%) | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| No. of patients | 4 | 23 | 15 | 2 | 5 | 8 | 7 | 4 | 1 |
| No. of treatments | 4 | 23 | 16 | 3 | 5 | 8 | 8 | 4 | 1 |

To test capacity to produce TNF-α, the isolated mononuclear cells ($2 \times 10^6$/ml) were cultured either unstimulated or in the presence of *Staphylococcus aureus* for 16 h. TNF-α mRNA expression was measured by dot blot analysis with a TNF-α specific cDNA probe [11, 12].

In 4 patients, the natural killer cell activity of isolated mononuclear cells from the ascites fluid was measured before and 24 h after the infusion [10].

*MTD and concomitant medication*

MTD was defined as the dose of intraperitoneal rhTNF-α that induced reversible grade III or irreversible grade II toxicity. For the suppression of side-effects, such as fever and chills, most patients received 1 g paracetamol 1 h before and every 6 h postinfusion, for up to 24 h postinfusion.

## RESULTS

*Patients' characteristics*

Out of 29 patients who entered this study, 28 were evaluable for toxicity and response (Table 1). 1 patient with peritoneal carcinosis from a colonic adenocarcinoma died 1 week after he had received his first intraperitoneal rhTNF-α infusion, of a cause unrelated to treatment (cardiorespiratory and renal failure).

*Courses of treatment*

The total number of treatments as well as the number of treatments received by each patient are listed in Table 2. Except for 1 patient, who was not eligible for evaluation because of early death, the only reason for discontinuation of therapy was treatment response. The first 4 patients entered the trial at a starting dose of 20 μg/m² rhTNF-α. Because of excellent tolerance at this dose, the starting dose was increased to 40 μg/m². In 4 patients, the starting dose was 200 μg/m² and in 1 patient 250 μg/m². Because of a rapidly resolving ascites, this patient had no further dose escalation when receiving his second intraperitoneal infusion.

*Toxicity*

The most prominent rhTNF-α related side-effects are listed in Table 2. There was no correlation between dose and adverse events. As rhTNF-α related side-effects never led to discontinuation of therapy, the MTD has to be defined as being greater than the maximum dose given in this study (350 μg/m²).

There were no significant changes in leucocyte and platelet counts during the study. 2 patients, both with an extensive tumour load, required one blood transfusion each. No relevant changes in clotting indices, serum electrolytes and renal function were observed.

In patients with liver metastases, where liver function tests, gamma-glutamyl transferase and alkaline phosphatase were already elevated before entering the study, further changes related to rhTNF-α did not occur during the test period. Except for 2 patients with extensive liver metastases who showed an increase in serum bilirubin to a maximum of 4 mg/dl (WHO grade II), no changes in bilirubin were observed. Urine analysis did not reveal any abnormalities related to the test drug.

3 patients developed local erythema and pain at the site of the abdominal puncture; in 1 patient this was clearly caused by dislocation of the infusion cannula leading to a partly subcutaneous infusion. Under systemic non-steroid antiphlogistic treatment all changes returned to normal within 48 h.

*Pharmacokinetic studies*

In all patients, no serum levels of rhTNF-α were measurable before, or at 1 and 24 h after the infusion. This also applied to the measurement of anti-TNF-α serum antibodies.

The levels of the rhTNF-α determination in the ascites ($n = 16$) before, and at 1 and 24 h after the infusion are shown in Fig. 1. 24 h after the end of the infusion, up to 25% of the maximum rhTNF-α levels was still detectable in the ascites. From these data, an ascites half-life of 8–12 h of rhTNF-α can be estimated.

