

Effects of Recombinant Human Interleukin-6 in Cancer Patients: A Phase I-II Study

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To define the toxicity profile of recombinant human interleukin-6 (rhIL-6) and to study its effect on hematopoiesis, biochemical parameters and other cytokines, rhIL-6 was administered in a phase I-II study to 20 patients with breast carcinoma or nonsmall cell lung cancer. RhIL-6 doses were 0.5, 1.0, 2.5, 5.0, 10, and 20 $\mu\text{g}/\text{kg}/\text{d}$, with at least three patients per dose level. RhIL-6 was administered 24 hours by continuous intravenous infusion followed by subcutaneous (SC) administration for 6 days, partly on an outpatient basis. RhIL-6-related side effects were fever, headache, myalgia, and local erythema. Starting at 2.5 $\mu\text{g}/\text{kg}/\text{d}$, these side effects were compounded by nausea, reversible increase in liver enzymes, and anemia. Flu-like symptoms were controllable up to and including 10 μg rhIL-6/kg/d with acetaminophen. RhIL-6 increased platelet counts with a decrease in mean platelet volume and increased leukocytes caused by

neutrophil, monocyte, and lymphocyte increase, with an increase in T cells and natural killer cells at 1.0 and 2.5 μg rhIL-6/kg/d. The reversible anemia was characterized by a decrease in serum iron, and an increase in ferritin and erythropoietin without reticulocytosis. RhIL-6 reduced total cholesterol levels and a dose-related increase of C-reactive protein and serum amyloid A plasma levels was observed. Serum IL-6 levels were increased, especially at 10 and 20 $\mu\text{g}/\text{kg}/\text{d}$, whereas no change in IL-1 β and tumor necrosis factor α levels was observed. RhIL-6 can be administered with controllable side effects in this setting, up to and including a SC dose of 10 $\mu\text{g}/\text{kg}/\text{d}$ on an outpatient basis, and has a promising stimulating effect on leukopoiesis and thrombopoiesis.

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INTERLEUKIN-6 (IL-6) is a 19- to 30-kD protein, which in the past has also been referred to as interferon- β 2 (IFN β 2), B-cell differentiation factor, and hepatocyte-stimulating factor.¹⁻³ It is produced by T cells, monocytes, fibroblasts, endothelial cells, and keratinocytes.⁴ IL-6 generates T-cell differentiation⁵ and proliferation,^{6,7} has a stimulatory effect on B cells with production of Ig,^{2,8} and induces the hepatic acute phase response,⁹ both in vitro and in animals. Once the complementary DNA for this cytokine was cloned,^{2,10} recombinant human IL-6 (rhIL-6) could be produced. On the one hand, this showed both a direct and an indirect antitumor capacity, demonstrated in vitro by growth inhibition of breast carcinoma and leukemia cell lines^{11,12} and in vivo by the suppression of solid tumors in mice.¹³ On the other hand, IL-6 supports the growth of plasmacytoma, for example.¹⁴

The striking effect of IL-6 on hematopoiesis concerns megakaryopoiesis. In vitro IL-6 proved to be a differentiation factor for megakaryocytes,¹⁵⁻¹⁸ and a megakaryocyte proliferation stimulator.¹⁹ This proliferative effect is synergistically influenced by IL-3.^{15-18,20} Administration of rhIL-6 to mice and monkeys resulted in maturation of megakaryocytes,^{21,22} with²³ or without²⁴ an effect on the number of bone marrow (BM) megakaryocyte colony forming cells and with an increase in platelet counts.^{21-23,25,26} This effect of rhIL-6 on the peripheral platelet count was also observed in mice after 5-

fluorouracil treatment²⁷ and in dogs and baboons after irradiation.^{28,29} There are only limited data available on the effects of rhIL-6 in humans.³⁰

To investigate the feasibility of this thrombopoietic drug in a clinical setting, a phase I-II study with rhIL-6 in patients during the prechemotherapy phase was performed to define its toxicity profile and its effect on hematopoiesis, biochemical parameters, and other cytokines.

MATERIALS AND METHODS

Patients. In this phase I-II study, 20 patients with either stage III or IV breast cancer or nonsmall cell lung cancer received rhIL-6 in a prechemotherapy phase. Patients between the ages of 18 and 70 years and with a Karnofsky performance score of 60% or more were eligible. At entry, a white blood count (WBC) of greater than $3 \times 10^9/\text{L}$ and a platelet count greater than $100 \times 10^9/\text{L}$ were required. Patients with severe heart, lung, liver (serum total bilirubin greater than $40 \mu\text{mol}/\text{L}$), or kidney impairment (serum creatinine greater than $150 \mu\text{mol}/\text{L}$) were excluded, and no history of serious allergic reactions, rheumatoid arthritis, generalized psoriasis, membranous glomerulonephritis, or other autoimmune disease was allowed. Finally, those concomitantly treated with another hematopoietic growth factor, cytokine, steroids, aspirin, or radiotherapy were not eligible for this study. The study was approved by the Medical Ethical Committee of the University Hospital of Groningen. All patients gave written informed consent.

