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A Phase II Combination Trial with Recombinant Human Tumor Necrosis Factor and Gamma Interferon in Patients with Colorectal Cancer*

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Summary. Recombinant human tumor necrosis factor (TNF) is a cytotoxic monokine with immunomodulatory functions. Gamma interferon (g-IFN) synergizes with TNF in many ways. We therefore conducted a Phase I/II combination trial with TNF and g-IFN at an immunomodulatory dose level in 16 patients with colorectal cancer. TNF (50 μg/m^2 in a 30 min infusion) and g-IFN (100 μg in subcutaneous injections) were administered daily Monday through Friday for 4 weeks. Two cases of major toxicity, one acute renal failure and one case of severe thrombocytopenia, led to discontinuation of study medication in these patients. Toxicities in remaining patients were manageable with conservative treatment. Changes in laboratory values included leukopenia, anemia and thrombocytopenia. Alterations in lipid metabolism and changes in serum levels of acute phase proteins were observed. Increase in both total lymphocytes and a Leu 11 positive subpopulation, as well as an induction of measurable interleukin 2 serum levels in a subgroup of patients, were noted. Response results of 14 evaluable patients were one patient with a mixed response, 4 with stable disease and 9 with disease progression. Median survival was 23.5 weeks with only one patient alive after 71 weeks. Therefore the drug combination of TNF/g-IFN in the chosen regimen cannot be recommended for the treatment of patients with colorectal cancer.

Key words: Tumor necrosis factor – Interferon gamma – Colonic neoplasms

Colorectal cancer ranks second as a cause of cancer death in industrialized countries with an incidence of approximately 50 new cases per 100000 individuals [42]. In advanced stages of this disease current treatment regimens rarely result in complete remission or cure. Therefore new innovative approaches have to be evaluated. Recently a new class of therapeutic agents, the biological response modifiers, have become available for clinical trials because they can be produced in large quantities by recombinant DNA technology. These agents include tumor necrosis factor-alpha (TNF) and interferon-gamma (g-IFN).

TNF possesses a direct cytotoxic activity against a variety of tumor targets, including colon carcinoma cell lines in vitro and causes hemorrhagic necrosis of tumor xenografts transplanted into immunosuppressed mice [3, 17, 30]. Furthermore TNF is an important mediator of monocyte cytotoxicity [13]. G-IFN, a major activator of resting monocytes, synergizes with TNF in this action [19]. One reason for this synergy may reside in the up-regulation of TNF receptors by g-IFN [2]. Besides this direct cytotoxic activity, TNF exerts pleiotropic effects on different tissues. TNF is the mediator of the toxic shock syndrome [41]. It is identical with cachectin [6] which causes inhibition of lipoprotein lipase and cachexia in tumor patients. Bone resorption is promoted by TNF [5].

In vivo g-IFN followed a bell shaped dose response curve in phase I and II trials [11]. Immunostimulation peaked at about 0.1 mg administered intramuscularily or subcutaneously. Higher doses

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Abbreviations: TNF = tumor necrosis factor; g-IFN = gamma-interferon; TGF = transforming growth factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL-1 = interleukin-1; IL-2 = interleukin-2; MHC = Major histocompatibility complex; MTD = maximally tolerated dose; Ig = immunoglobulin

resulted in inhibition of macrophage effector functions. On the other hand, TNF has a linear dose response curve and shows its most pronounced antitumor action at high doses in animal models [9]. The maximal tolerated dose (MTD) of TNF in a phase I clinical trial was 150 $\mu g/m^2$ administered intramuscularily [21]. In two phase I trials MTD of TNF in combination with administration of g-IFN ranged between 100–150 $\mu g/m^2$ [1, 10]. A TNF dose of 80 $\mu g/m^2$ has been suggested by a different group as the most efficient immunomodulatory dose level in humans [27]. To make use of the synergy in anti-proliferative and immunostimulatory effects of TNF and g-IFN we conducted a phase II trial with a combination of both lymphokines. G-IFN was given at the immunostimulatory dose level concomitant with a 30 min infusion of 50 $\mu g/m^2$ TNF. Both drugs were administered daily Monday through Friday for 4 consecutive weeks. This study was aimed at the determination of toxicity and tumor response in patients with colorectal cancer.

