Eur J Cancer Clin Oncol, Vol. 25, No. 2, pp. 287–291, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Intralesional Application of Recombinant Human Tumor Necrosis Factor Alpha Induces Local Tumor Regression in Patients with Advanced Malignancies

HELGE H. BARTSCH,* KLAUS PFIZENMAIER,† MICHAEL SCHROEDER‡ and GERD A. NAGEL*

**Division of Hematology/Oncology, Department of Internal Medicine, University of Goettingen, Robert-Koch-Str. 40, 3400 Goettingen, F.R.G., †Clinical Research Group 'Biological Regulation of Host-Tumor Interaction', Max-Planck-Society, Gosslerstr. 10 d, 3400 Goettingen, F.R.G. and ‡Department of Otorhinolaryngology, University of Goettingen, Robert-Koch-Str. 40, 3400 Goettingen, F.R.G.*

Abstract—*Fourteen patients with different advanced solid tumors were treated by intratumoral application of recombinant human tumor necrosis factor alpha. In five patients, local tumor regression occurred. However, the duration of response was short, implying a rapid development of resistance to rTNF-alpha application. The main clinical side-effects, including chills, fever, anorexia and fatigue, were similar to systemic rTNF-alpha treatment. Cardiovascular, pulmonary or metabolic toxicities were not observed. This study demonstrates that a high concentration of rTNF-alpha at the tumor site has the potential to induce local tumor regressions and, therefore, seems more reasonable for further clinical investigations, especially in combination with other cytokines.*

INTRODUCTION

TUMOR necrosis factor alpha (TNF-alpha), a cytokine predominantly produced by activated macrophages, exerts pleiotropic biological activities in normal and malignant human tissue [1–4]. Its cytostatic and cytolytic potential against malignant cells *in vitro* and *in vivo* [5, 6] created much interest in this cytokine for use as antitumoral substance in humans. Following purification and cloning of the human gene for TNF-alpha [7, 8], sufficient amounts of recombinant TNF-alpha are now available for conducting clinical trials. So far, phase I trials in patients with advanced malignancies receiving rTNF-alpha either i.v., i.m. or s.c. revealed only a low incidence of tumor responses [9–11]. We here report our experience from a clinical trial of rTNF-alpha administered as intratumoral injections in patients with different advanced solid tumors.

Accepted 21 September 1988.
Correspondence and reprint requests should be sent to: Dr. med. H.H. Bartsch, Division of Haematology/Oncology, Department of Internal Medicine, University of Goettingen, Robert-Koch-Str. 40, 3400 Goettingen, F.R.G.
This work was in part supported by Stiftung Volkswagenwerk and Deutsche Krebshilfe e.V.

MATERIALS AND METHODS

Patient characteristics

Fourteen patients were included in this trial after informed consent was obtained. Only patients with palpable, histologically proven malignant tumor, who had failed previous standard antitumor therapies, were eligible for the study. Patient characteristics are summarized in Table 1. The main exclusion criteria were the existence of CNS metastases, respiratory insufficiency with VC and/or FeV1 < 70% of predictive value, significant cardiac disease (NYHA II–IV), serious infections, pregnant and lactating women, patients with known history of allergic reactions, hemorrhagic diathesis or lipoprotein disorders.

Material and treatment schedule

Recombinant TNF-alpha was produced by Genentech, San Francisco, U.S.A., and kindly provided by Thomae GmbH, Biberach/Riss, F.R.G. The specific activity was 4×10^7 U/mg protein as defined by an *in vitro* cytotoxicity assay on actinomycin D treated mouse L929 cells [12]. Purity was >99% as detected by HPLC and isoelectric focussing. Endotoxin content was <1 ng/mg of protein (limulus test). Patients received rTNF-alpha intra-