Study design. rhIL-6 was administered for 7 consecutive days, followed by a rest period of 7 days. The patients received rhIL-6 on day 1 for 24 hours by continuous intravenous (IV) infusion that could be discontinued immediately if untoward side effects occurred. On days 2 through 7, the patients received the rhIL-6 once daily subcutaneously (SC). *Escherichia coli*-derived IL-6 (10^8 U/mg protein) was provided by Sandoz Pharmaceutical Ltd (Basel, Switzerland). For the infusion on day 1, polypropylene syringes were used for each of the two 12-hour infusions. Each infusion contained half of the daily rhIL-6 dose, 96 mg (2 mg/mL) human serum albumin and 0.9% saline in a total volume of 48 mL. For SC injection, the rhIL-6 vial was reconstituted with 1 mL sterile water. After instructions by the oncology nurse, rhIL-6 was injected in the upper leg, only up to day 4 on an in-patient basis. RhIL-6 treatment schedule consisted of increasing dose levels, ie, 0.5, 1.0, 2.5, 5, 10, and 20 $\mu\text{g}/\text{kg}/\text{d}$ with at least three patients per dose level. The maximum tolerated dose was defined as the dose level preceding that at which

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at least two patients experienced World Health Organization (WHO) grade III or IV toxicity or life-debilitating toxicity leading to discontinuation of the rhIL-6 treatment. Blood pressure, pulse rate, and temperature were controlled frequently during the first 4 days; body weight was measured once daily. Acetaminophen, with a maximum of 3 g/d, was administered for fever ($>38.5^{\circ}\text{C}$, measured axillary) or flu-like symptoms. Antiemetics were prescribed if necessary, for nausea.

Blood counts, mean platelet volume, and differential counts were obtained at days 1, 3, 5, 8, 10, 12, and 15. Liver and renal function and serum levels of sodium, potassium, calcium, total protein, albumin, glucose, total cholesterol, serum iron, and serum ferritin at dose level $20\ \mu\text{g}/\text{kg}/\text{d}$ were determined on days 1, 3, 8, and 15. Serum erythropoietin levels were determined by radioimmunoassay (RIA; Sorin Biomedica, Stillwater, MN). BM aspiration was performed on day 1 and day 8 for BM culture; granulocyte-macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) were cultured as previously described.³¹ In BM smears the myeloid-erythroid ratio (M:E ratio) was determined. Immunophenotyping of peripheral lymphocyte populations was performed on days 1 and 8. CD3, CD4, CD8, CD19, CD20, CD56, CD57, and CD25 positive cells were determined by Fluorescence-activated cell sorting analyses (Becton Dickinson, Sunnyville, CA). Samples for fibrinogen, Ig levels (IgG, IgM, IgA) measured by Behring nephelometer (Behringwerke AG Diagnostica, Marburg, Germany), and antinuclear antibodies (ANA) measured by immunofluorescence technique were taken on days 1, 8, and 15. On days 1 through 5 and days 8, 10, 12, and 15, plasma samples were obtained before rhIL-6 administration, for C-reactive protein (CRP), serum amyloid A (SAA), and IL-6, IL-1 β , and tumor necrosis factor α (TNF α). CRP (normal value, $<2\ \text{mg}/\text{L}$) and SAA (normal value, $<3\ \text{mg}/\text{L}$) levels were obtained using enzyme-linked immunosorbent assays (ELISA)³² and IL-6 (normal value, $<10\ \text{ng}/\text{L}$) by using the B9 bioassay and ELISA³³. TNF α (detection limit, $15\ \text{ng}/\text{L}$) was measured by enzyme-amplified sensitivity immunoassay (Medgenix Diagnostics SA, Fleurus, Belgium) and IL-1 β (detection limit $10\ \text{ng}/\text{L}$) by ELISA (Cistron Biotechnology, Pinebrook, NJ).

Statistical analysis. The two-tailed Student's *t*-test and the Spearman rank analysis were used for statistical analysis. *P* values $<.05$ were considered significant. Unless otherwise stated the two-tailed Student's *t*-test was used.

RESULTS

Patient characteristics. As shown in Table 1, 20 patients, with a mean age of 44.9 years (range, 24 to 63), were entered. Twelve received both chemotherapy and radiotherapy, 3 received only chemotherapy, and 5 received no previous treatment before rhIL-6. Radiotherapeutic treatment of a large part of the pelvis was given to 1 patient 3 months before entry.

Toxicity. Side effects, summarized in Table 2, consisted mainly of fever and flu-like symptoms, which started at the lowest dose level of $0.5\ \mu\text{g}/\text{kg}/\text{d}$. Three out of seven patients at 0.5 and $1.0\ \mu\text{g}/\text{kg}/\text{d}$ dose levels needed up to 3 g acetaminophen for these complaints. At higher dose levels, all patients were treated with 3 g/d acetaminophen. Fever tended to get higher at escalating dose levels up to and including $20\ \mu\text{g}/\text{kg}/\text{d}$, but did not exceed 40°C . The rise in temperature was observed 7 to 8 hours after the start of IV rhIL-6 at doses ranging from 0.5 to $5.0\ \mu\text{g}/\text{kg}/\text{d}$, and after 3 to 4 hours at doses of 10 and $20\ \mu\text{g}/\text{kg}/\text{d}$. After SC rhIL-6 administration the temperature increase occurred after 8 hours at the lower dose levels and after 4 hours at the 10 and $20\ \mu\text{g}/\text{kg}/\text{d}$ dose levels. This increase subsided after 6 hours for all patients