Methods

Patients

All 16 patients had histologically proven, advanced colorectal cancer. Disease progression was documented in the preceding two months before entry in all patients. Eligibility criteria included performance status (Karnofsky) of at least 50, measurable lesions, adequate renal function (creatinine <2.0 mg/dl), adequate hepatic function (bilirubin <1.5 mg/dl), and adequate hematopoietic function (WBC count $>3500/mm^2$, platelet count $>100000/mm^2$). Exclusion criteria were chemotherapy during the last 3 weeks (6 weeks for nitrosoureas and mitomycin C), brain or leptomeningeal disease, previous or concurrent malignancies other than colorectal cancer and severe concurrent nonmalignant diseases. The study protocol had been approved by the local ethical committee. All patients had been informed of experimental nature of this program and had signed an informed consent form.

Characteristics of TNF and g-IFN

TNF and g-IFN were supplied by Boehringer, Ingelheim, West Germany). G-IFN had a specific activity of 2×10^2 U/mg (using the EMC/A549 bioassay referenced to the NIH g-IFN standard Gg 23-901-530) and TNF of approximately 3×10^7 U/mg (L cell assay with actinomycin D added). Both products were $>98\%$ pure as determined by SDS polyacrylamide gel electrophoresis and contained <1.0 ng endotoxin per milligram protein as tested by the limulus amebocyte lysate assay.

Treatment with TNF and g-IFN

Patients received TNF (50 $\mu g/m^2$) as a 30 min i.v. infusion and g-IFN (0.1 mg) s.c. daily Monday through Friday for 4 weeks. If no progressive disease occurred, an identical second 4 week cycle was given after a two week rest period.

Concomitant Medications

At the beginning of the trial, 1000 ml physiologic sodium chloride solution were given before TNF infusion as prehydration. Later, this was deemed unnecessary and reduced to 500 ml. All patients received either indomethacine (25 mg) or diclofenac (50 mg) thrice daily to ameliorate side effects of the treatment. To reduce gastrointestinal symptoms most patients were given 300 mg rantidine once or twice daily. Pethidine was administered i.v. to patients with severe shaking chills. No glucocorticoid medication was permitted during treatment.

Patient Monitoring

Vital signs were monitored frequently on treatment days. Complete blood counts with differential and standard chemistry profiles were obtained before treatment and twice weekly during the treatment period. Coagulation studies, tumor markers CEA and CA 19-9, serum immunoglobulin levels, cell surface marker analysis and IL-2 assays were done before therapy and once weekly thereafter. ECG and chest x-ray were ordered before treatment and thereafter, if indicated.

Cell Surface Marker Analysis

Heparinized blood samples were obtained from the patients before therapy and on Mondays weekly thereafter at 8 a.m. Mononuclear cells were isolated by gradient centrifugation over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Aliquots of mononuclear cells were stained with one of the following monoclonal antibodies: OKT 11, OKT 4, OKT 8 (Ortho, Raritan, New Jersey) Tac, Leu 11 (Becton Dickinson, Mtn. View, California) or B4, My4 (Coulter, Hialeah, Florida). Antibodies were either directly labelled with FITC or

a FITC conjugated goat anti-mouse antibody (Tago, Burlingame, California) was employed. Fluorescent cells were determined with a UV microscope (Zeiss, Wetzlar, West Germany).

IL 2 Assays

IL-2 Elisa kits were purchased from Genzym, Boston, MA. Serum samples were stored at −70° C until use. Elisa was performed according to the supplier's directions. The lower limit of detection is 0.05 U/ml.