Table 1. Patient characteristics

No.	Age	Sex	Diagnosis	Pretreatment	rTNF-alpha dose	Applications/ week	Total No. of applications	Response	Present status
1	31	f	Melanoma	S, BT	25	1	5	NC	Alive
2	62	m	Adenoca., lung	S	50	2	10	MR	Died of disease
3	16	m	Chondrosarcoma	S, CHT	50	2	8	PD	Alive
4	52	f	Adenoca., colon	S, CHT	50	3	22	PD	Alive
5	56	f	Breast ca.	S, CHT, HT, RT	75	3	6	PD	Died of disease
6	54	f	Liposarcoma	S, CHT	75	3	10	PD	Died of disease
7	57	f	Melanoma	S, CHT	100	1	4	PD	Alive
8	59	m	Squamous cell ca., oropharynx	S, CHT, RT	100	1	5	PR	Died of disease
9	63	f	Mucoepidermoid ca.	S	100	2	9	NC	Alive
10	61	m	SSLC	S, CHT, RT	100	3	10	NC	Died of disease
11	47	f	Breast ca.	S, CHT, HT, RT	150	2	19	PD	Died of disease
12	54	m	Histiocytoma	S, CHT, RT	150	3	32	PR	Alive
13	37	m	Melanoma	S, CHT	200	2	40	PR	Alive
14	67	m	Osteosarcoma	S	300	3	24	MR	Alive

S = surgery; BT = biological therapy; HT = hormone therapy; RT = radiotherapy; CHT = chemotherapy.

tumoral 1–3 × week, depending on the degree of local inflammation, for at least 4 weeks. Doses were escalated in different patients starting from 25 to 300 μg per injection. Physical examination, documentation of constitutional symptoms and vital signs as well as complete blood count, whole blood chemistry and coagulation profile were performed weekly. Photodocumentation of the tumor site and assessment of response, according to UICC criteria, was done at 4 week intervals. Local and systemic toxicity was documented for each rTNF-alpha injection and classified according to the WHO toxicity scale.

RESULTS

Response to intratumoral rTNF-alpha therapy

PR was achieved in three patients, MR in two patients, and stable disease in three patients. All responses were restricted to the site of TNF application. In patient 8, a remarkable decrease of the palpable tumor mass was seen after three injections of rTNF-alpha. Due to the clinical side-effects of rTNF-alpha therapy, the patient refused further treatment after a total of five injections and subsequent tumor growth was found. Patient 12 had undergone 4 × surgical removement, intensive chemotherapy and radiotherapy of an exophytic growing malignant histiocytoma located on the back (Fig. 1). After 3 weeks of rTNF-alpha treatment, the tumor necrotized and the skin lesion began to heal (Fig. 2). However, after 12 weeks of therapy, tumor growth was noted again and rTNF-alpha applications were discontinued. Patient 13 with a large tumor mass of malignant melanoma at the right upper chest not amenable to surgical treatment had received locoregional chemotherapy, without response. After 4 weeks of rTNF-alpha treatment, the tumor mass was not further palpable, the lymphedema of the right arm resolved completely, although a CT scan still demonstrated residual tumor within the pectoralis muscle. In two patients (2 and 14), the local regression of the tumor was ≥50%, but the size of pulmonary metastases did not change and, therefore, these patients did not qualify as PR.