Table 1. Patient Characteristics

Patient No.	Female/Male	Age (yrs)	Tumor	Previous Treatment	
				Chemotherapy	Radiotherapy
1	F	40	Breast	+	+
2	F	30	Breast	+	+
3	F	46	Breast	+	+
4	F	54	Breast	+	+
5	F	39	NSCLC	-	-
6	F	51	Breast	+	+
7	F	44	Breast	+	+
8	M	63	NSCLC	-	-
9	F	29	Breast	+	+
10	F	46	NSCLC	-	-
11	F	43	Breast	+	+
12	F	42	Breast	+	-
13	F	48	Breast	+	+
14	F	24	NSCLC	+	+
15	F	48	NSCLC	-	-
16	M	57	NSCLC	-	-
17	F	42	Breast	+	+
18	M	51	NSCLC	+	-
19	M	61	NSCLC	+	-
20	F	40	Breast	+	+

Abbreviations: NSCLC, nonsmall cell lung cancer; +, yes; -, no.

receiving doses up to and including $10\ \mu\text{g}$. In two patients, at the $20\ \mu\text{g}$ dose, the temperature tended to stay higher ($>38^{\circ}\text{C}$) until the next injection, despite 3 g/d acetaminophen. At the other dose levels, fever and the flu-like symptoms responded to treatment with acetaminophen. All patients had local erythema at the injection site, which subsided within 48 hours. A small local infiltrate was sporadically observed after IV treatment. Ten patients had chills starting 30 minutes after injection and/or several hours later starting at the $2.5\text{-}\mu\text{g}$ dose with a duration of 15 minutes. Nausea occurred without a clear time relationship to rhIL-6 administration also starting at the $2.5\text{-}\mu\text{g}$ dose. Three of the seven patients with this complaint needed metoclopramide and responded well to this treatment. No effect of rhIL-6 was observed on blood pressure and body weight. One patient suffered from a transient ischemic attack in cerebro. This was caused by tumor embolization originating from tumor infiltrations in the pulmonary vein and on the mitral valve.

Hematologic effects. After an initial decrease at day 3, an elevated platelet count was noticed especially after cessation of rhIL-6. The highest value was reached between day 8 and 15 in all patients (Fig 1) and was significant at the 1.0- , 2.5- , 5.0- , and $20\text{-}\mu\text{g}$ dose levels when compared with baseline values (Table 3). The increase in platelets was dose related when the maximum percentage of increase was compared with day 1 ($r = .84$, $P < .002$, Spearman rank analysis, Fig 2). The mean platelet volume decreased during rhIL-6 treatment. The lowest values were reached during the increase in platelet counts (mean \pm SE; day 1, 8.25 ± 0.26 fL; day 15, 7.11 ± 0.30 , $P < .001$) without a dose-effect relationship.

There was a slight and early increase in leukocytes compared with baseline values starting at the $2.5\text{-}\mu\text{g}$ dose and continuing up to and including the highest rhIL-6 dose at day 3 and 5 and a second smaller increment on day 15 (mean

Table 2. Side Effects

	rhIL-6 Doses ($\mu\text{g}/\text{kg}/\text{d}$)											
	0.5		1		2.5		5		10		20	
	IV	SC	IV	SC	IV	SC	IV	SC	IV	SC	IV	SC
No. patients	3	3	4	4	4	4	3	3	3	3	3	3
Fever												
WHO grade I	1	3	1	1	1	—	—	—	—	—	—	—
WHO grade II	1	—	2	2	1	4	3	3	3	3	2	3
Chills	—	—	—	—	3	4	2	—	2	1	2	2
Headache	1	2	1	1	3	2	2	3	—	2	1	2
Myalgia	—	1	—	1	—	1	—	—	2	2	—	—
Local erythema	1	2	3	3	1	3	—	3	1	3	—	3
Local infiltrate	1	1	1	—	—	—	—	—	—	—	—	—
Nausea	—	—	—	—	2	2	1	2	1	1	—	1
Hepatotoxicity WHO grade I-II	NA	—	NA	—	NA	1	NA	1	NA	2	NA	—

Abbreviation: NA, not applicable.