Statistical Analysis

The nonparametric, signed-rank Wilcoxon test was used for statistical analysis. The level of significance was set to 5%. To compensate for multiple testing the predefined significance level of $a = 5\%$ has been corrected by the method of Bonferroni-Holm [42].

Tumor Response

Standard WHO criteria were used to evaluate the therapeutic response in terms of complete or partial response, stable or progressing disease.

Results

Patients

Sixteen patients were studied. Patient characteristics are summarized in Table 1. The median performance status was 70 and median age 57 years. Fifteen patients had received prior chemotherapy with 5-FU based regimens, usually in combination with methotrexate or leucoverin. Five patients were switched to alternative regimens thereafter. Local perfusion of liver metastases with chemotherapeutic agents was performed in two cases.

Ten patients received one and four patients two four week courses of treatment with TNF/g-IFN separated by a two week rest period. In 2 patients treatment was terminated prematurely because of toxicity.

Table 1. Patient characteristics

Number	16
Male/Female	9/7
Age (Median)	57 years (44–67)
Karnofsky (Median)	70% (50–90%)
Colon/Rectum Cancer	12/4
Previous Therapy:	
– Surgery	16
– Chemotherapy	15
– Radiotherapy	5
– g-IFN	2
Site of Metastasis:	
– Liver	14
– Lung	3
– Locoregional	3
– Bone	1
– Subcutanous	1

Toxicity

Two cases of major toxicity were observed. One patient suffered from acute tubular necrosis after 3 days of treatment with a 4 kg weight gain and an increase in serum creatinine to 3.5 mg/dl. The second patient had a decrease of platelet count to 21×10^3 µl on day 10 of treatment. He had received spinal irradiation for bone metastasis, and bone marrow examination revealed infiltration by tumor cells. After discontinuation of the study medication both, patients recovered with conservative treatment.

Remaining toxicities were tolerable and manageable. They included shaking chills of approximately 30 min duration in 13 patients, requiring pethidine injections in most patients. Fever up to 39.5° C was rarely observed and occurred only during the first treatment day due to regular use of cyclooxygenase inhibitors. Nausea and vomiting was noticed in 8 patients and might have been induced by the pethidine medication. Headaches in 6 patients were usually relieved by ergotamine preparations. A burning sensation occurred in the eyes of four patients. Back pain and pain in tumor bearing areas was noticed in 4 patients. One patient with probable lumbar plexus infiltration required regular pethidine injections for pain relief. Weight gain of more than 4 kg due to fluid retention resulting in ascites and leg edema occurred in 4 patients who had massive liver metastasis. One patient developed a bullous exanthema on the left lower arm. Histological examination revealed leukocytoclastic vasculitis with epidermal necrosis; no deposits of IgA, IgM, IgG or C3 were detected. Discontinuation of the study medication was not necessary and the skin lesion resolved during continuing therapy. One case of grade I renal toxicity and one case of asymptomatic pancreatitis were observed, but did not necessitate termination of the treatment. Three patients developed grade I or II hepatic toxicity with elevation of bilirubin and transaminases. But hepatic toxicity was usually short lived and subsided spontaneously during continuing treatment or shortly thereafter. Blood

pressure swings with systolic values under 90 mmHg and over 200 mmHg which were mainly asymptomatic, occurred during the beginning of the TNF/g-IFN treatment period.