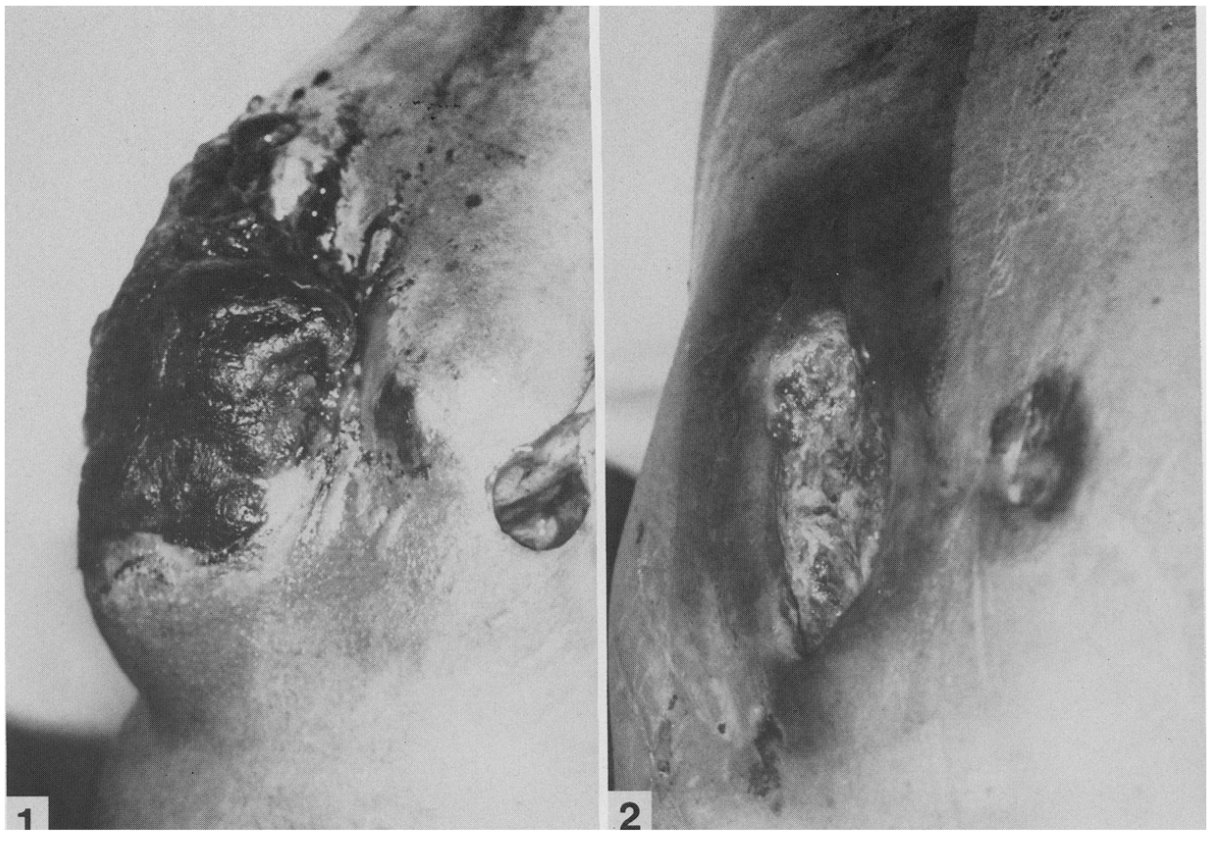
We were able to histologically re-examine the rTNF-alpha treated metastases of a small cell lung caner in patient 10. As Fig. 3 shows, there was a substantial number of degenerated cells, representing >50% of the whole tissue, whereas the CT scan demonstrated an unchanged volume of the metastases.

Toxicity of rTNF-alpha

The clinical side-effects of intralesional rTNF-alpha therapy were similar to our data reported from i.m. application of rTNF-alpha [11]. The main symptoms were chills, fever, local edema and pain at the site of injection. The severity and duration of symptoms were dose related. At rTNF-alpha doses >25 μg, chills appeared 10–25 min after injection with a duration of between 10 and 30 min. The maximal temperature elevations were measured 1–3 h following injection and most patients received antipyretic medication (paracetamol). Twenty-four hours after rTNF-alpha application local inflammation was noted and, in some patients, signs of hemorrhagic necrosis appeared (Fig. 4). No cardiovascular, pulmonary or CNS toxicity was observed. At the day of injection, all patients complained of anorexia and fatigue (WHO I–II). The laboratory investigations revealed no evidence of cumulative hematologic toxicity as well as unchanged liver and renal function. Within the dose range of this study, we found no influence on coagulation parameters.

DISCUSSION

The mechanisms responsible for the cytostatic or cytolytic action of TNF-alpha on malignant cells still need to be elucidated. There is evidence from *in vitro* studies that TNF-alpha selectively inhibits expression of certain oncogenes, which may play