\pm SE; day 1, $6.33 \times 10^9/\text{L} \pm 0.46$; day 3, 9.38 ± 0.52 , $P < .001$; day 5, 6.88 ± 0.55 , $P < .02$; day 15, 7.12 ± 0.61 , $P < .05$ Fig 3). The increase in leukocytes initially consisted of an increase in neutrophils (day 3) and monocytes (days 3 and 8), whereas a significant increase in lymphocytes (dose 0.5 to 20 μg , $n = 18$, $P < .05$ v day 1) occurred on day 15. The effect of rhIL-6 on lymphocytes was characterized, especially at the lower dose levels, by a maximum increase 7 days after cessation of rhIL-6. On day 8, immunophenotyping (Fig 4) showed an increase in T cells, natural killer (NK)

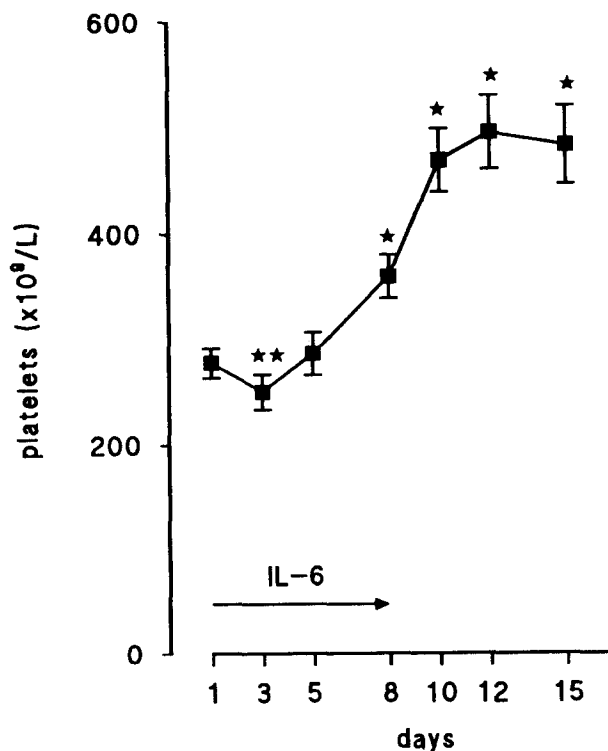


Fig 1. Effect of rhIL-6 on platelet counts. Each point represents the mean \pm SE of pooled data at all dose levels. P values were $< .001$ (*) and $< .002$ (**).

cells, and cells expressing the IL-2 receptor at 1.0 to 2.5 $\mu\text{g}/\text{kg}/\text{d}$. This increase was not consistent at 5.0 to 10 $\mu\text{g}/\text{kg}/\text{d}$ and there was a significant reduction of these cells compared with day 1 (CD4, CD8, CD25, $P < .05$; CD57, $P < .02$) at 20 $\mu\text{g}/\text{kg}/\text{d}$. There was no alteration in the levels of the immunoglobulins IgG and IgM, but there was a slight increase in IgA reaching a maximum level on day 15 (mean \pm SE: 2.87 ± 0.53 g/L v 3.33 ± 0.83 , $P < .01$). There was no effect observed for rhIL-6 on basophils or eosinophils.

Figure 5 shows the effect of rhIL-6 on hemoglobin (Hb). There was a dose-dependent rapid decrease in the Hb level that could not be explained by the amount of blood drawn from these patients. Serum Fe was not affected at the lowest rhIL-6 doses, but there was a rapid drop in serum Fe at higher doses. This drop in Hb and serum Fe was reversible after cessation of rhIL-6, without concomitant blood transfusion. Iron staining of the BM at the 10 $\mu\text{g}/\text{kg}/\text{d}$ dose did not show any changes, and there was also no change observed in the percentage of sideroblasts ($< 3\%$). There was no reticulocytosis during or after cessation of rhIL-6 with maximum (for anemia-corrected³⁴) values of reticulocytes of 0.5% to 1.3% at the 10 to 20 $\mu\text{g}/\text{kg}/\text{d}$ dose levels. Serum lactic dehydrogenase (LDH) levels were unchanged. Erythropoietin levels showed an increase at all dose levels at day 3 versus day 1 ($P < .05$) and they increased until day 8 ($P < .01$). This effect was dose dependent (day 8, $r = .56$, $P < .05$, Spearman rank analysis). The mean percentage increase of erythropoietin at 0.5 $\mu\text{g}/\text{kg}/\text{d}$ on day 8 compared with day 1 was

Table 3. Increase in Platelet Count per Dose Level rhIL-6

Dose rhIL-6 ($\mu\text{g}/\text{kg}/\text{d}$)	Baseline Value \pm SE ($\times 10^9/\text{L}$)	Maximum Value \pm SE ($\times 10^9/\text{L}$)	P Value
0.5	352 ± 35	444 ± 62	NS
1.0	266 ± 44	421 ± 59	$< .01$
2.5	284 ± 34	505 ± 69	$< .02$
5.0	238 ± 6	480 ± 43	$< .05$
10	273 ± 15	577 ± 86	NS
20	256 ± 32	743 ± 119	$< .05$

Abbreviation: NS, not significant.

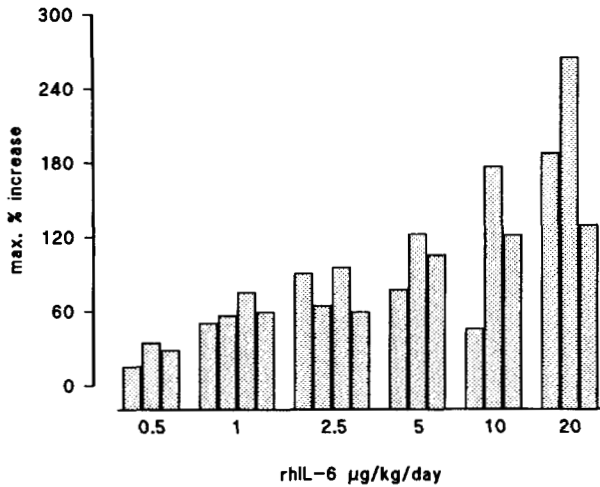


Fig 2. The maximum percentage platelet increase compared with baseline in all patients. Doses of rhIL-6 were 0.5, 1.0, 2.5, 5.0, 10, and 20 µg/kg/d. Each bar represents one patient. The response was dose related ($r = .84, P < .002$, Spearman rank analysis).