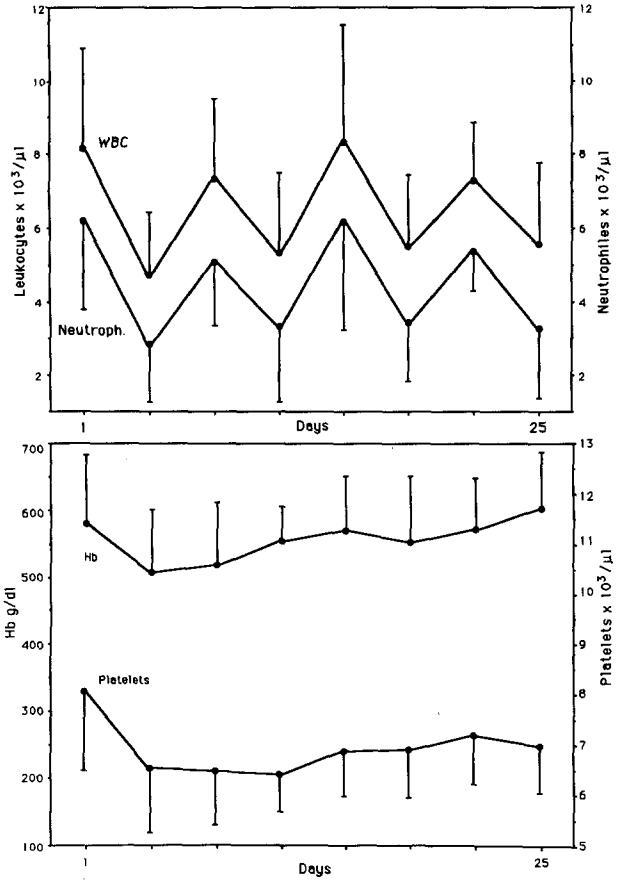

Fig. 1. Mean changes of hematological parameters of 14 patients during TNF/g-IFN treatment

Changes in Hematologic Parameters

During TNF/g-IFN treatment, several changes in laboratory values were observed. TNF/g-IFN had a pronounced effect on hematologic parameters. The platelet count dropped from a pretreatment mean of $329 \times 10^3/\mu l$ to an average of $205 \times 10^3/\mu l$ after two weeks ($p = 0.0015$, $a_B = 0.005$) and increased thereafter, despite continuous treatment, to a mean of $249 \times 10^3/\mu l$ after 4 weeks (Fig. 1). The mean leukocyte count fell rapidly during the first 4 days from a mean of 8160/µl to 4720/µl ($p = 0.001$, $a_B = 0.0045$), mainly reflecting a decrease in neutrophil granulocytes from 6200/µl to 2830/µl ($p = 0.0015$, $a_B = 0.0055$). During the treatment-free weekends, the leukocyte and granulocyte counts returned to the pretreatment ranges and decreased again when TNF/g-IFN administration was restarted (Fig. 1). Average hemoglobin concentration and erythrocyte counts fell during the first week by 0.81 g/dl and $0.21 \times 10^6/\mu l$ ($p = 0.02$, $a_B = 0.0125$ and $p = 0.05$, $a_B = 0.025$) respectively and increased thereafter to the pretreatment level (Fig. 1).

Treatment with TNF/g-IFN had a major impact on lipid metabolism. Mean triglyceride levels rose after institution of therapy from 151 mg/dl to 225 mg/dl after 4 days ($p = 0.0069$, $a_B = 0.00714$) and remained elevated at this level. HDL cholesterol fell from an average of 36.7 mg/dl to 27.2 mg/dl in the first week ($p = 0.0093$, $a_B = 0.0083$) and stayed depressed during the continuing 4 week treatment period (Fig. 2). LDL cholesterol levels did not change significantly during the observation time. Total cholesterol values showed a fluctuating