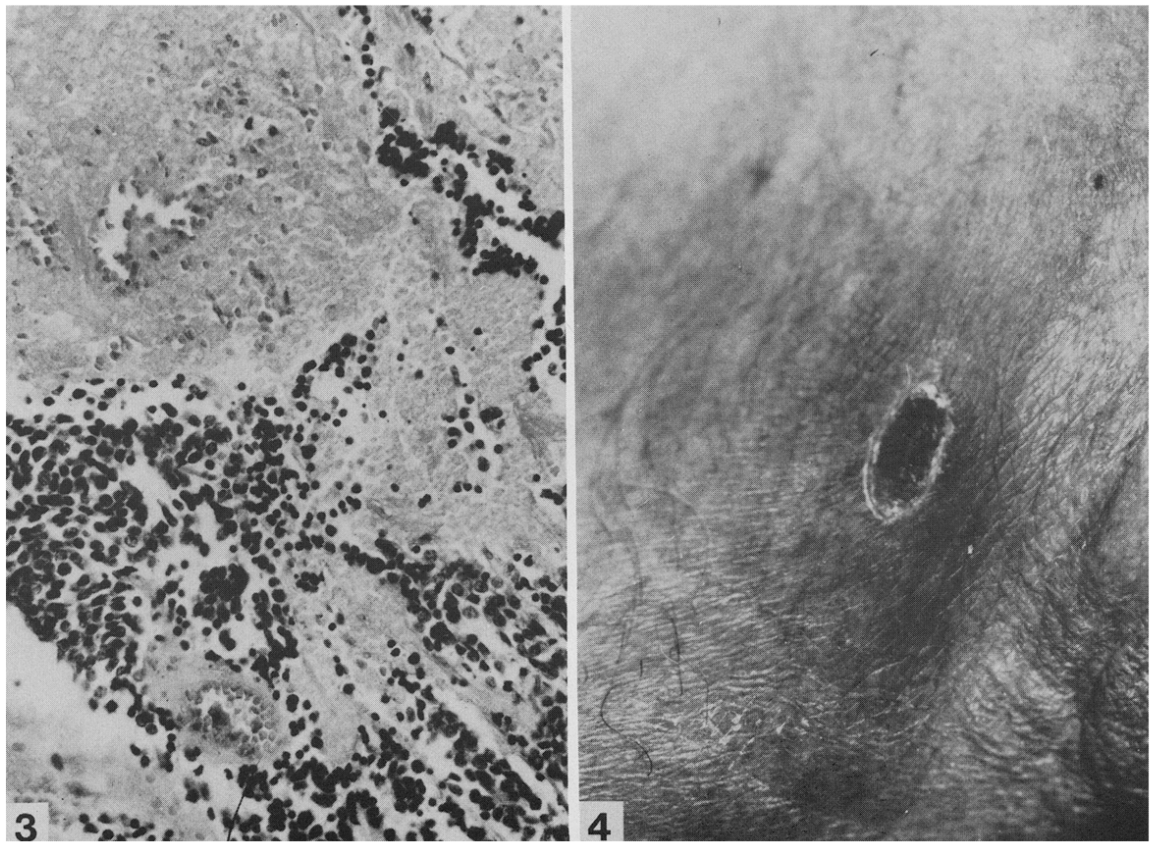


Fig. 1. *Ulcerating malignant histiocytoma* (18 × 15 cm) *on the back of patient* 12 *before starting treatment with rTNF-alpha.*

Fig. 2. *Three weeks after intratumoral rTNF-alpha therapy* (3 × 150 μg/*week*).

Fig. 3. *Large section of degenerating tumor cells beside residual viable cells of a small cell lung cancer* ('*oat cell carcinoma*') *metastasis following* 10 *intratumoral applications of rTNF-alpha* (*hematoxylin–eosin stain* × ca 150).

Fig. 4. *Hemorrhagic necrosis of a lymph node metastasis of adenocarcinoma following three intranodal injections with* 50 μg *rTNF-alpha.*

an important role in the control of growth and differentiation of malignant cells [13, 14]. In addition, TNF-alpha influences a variety of normal tissues and exerts strong and multiple immunomodulatory activities [1–4]. These actions of TNF might also contribute to the antitumor effects seen *in vivo* [6, 15]. Based on our data from a phase I trial of rTNF-alpha in patients with advanced malignancies [11] and observations made in an animal model [6] that the intratumoral application of partially purified TNF-alpha was more effective than intravenous injection, we investigated the antitumor effect of intralesional rTNF-alpha therapy in patients with advanced solid tumors.