(mean ± SE) 47 ± 25 and at 20 µg/kg/d, 113 ± 65 . There was a decrease of erythropoietin levels after cessation of rhIL-6 at all doses. Serum ferritin levels at the 20 µg/kg/d dose were increased $440\% \pm 284\%$ at day 8, and decreased at day 15. The number of BFU-E and CFU-GM was not affected by 7 days rhIL-6 treatment. There was an observed increase of the M:E ratio (mean ± SE: day 1, 3.21 ± 0.45 ; day 8, 4.05 ± 0.59 ; $P < .02$).

Biochemical effects. Of 20 evaluative patients, 4 had a 2.7- to 6.1-fold increase in AST (serum glutamic oxaloacetic transaminase [SGOT]) and/or ALT (serum glutamic pyruvic

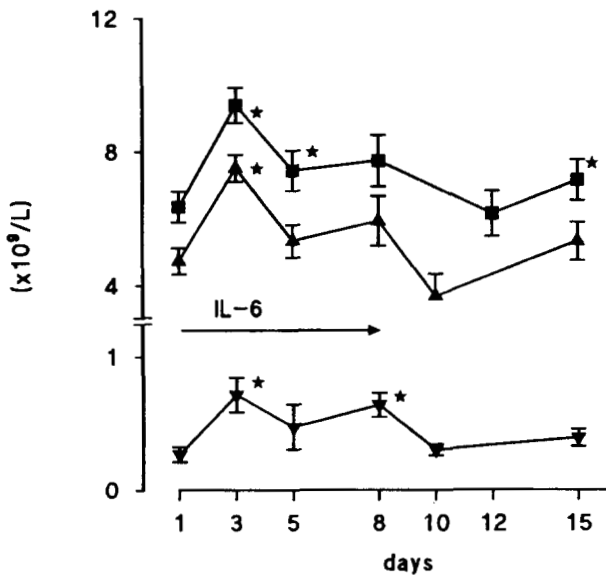


Fig 3. Effect of rhIL-6 on leukocytes (■), neutrophils (▲), and monocytes (▼). Represented is the mean ± SE at 2.5- to 20-µg/kg/d dose levels. An asterisk indicates a significant difference versus baseline.

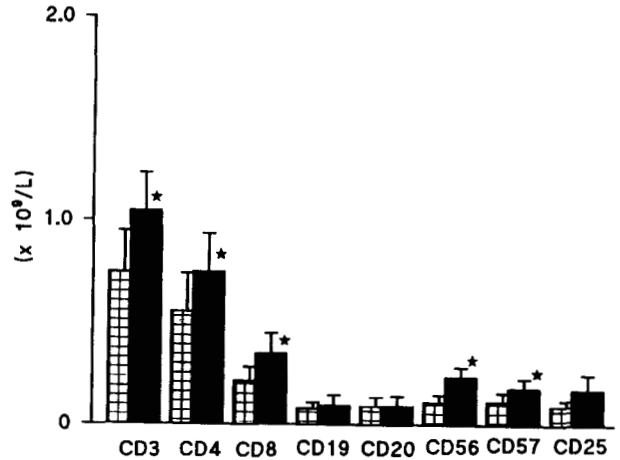


Fig 4. Effect of rhIL-6 on lymphocyte subsets. Represented are pooled results of the mean ± SE of the 1.0- and 2.5-µg/kg/d doses ($n = 5$). (▤), Results at day 1; (■) results at day 8. An asterisk indicates a significant difference versus baseline: for CD3, $P < .05$; CD4, $P < .01$; CD8, $P < .02$; CD56, $P < .02$; CD57, $P < .001$.

transaminase [SGPT]). A 1.4- to 3.4-fold increase of alkaline phosphatase occurred in 5 and γ GT increased 1.6- to 8.0-fold in 11 patients. In 5 of these patients, this was a solitary increase. The highest increase was observed at day 8; the increases were reversible after cessation of rhIL-6. Only 1 of the patients had liver metastases and of the 9 patients showing no changes in liver enzymes, 3 had tumor lesions in the liver. There was no relationship between the pretreatment