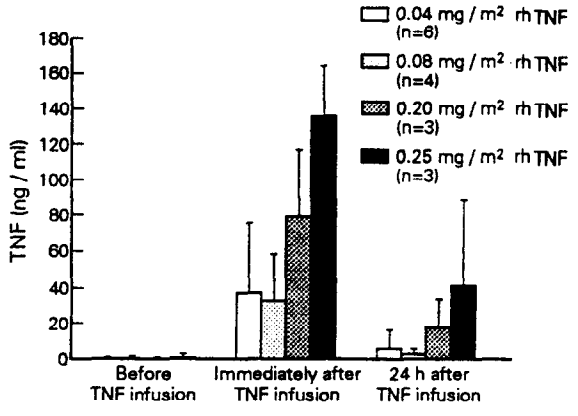


**Fig. 1. Mean (S.D.) TNF-α levels in ascites fluid.**

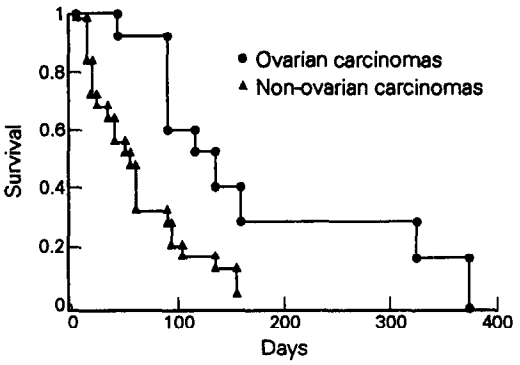


**Fig. 2. Kaplan–Meier survival curve.**

*Cytological and immunological studies*

In all patients intraperitoneal tumour cells were found on cytological examination. Follow-up examinations revealed an inconsistent pattern showing a complete disappearance or persisting presence of tumour cells unrelated to the resolution of ascites.

Differential count of cells isolated from the ascites fluid of 16 patients before and at 2 and 24 h after infusion revealed a uniform pattern. In absolute numbers monocytes/macrophages remained unaltered, granulocytes increased massively, and lymphocytes decreased 24 h after infusion. No significant changes in TNF-α production of cultured mononuclear cells, either stimulated or unstimulated, were seen during treatment. Analysis of rhTNF-α mRNA expression of these cells did not show any changes due to the application of rhTNF-α.

Natural killer activity levels of isolated ascites mononuclear cells in 4 patients remained unaltered under rhTNF-α treatment.

*Response, survival and long-term effects*

Out of 29 patients, 28 were evaluable for response: anti-ascites and antitumour effect of intraperitoneal rhTNF-α. 22 patients had a complete disappearance of ascites within the treatment period (CR + PR = 76%; 95% CI 56–90%). All 10 patients with ascites due to ovarian carcinoma responded and died without relapsing ascites formation within 16–360 days (CR = 100%; 95% CI 69–100%). In 15 patients with underlying gastrointestinal tract cancer there were 5 CRs and 4 PRs. In 2 patients with metastatic breast cancer there was 1 CR and 1 PR, and 1 PR occurred in a patient with metastatic endometrial carcinoma. The group of 6 non-responders could be subclassified into patients with less than 50% reduction of ascites (4 MR), no increase (1 SD), or progressive ascites formation (1 PD).

2 patients, who had an objective response initially (1 CR, 1 PR) showed recurrent ascites at the time of death. In 7 patients with an incomplete resolution of ascites after the scheduled treatment period, no ascites was found on ultrasound examinations before death (6–112 days after final infusion). Including these late results, the response rate in the non-ovarian cancer group was 78% (95% CI 49–91%).

In the time from the first intraperitoneal rhTNF-α infusion to the resolution of the ascites, there was no difference between ovarian carcinomas and tumours of other origin (15 vs. 20 days, respectively). The Kaplan–Meier estimate of survival from the start of treatment to death for all patients is shown in Fig. 2. Median survival of the patients with ovarian cancer was 139 (16–360) days compared with 56 (6–476) days in patients with gastrointestinal tract and other cancers.

## DISCUSSION

Intraperitoneal treatment of a metastatic tumour spread to the peritoneal cavity has been investigated with a wide range of antineoplastic agents as well as biological response modifiers [13]. Objective tumour responses have been described after intraperitoneal application of compounds such as *Corynebacterium parvum* and lymphokines such as recombinant alpha-2 interferon (IFN-$\alpha_2$) and interleukin 2 (IL-2) in patients with ovarian and colorectal cancer [14–17].

In this phase I study, intraperitoneal rhTNF-α was an effective palliative treatment for malignant ascites due to metastatic adenocarcinomas of various origins. Measurable tumour response, however, was not observed in our patients. This is probably due to the massive tumour load of patients entering phase I trials and the average number of only 2.5 intraperitoneal treatment cycles in patients responding with a complete resolution of their ascites.