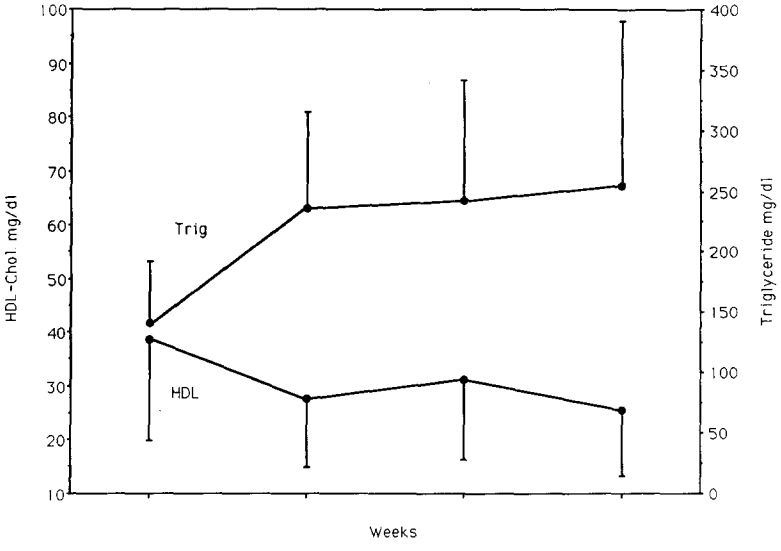

Fig. 2. Mean changes of blood lipid concentrations of 14 patients during TNF/g-IFN treatment

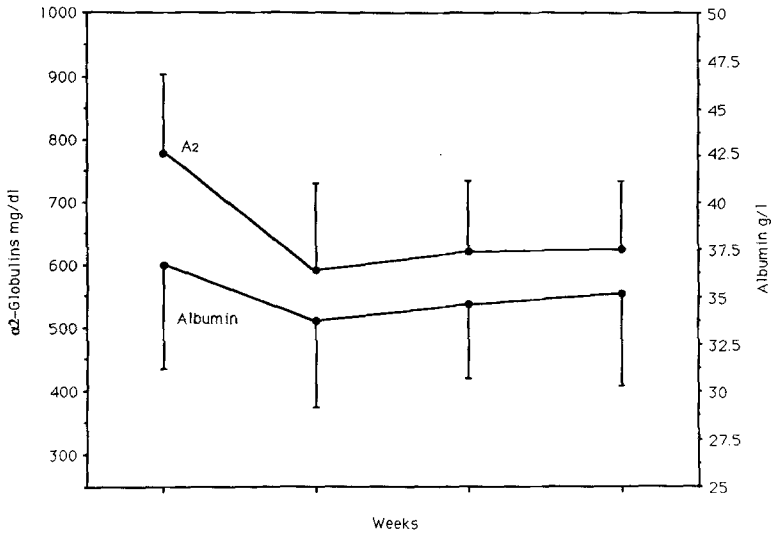

Fig. 3. Mean changes of acute phase proteins of 14 patients during TNF/g-IFN treatment

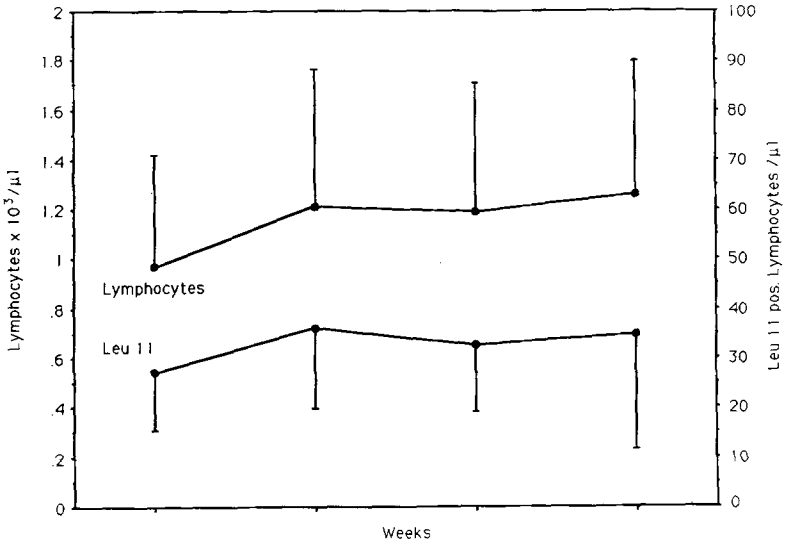

Fig. 4. Mean changes of blood levels of lymphocytes ($n=14$) and Leu 11 positive cells ($n=7$) during TNF/g-IFN therapy

pattern with decreases during the treatment week and increases during the treatment free weekends.