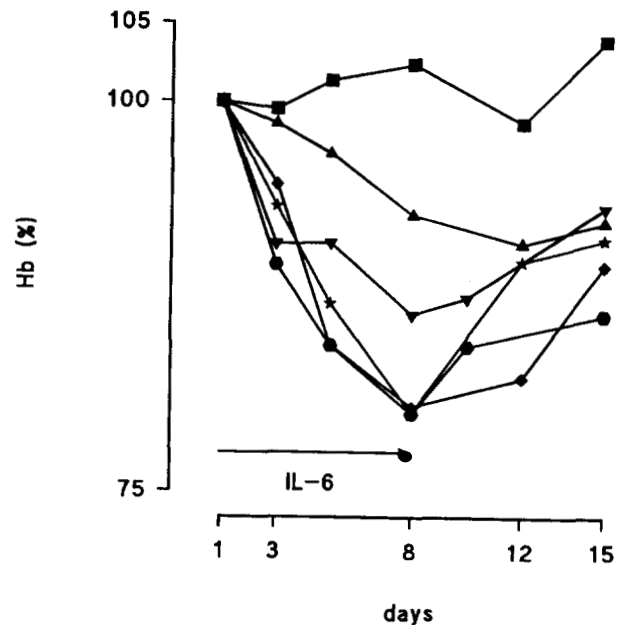


Fig 5. Effect of rhIL-6 on Hb level. Values are expressed in percentage, compared with baseline level of 100%. Represented are mean values at 0.5 µg (■), 1.0 µg (▲), 2.5 µg (▼), 5.0 µg (◆), 10 µg (⊕), and 20 µg (●). The response was dose related ($r = .73, P < .002$, Spearman rank analysis).

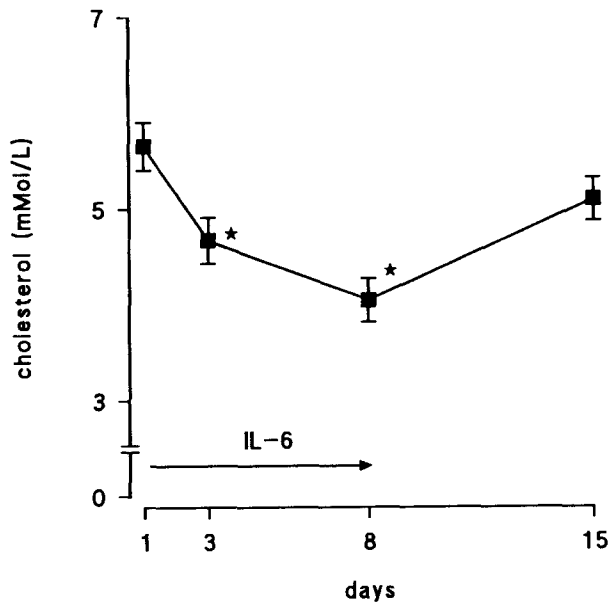


Fig 6. Effect of rhIL-6 on total cholesterol level. Represented is the mean \pm SE at the doses 1.0 to 20 μ g/kg/d. The asterisk indicates a significant difference versus baseline ($P < .001$).

liver function and the increases in liver enzymes during rhIL-6 treatment. Serum albumin decreased at day 8 with 5.3 ± 0.7 g/L (mean \pm SE) at 10 μ g/kg/d and 10.0 ± 1.0 g/L at 20 μ g/kg/d. Levels were normalizing at day 15. No effect was observed on serum creatinine. ANA increased in 1 patient at 0.5 μ g/kg/d rhIL-6. This increase persisted in the months after cessation of rhIL-6. RhIL-6 markedly reduced total cholesterol (Fig 6). This effect is rapid, pronounced, dose related (day 1 v day 8; $r = .69$, $P < .01$, Spearman rank analysis), and reversible.

Effects on acute phase proteins. rhIL-6 induced a rapid dose-dependent increase in CRP levels (Fig 7). The CRP levels showed a slight decrease during rhIL-6 treatment. The CRP levels in all patients at the highest dose level (20 μ g/kg/d) were lower than at 10 μ g/kg/d rhIL-6. The same pattern was observed for the acute phase protein SAA (Fig 8). RhIL-6 also increased fibrinogen at all dose levels, with a $74\% \pm 14\%$ increase on day 8 (mean \pm SE; $n = 12$; 4.87 ± 0.46 g/L v 8.28 ± 0.29 g/L, $P < .001$).

Effects on cytokines. IL-6 was detected in plasma during IV administration starting at 1.0 μ g/kg/d and incidentally during SC treatment (measured 24 hours after administration) at 5.0 μ g/kg/d and at 10 and 20 μ g/kg/d in levels up to 3,000 ng/L (Fig 9). There was no increase measured in IL-1 β and TNF α in any patient.

DISCUSSION

This study shows that rhIL-6 is relatively well tolerated by cancer patients at doses up to and including 10 μ g/kg/d with the following side effects: fever, chills, headache, myalgia, minimal local erythema at the injection side, nausea, reversible increase in liver enzymes, and anemia.