Ascites-related responses were higher in patients with ovarian cancer. Recurrent ascites after pretreatment with intraperitoneal chemotherapy obviously did not influence this favourable outcome (Table 1). It is interesting, however, that the number of treatment cycles leading to response was the same in ovarian and non-ovarian cancer patients. The difference in response rates was probably related to the peritoneal tumour growth pattern of ovarian and non-ovarian cancer. Although a considerable peritoneal tumour load may be diagnosed at operation, in ovarian cancer it is usually distributed in fine nodules all over the peritoneal cavity and the omentum. In contrast, in non-ovarian cancer, involvement of intraperitoneal structures very often leads to palpable bulky tumours. Thus, given an identical tumour burden, the resulting contact surface per unit tumour volume available for a compound administered intraperitoneally can be considerably larger in ovarian cancer [18]. This may result in a higher therapeutic efficacy of the intraperitoneal drug.

Intraperitoneal rhTNF-α at the dose levels given was well tolerated with fully reversible moderate side-effects. The twofold increase in serum bilirubin in 2 patients with liver metastases from colorectal cancer was clearly related to rapid intrahepatic tumour progression. Since the toxicities observed were never dose-limiting, it was not possible to establish individual MTDs for intraperitoneal rhTNF-α. This is in contrast to our experience and that of others with intravenously applied rhTNF-α at similar dose levels [4–6] and findings from intraperitoneal IL-2 treatment in which well-defined MTDs were reached [16].

In animal experiments, approximately 20% of the intraperitoneal TNF concentration is found in the systemic circulation (E.S.). In this clinical trial, however, TNF-α plasma levels and antibodies to TNF-α were not detectable. With the assay used (lower detection limit 5 pg/ml) this was surprising and contrasts with other studies, where intraperitoneal IL-2 or IFN-$\alpha_2$ led to measurable serum concentrations [15, 16]. rhTNF-α has a similar molecular weight (17 kD) to IL-2 (17.5 kD) or IFN-$\alpha_2$ (19 kD). However, it is mainly trimeric, which may well result in a lower permeability of the peritoneal surface to this molecule [18] and in part explain the remarkably prolonged intraperitoneal half-life of rhTNF-α we found. In addition, the short plasma half-life of rhTNF-α and the low intraperitoneal doses given in this study may well contribute to extremely low non-measurable rhTNF-α serum concentrations. Obviously the concentrations achieved were high enough to trigger lymphokine-related systemic side-effects.

Several in part paradoxical effects of rhTNF-α in experimental ovarian cancer have been described: prevention of ascitic disease,

the induction of an inflammatory response, necrosis of omental tumours, increase in mouse survival time and promotion of the adhesion of tumour cells to the peritoneum [19].

Although the effect of intraperitoneal rhTNF-α on ascites formation was dramatic in our trial, the mechanisms of action remained unclear. A consistent pattern of massively increased intraperitoneal granulocyte counts in combination with a decreased number of lymphocytes 24 h after rhTNF-α application may indicate the induction of an inflammatory process. In addition TNF-α as an inflammatory mediator can significantly decrease the fibrinolytic activity of human omental tissue mesothelial cells [20]. All this could lead to subsequent fibrotic changes and sealing of the peritoneal surface. The results of our cytological examinations revealed no clear-cut cytotoxic action although in a number of patients we observed a decrease in the Papanicolaou score during treatment.

No enhancement of intraperitoneal TNF-α production of cultured peritoneal mononuclear cells, either stimulated or unstimulated, was observed and TNF-α mRNA expression by these cells remained unchanged during treatment. This was in contrast to our findings in isolated mononuclear cells from the peripheral blood after 24 h of intravenous rhTNF-α infusion [11].

Whilst others observed increased natural killer activity after intraperitoneal administration of *C. parvum*, IL-2 and IFN-$\alpha_2$, we found no changes in natural killer activity levels of intraperitoneal mononuclear cells after treatment [14–16]. Natural killer activity, however, was only assayed in a small subset of 4 patients and therefore might not be representative for our patient population as a whole.

Although no survival benefit was evident, our patients gained considerably from the palliative effect of intraperitoneal TNF-α. Patients who had to be admitted because of resistant gross ascites became free of concomitant clinical symptoms for their remaining life. Due to the design of this study, we only treated patients with advanced, therapy-resistant disease. We therefore believe that this mode of treatment merits further investigation in patients without heavy pretreatment and with smaller tumour burden. This especially refers to patients with ovarian cancer where often only minimal metastatic remnants in the peritoneal cavity are present after operative treatment.

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