Alterations in acute phase proteins were noticed. Although a rise in alpha$_2$-globulins was expected we found a decrease from 768 mg/dl to 626 mg/dl after one week of treatment ($p=0.022$, $a_B=0.0167$, Fig. 3). Serum albumin dropped from an average of 36.7 g/l to 33.7 g/l after one week ($p=0.0035$, $a_B=0.00625$) and increased again to a mean of 35.2 g/l after 3 weeks (Fig. 3).

Immune Status

During the 4 week observation interval several parameters of the immune system were measured regularly. An augmentation of total lymphocytes was noticed from a pretreatment mean of 971/µl to 1260/µl after 3 weeks ($p=0.011$, $a_B=0.01$). About half of this increase was accounted for by a rise in Leu 11 positive natural killer cells from a mean of 281/µl to 382/µl after one week ($p=0.063$, $a_B=0.05$, Fig. 4). No major changes in CD4, CD8, CD2 positive T-cell populations were observed. No activation of T-cells was detected as indicated by absence of elevation of the IL2-receptor positive subpopulation. Also no alteration of the amount of B1 and My4 positive lymphocytes was found.

Interleukin 2 serum levels were determined weekly by ELISA technique. Of nine patients studied, only 3 had measurable IL2 serum levels before treatment. In these 3 patients IL2 serum concentration rose during TNF/g-IFN administration. Of

Table 2. Interleukin 2 Serum Concentrations in U/ml

Patient No.	Pretreatment	after 3 Weeks
3	0.65	0.95
4	0.53	0.68
7	ND	0.05
8	ND	ND
9	ND	0.5
11	1.12	75
12	ND	ND
14	ND	ND

ND not detected

the remaining 6 patients with initially undetectable serum IL2, 3 produced detectable amounts of IL2 after 3 weeks of therapy (Table 2).

Response Results

Of 16 patients entered into our TNF/g-IFN trial, two had to be excluded from further therapy because of major toxicity. 10 patients received one 4-week treatment course and four patients a second therapy course after a two week rest interval. These 14 patients were evaluable for response. One patient with rectal cancer exhibited a mixed response with a partial response in loco-regional disease and disease progression of pulmonary metastases. Four patients had stable disease and nine patients had disease progression. The four patients with stable disease had renewed disease progression one to two months after cessation of therapy. One patient was not available for follow up. The remaining 13 patients were evaluable for survival. Median survival for this group was 23.5 weeks. Only one patient is still alive after 71 weeks.

Interestingly, reductions of more than 20% of elevated LDH serum levels were observed in 4 patients during the first one to two weeks of therapy. In one of these patients this reduction was preceded by an initial rise in LDH serum concentration. Four patients showed a greater than 10% decrease in CEA and none in CA 19-9 tumor markers. No correlation between decreases in serum LDH or CEA values and arrest of disease progression was noted.

Discussion

In tumor bearing animals TNF administration has resulted in cures only at sublethal doses [18]. In the most commonly used animal model where Meth A sarcoma is transplanted into mice, direct cytotoxicity of TNF does not seem to induce major tumor regression [31]. Instead TNF appears to mediate its antitumor effect via damage to the tumor's vasculature. TNF has been shown to activate the coagulation cascade [7] and to have a direct effect on endothelial cells [34, 36]. These processes ultimately lead to intravascular fibrin formation and to hemorrhagic necrosis [28]. In animal models, extravasation of red blood cells can be observed in tumor nodes histologically. Injection of ^{51}Cr labelled erythrocytes 30 min before TNF administration into SA1 sarcoma bearing murine hosts resulted in accumulation of the radioactive tracer in tumor locations [18]. In one patient treated with TNF, hemorrhage into a superficial tumor manifestation has been documented by direct observation [35].