The fever and flu-like symptoms at doses of 0.5 to 10 μ g/kg/d were manageable with acetaminophen and nausea was

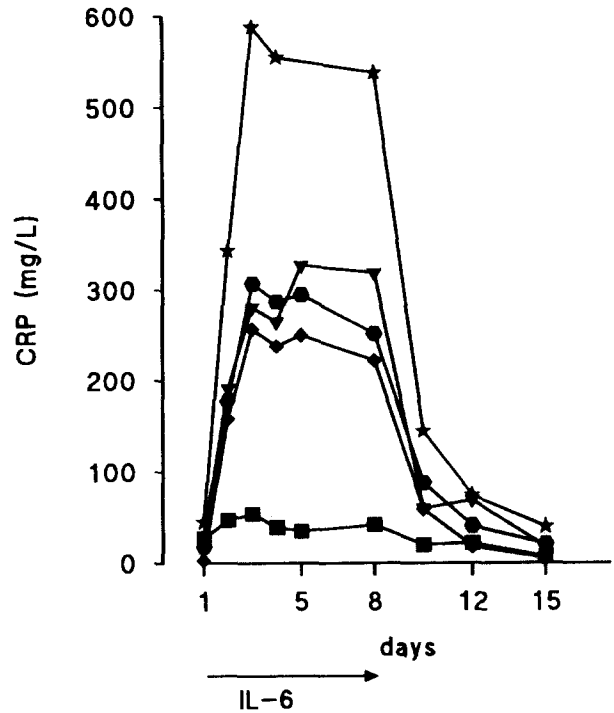


Fig 7. Effect of rhIL-6 on CRP. Represented are mean values at 0.5 μ g (\blacksquare), 2.5 μ g (\blacktriangledown), 5.0 μ g (\blacklozenge), 10 μ g (*), and 20 μ g (\bullet). The response was dose related up to 10 μ g ($r = .87$, $P < .002$, Spearman rank analysis).

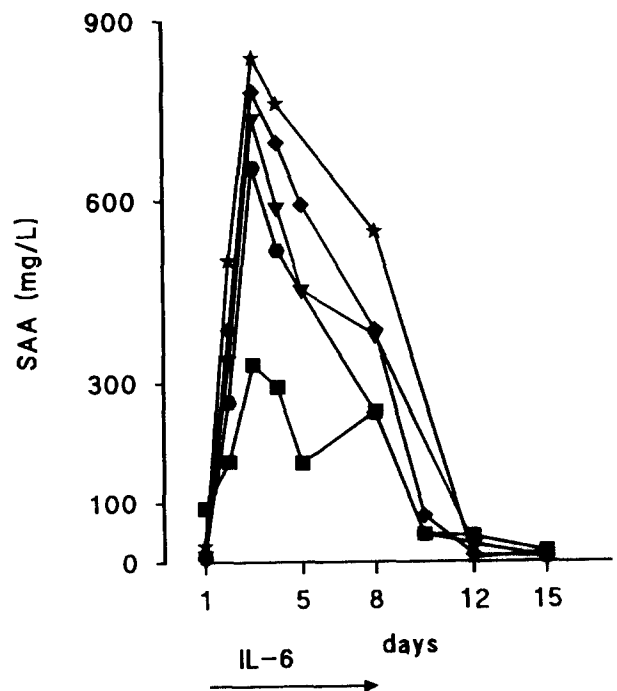


Fig 8. Effect of rhIL-6 on SAA. Represented are mean values at 0.5 μ g (\blacksquare), 2.5 μ g (\blacktriangledown), 5.0 μ g (\blacklozenge), 10 μ g (*), and 20 μ g (\bullet). The response was dose related up to 10 μ g ($r = .80$, $P < .002$, Spearman rank analysis).

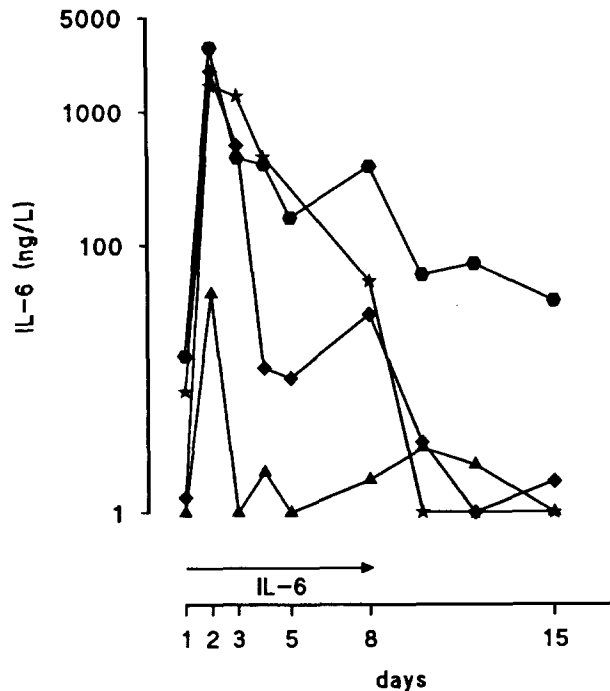


Fig 9. Serum IL-6 levels. Represented are mean values at 1.0 μg (■), 5.0 μg (◆), 10 μg (*), and 20 μg (●) on an exponential scale. The serum levels of IL-6 were measured by ELISA.