The local tumor regression observed in five out of 14 patients suggests a higher efficacy of local versus systemic TNF treatment, where such dramatic tumor responses were not seen even at higher total doses [9–11]. It is possible that a critical local TNF-alpha concentration at the tumor site is important for the induction of a tumor response. However, the short duration of responses suggests rapid development of TNF-alpha resistance of the tumor. These findings are in agreement with recent *in vitro* data obtained from established tumor cell lines showing ligand-induced acquisition of TNF-alpha resistance [14]. The clinical observation of rapid development of resistance under TNF-alpha treatment, therefore, stresses the need for definition of treatment modalities to maintain TNF sensitivity, e.g. by combining TNF-alpha with other cytokines. The toxicity pattern associated with local rTNF-alpha treatment is comparable to systemic application. The local inflammation appearing 24 h after i.t. rTNF-alpha injection indicates infiltration of mononuclear cells, which was confirmed by cytologic examination (data not shown). The degree of inflammation and the concomitant pain at the site of injection were limiting for dose and frequency of rTNF-alpha treatment. Based on this definite, but transient, antitumoral activity of rTNF-alpha shown here, we are currently investigating whether a combination of rTNF-alpha with other cytokines (rIFN-gamma and rIFN-alpha) will improve the frequency and duration of responses.

Acknowledgements—We are indebted to Prof. Schauer and Dr. Bergholz, Department of Pathology, University of Gœttingen, for providing us histological slides of our patients. We also thank G. Schmidt for assistance in the preparation of the manuscript.

REFERENCES

1. Old LJ. Tumor necrosis factor (TNF). *Science* 1985, **230**, 630–632.
2. Beutler B, Cerami A. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* 1986, **320**, 584–588.
3. Pfizenmaier K, Krönke M, Scheurich P, Nagel GA. Tumor necrosis factor (TNF) alpha: control of TNF sensitivity and molecular mechanisms of TNF-mediated growth inhibition. *Blut* 1987, **55**, 1–10.
4. Le J, Vilcek J. Biology of disease. Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. *Lab Invest* 1987, **56**, 234–248.
5. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA Jr, Shepard HM. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells *in vitro*. *Science* 1985, **230**, 943–945.
6. Haranaka K, Satomi N, Sakurai A. Antitumor activity of murine tumor necrosis factor (RNF) against transplanted murine tumors and heterotransplanted human tumors in nude mice. *Int J Cancer* 1984, **34**, 263–267.
7. Aggarwal BB, Kohr WJ, Hass PE *et al.* Human tumor necrosis factor: production, purification, and characterization. *J Biol Chem* 1985, **260**, 2345–2354.
8. Pennica D, Nedwin GE, Hayflick JS *et al.* Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984, **312**, 724–729.
9. Blick M, Sherwin SA, Rosenblum M, Gutterman I. Phase I study of recombinant tumor necrosis factor in cancer patients. *Cancer Res* 1987, **47**, 2987–2989.
10. Creaven PJ, Player JE, Dupere S *et al.* Phase I clinical trial of recombinant tumor necrosis factor. *Cancer Chemother Pharmacol* 1987, **20**, 137–144.
11. Bartsch HH, Nagel GA, Mull R, Flener R, Pfizenmaier K. Phase I study of recombinant human tumor necrosis factor-alpha in patients with advanced malignancies. *Mol Biother* 1988, **1**, 21–29.
12. Kramer SM, Crver HG. Serum-free *in vitro* bioassay for the detection of tumor necrosis factor. *J Immunol Meth* 1986, **93**, 201–204.
13. Krönke M, Schlüter C, Pfizenmaier K. Tumor necrosis factor inhibits MYC expression in HL-60 cells at the level of mRNA transcription. *Proc Natl Acad Sci USA* 1987, **84**, 469–473.
14. Schütze S, Scheurich P, Schlüter C, Ücer U, Pfizenmaier K, Krönke M. Tumor necrosis factor-induced changes of gene expression in U937 cells. Differentiation-dependent plasticity of the responsive state. *J Immunol* 1988, **140**, 3000–3005.
15. Shklar G, Schwartz J. Tumor necrosis factor in experimental cancer regression with alpha-tocopherol, beta-carotene, canthaxanthin and algae extract. *Eur J Cancer Clin Oncol* 1988, **24**, 839–850.
16. Nophar Y, Holtman H, Ber R, Wallach D. Dominance of resistance to the cytocidal effect of tumor necrosis factor in heterocaryons formed by fusion of resistant and sensitive cells. *J Immunol* 1988, **140**, 3456–3460.