In the SA1 sarcoma system complete remission of tumors after TNF application depends on the immunocompetency of the murine host [29]. A rim of living tumor cells appears to resist the initial attack of TNF and must be eliminated by tumor infiltrating host immune cells. In patients with colorectal cancer only less than 1% of tumor infiltrating macrophages are activated and produce endogenous TNF as shown by in situ hybridization [4]. TNF and g-IFN are immunomodulators and synergistically activate monocytes, granulocytes and lymphocytes [19, 24, 37, 40]. TNF and g-IFN cause expression of class II MHC antigens on different cell types [32]. Induction of lymphokines such as GM-CSF, M-CSF and IL-1 is promoted by TNF [23]. Exogenous TNF induces its own production in macrophages [33] and g-IFN synergizes in this regard [8]. This effect can be antagonized by TGF-beta [12], prostaglandin E_2 [26] and alpha-globulins such as $alpha_1$-antitrypsin or $alpha_2$-macroglobin [38] in vitro. We also observed that during therapy numbers of total lymphocytes and Leu-11 positive NK cells increased in peripheral blood. Furthermore, measurable IL2 serum levels could be induced in a subgroup of our patients possibly causing the observed rise in lymphatic cells. But these alterations in effector cell numbers obviously did not translate into a clinically relevant antitumour effect.

TNF/g-IFN treatment influenced a variety of hematologic parameters. Rapid decreases in granulocyte concentrations in peripheral blood were observed. Margination is one probable mechanism because TNF is known to augment stickiness of endothelial cells to granulocytes in vitro [15]. Furthermore TNF has a direct inhibitory action on bone marrow progenitor cells [22]. This effect may have been involved in the gradual decline in platelet and erythrocyte concentrations.

During TNF/g-IFN therapy several toxic effects subsided and laboratory alterations returned towards normal despite continuing treatment. For

example fever and blood pressure alterations were most pronounced on the first treatment day. Laboratory changes such as a decrease of platelet and erythrocyte counts and serum albumin levels were observed only temporarily. Therefore attenuation to the action of TNF/g-IFN must be supposed [14]. Only a few laboratory values remained at the altered niveau, e.g. like triglyceride levels. The persistent effect of TNF on lipoproteinlipase has been documented in animal models [16].

Several explanations for the attenuation effects of TNF/g-IFN treatment are possible. Downregulation of receptors or exhausted release of secondary lymphokines could be anticipated. Inhibitors of TNF action have been described which might be induced by prolonged TNF administration [39]. Immunosuppressive substances, like TGF-beta, prostaglandine E_2 or alpha globulins [12, 26, 38] might inhibit effector functions of macrophages. To prevent attenuation in future trials, TNF could be administered after longer treatment free intervals. Alternatively, daily dose escalation could be tried to achieve continued efficacy, especially because many toxic side effects subside during therapy.

Cyclooxygenase inhibitors, such as indomethacin, reduce side effects of TNF administration and might be livesaving in TNF overdosage in animal systems [25]. In this trial indomethacin or diclofenac decreased fever episodes and other systemic effects described in phase I trials and probably contributed to the low rate of severe toxicity and to the acceptable tolerability of the study medication. Also hematological and hepatic toxicity, the two most frequently occurring complications, were usually mild and did not necessitate discontinuation of the study drugs.

Evaluation of response results revealed only one mixed response, a partial remission in locoregional cancer with progression of lung metastases, and 4 patients with stable disease. All these patients progressed within one to two months after cessation of therapy. Median survival for our group of 14 patients with initially progressive colorectal cancer was only 23.5 weeks. Only one patient was alive after 71 weeks. According to our study design, the chance to achieve a 20% response rate after 14 consecutive, nonresponding patients was considered to be below 5%, and therefore the trial was terminated. In a different, already published phase I study, TNF/g-IFN combination therapy did not result in any responses in patients with colorectal cancer, even at maximal tolerated doses of TNF and gamma-Interferon [1]. In conclusion, combination therapy with TNF/g-IFN cannot be recommended for patients with colorectal carcinoma in the chosen dosis regimen. Whether TNF shows activity in conjunction with other biological response modifiers, such as IL2, has to be determined in future clinical trials.

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