controlled with metoclopramide. The complaint of nausea was noteworthy and was also noticed in patients during rhIL-1 treatment in which high levels of IL-6 were observed.³⁵ A clinically important side effect was a rapid, dose-related fall in Hb levels of 2.7% to 26.8% in 8 days. The anemia coincided with a reduction in serum Fe and an increase in ferritin and erythropoietin. However, BM iron staining did not show any changes indicating sufficient iron stores. The reticulocyte count of less than 1.5% could indicate that the origin lies probably partly in an interference in red blood cell production rather than in a peripheral cause such as hemolysis.³⁴ This is confirmed by an unchanged LDH level. There was a reduction in serum albumin at the higher rhIL-6 doses, but no change in body weight was observed. The reduction of albumin could be a part of the acute phase response, as prealbumin production is suppressed by IL-6.²⁶ However, it could also indicate a change in distribution volume because measurement of the body weight is not a sensitive method. This anemia is also observed in animal studies and studies in humans,^{28-30,36} and resembles the anemia of chronic disease (ACD).³⁷ IL-1 β and TNF α administration also results in an anemia resembling ACD with a suppressive effect on CFU-E and/or BFU-E formation as a possible cause.³⁸⁻⁴⁰ In the present study, no change was found in BFU-E numbers. Because there appeared to be no change in IL-1 β and TNF α levels, the observations are most likely not caused by these cytokines, and might be a direct effect of IL-6. The suppressing effect on CFU-E has already been reported several hours after rhIL-1 α administration⁴¹ and could be responsible for the rapid decline of the Hb level observed. Another explanation could be sequestration. Further investigations are

needed to resolve the yet-unknown underlying mechanisms of the anemia observed.

The maximum tolerated dose in this setting for outpatient treatment of cancer patients was considered to be 10 μg rhIL-6/kg/d. This was based on general malaise symptoms, fever, and anemia and not on the occurrence of WHO grade III or IV toxicity in two patients at the same dose level. These data are in accordance with the results of Weber et al.³⁰ Based on the 11 patients treated, they considered 10 μg /kg/d to be the safely tolerated dose. Dose-limiting toxicity consisting of arrhythmia and hepatotoxicity was observed at 30 μg /kg/d. rhIL-6 treatment was cautioned against especially in patients with pretreatment liver dysfunction or extensive tumor infiltration.³⁰ In the present study, reversible liver enzyme increase, unrelated to the presence of liver metastases or pretreatment liver dysfunction and no clinical evidence of arrhythmia, was observed in 11 of the 20 patients.

The rhIL-6 treatment resulted in a dose-related increase in platelets, reaching a maximum after cessation of rhIL-6. This increase was preceded by an initial decrease in platelets at day 3, as was also noticed by Weber et al.³⁰ The origin of this initial decrease is still unknown. The increase in platelets coincided with a reduction in platelet volume.

rhIL-6 did increase leukocytes because of an increase in neutrophils and monocytes and this was not associated with an increased number of CFU-GM. It is conceivable that the increase may be related to the induction of other cytokines or to the cooperative effect of different cytokines because in vitro culture IL-6 can promote the differentiation of the monocytic pathway.⁴² The effect on lymphocyte subpopulations was dose dependent. An increase in T cells, NK cells, and cells expressing the IL-2 receptor was observed at the lower dose levels (1.0 and 2.5 μg /kg/d) and a reduction of the number of these cells at the highest dose level. Because of the limited number of patients at each dose step, it is difficult to conclude that these effects are clearly dose related. No increase in the number of B cells was observed in the present study, but of the Igs, IgA showed an increase with a maximum effect 7 days after end of treatment. The dose-dependent, lowering effect of rhIL-6 on serum cholesterol levels has also been observed after administration of other growth factors such as rhIL-3,⁴³ granulocyte-macrophage colony-stimulating factor (GM-CSF),⁴⁴ and M-CSF.⁴⁵ This reduction was characterized by a decrease in low-density lipoprotein (LDL)-cholesterol level.^{43,45,46} Contrary results have been found in rabbits with an increase in LDL and very LDL cholesterol and a decrease in high-density lipoprotein cholesterol after rhIL-6 administration and a down-regulation of the LDL receptor.⁴⁷

An impressive effect on acute phase protein production was observed, which is in agreement with animal data²⁶ and the recent reports in humans.^{36,48} Regarding the SAA, CRP, and fibrinogen response, it is interesting that SAA levels—and to a lesser extent CRP levels—already decreased during the rhIL-6 treatment after having reached a maximum at 48 hours. Although similar findings were reported by Mayer et al.²⁶ in nonhuman primates with SC treatment only, this might be the result of the administration schedule with IV initially and then SC treatment. The initial acute phase response dur-

ing the first 48 hours was dominated by SAA, whereas the increase in SAA and CRP levels during later rhIL-6 treatment, especially at higher rhIL-6 doses, tended to be equal in all patients studied. This suggests that SAA may predominate over CRP in acute situations, whereas CRP predominates the response in chronic acute phase reactions.

In conclusion, we have shown that rhIL-6 can be safely administered up to and including a dose of 10 $\mu\text{g}/\text{kg}/\text{d}$ SC with side effects common to other biologic response modifiers. An important and unexplained side effect, anemia, warrants further study. The most important clinical effect of rhIL-6 in humans is thrombocytosis with a potentially beneficial effect on WBCs being observed in addition. Further studies into the efficacy of rhIL-6 alone and after chemotherapy should be performed.

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