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## Original Paper

# Phase I Study of Interleukin-6 in Children with Solid Tumours in Relapse

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**The aim of this study was to evaluate the feasibility, toxicity and efficacy of escalating doses of subcutaneous recombinant interleukin-6 (IL-6) in children with solid tumours in relapse. Recombinant IL-6 was administered subcutaneously once daily for 14 consecutive days, with a 14 day follow-up period. The starting dose for IL-6 was 1 μg/kg/day and was escalated in subsequent patients groups until 10 μg/kg. Doses were escalated every 3 patients, provided that grade III or IV organ toxicity did not occur at the preceding dose level. Twelve patients were treated, three at each dose level. No grade 3–4 major organ toxicity was observed. Flu-like symptoms and fatigue were the most common side-effects. All these symptoms resolved after the end of IL-6 administration. Significant increases in acute-phase proteins (CRP [C reactive protein], fibrinogen) and ESR (Erthrocyte sedimentation rate) were observed in all patients. Stimulatory effects on thrombocytopoiesis were observed at every dose level, and were maximal at 5 μg/kg and 10 μg/kg. There was no tumour response observed during IL-6 administration. Pharmacokinetic profiles performed in 3 patients are consistent with previous reports in adults. IL-6 is a promising new cytokine for paediatric oncology, in particular to increase thrombocyte counts. We recommend that further studies in children proceed at a dose of 5–10 μg/kg/day in a once or, better, twice daily administration.** © **1997 Elsevier Science Ltd.**

**Key words: interleukin-6, phase I, child, solid tumour, platelet**
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## INTRODUCTION

INTERLEUKIN 6 (IL-6) is a pleiotropic cytokine involved in multiple biological processes, including regulation of haematopoiesis, immune responses, and acute-phase reaction [1]. IL-6 has been reported to exert potent anti-tumour effects—in the mouse model, IL-6 inhibits the progression of methylcholantrene-induced tumours and transplanted human mammary and colorectal carcinomas [2]. These antitumour effects are probably secondary to the stimulation of T-cell activation and proliferation in these models [2]. IL-6 is a potent haematopoietic growth factor in particular for the megakaryocyte lineage in animals and in man [3–10]. It induces nuclear endoreduplication and maturation of megakaryocytes, which leads to an increase in size and to a shift of ploidy toward higher values. As a consequence, it may increase the release of a large number of platelets and induce an increase of circulating platelets [10]. In cancer patients, a strong correlation between circulating serum IL-6 and thrombocyte counts has been reported [11]. Anti-IL-6 administration has been reported to decrease circulating platelet counts in patients with normal counts as well as in patients with thrombocytosis [11, 12].

Taken altogether, these results suggest that IL-6 may be useful for increasing thrombocyte counts in clinical practice, in particular in patients receiving cytotoxic chemotherapy. Recently, IL-6 became available for clinical use in phase I–

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II trials in adults with cancer or haematological malignancies [13–22]. In humans, IL-6 administration has been reported to induce inconsistent antitumour effects, but to increase platelets counts in phase I–II trials, as well as in a randomised trial [13–22].

To evaluate the potential antitumour and thrombopoietic effects of IL-6 in children, a phase I study of human recombinant IL-6 was performed in children with solid tumours in first or second relapse.

## PATIENTS AND METHODS

*Patient selection*

Twelve patients entered the study. Eligibility criteria were: age less than 16 years, solid tumour in first or second relapse except hepatoblastoma and non-Hodgin's lymphoma, Karnofsky performance status over 50%, life expectancy of at least 3 months, written informed consent by parents or legal guardian. Patients were required to have a minimum platelet count of $100 \times 10^9$/l, a haemoglobin level >8 g/dl, and a white blood cell (WBC) count $>3 \times 10^9$/l. Patients also had to meet the following criteria: creatinine value within 2-fold of the normal range, AST, γGT and bilirubin within 3-fold of the normal range, normal coagulation parameters, no active infection. Concomitant chemotherapy, radiotherapy, corticosteriods, cytokines and other growth factors were not allowed. Patients could not have received corticosteriods for 4 weeks, and chemotherapy or radiotherapy or other cytokines for 8 weeks.

Patients were excluded from participation in the study when any of the above criteria were not met or when they presented with significant history of severe allergy, generalised psoriasis, glomerulonephritis or active autoimmune disease, evidence of serious active infection, or impairment of cardiac, pulmonary or central nervous system (CNS) function. Patients with hepatoblastoma and non-Hodgkin's lymphomas (including Burkitt's lymphoma) were not eligible.

*Interleukin-6 administration*

Interleukin-6 was an *E. coli*-derived recombinant human IL-6 supplied by Sandoz Pharma Ltd (Basel, Switzerland), lyophilised in 2 ml vials of 150 μg or 750 μg and to be reconstituted with 1 ml of the supplied sterile water. IL-6 was administered once daily in the morning, subcutaneously either in the abdomen, the thigh or the upper arm. The treatment was initiated in hospital for the first 3 days of administration. If the patient was clinically stable and IL-6 well tolerated, the treatment was then continued as an outpatient for 14 days. Patients then had 14 days off therapy before final evaluation unless significant tumour progression occurred.

The study plan was to treat patients at four dose levels of 1, 2.5, 5 and 10 μg/kg/day. Due to significant hepatic and cardiac toxicity at higher dosages during phase I studies in adults, no further dose escalation was planned. At each dose level, 3 patients were included unless grade 3 or greater WHO toxicity occurred (excluding fever). The maximum tolerable dose was defined as the dose level below that producing dose-limiting toxicity. Should the MTD be achieved, further patients, to a maximum of 12 in the completed study, could be recruited at that dose level. Paracetamol 15 mg/kg every 6 hours was administered to the patient if chills and/or fever occurred after the first dose. Proparacetamol was administered intravenously to several patients for severe chilling and high fever after the first dose.

*Study monitoring*

All patients' parents gave informed consent before initiation of therapy. The protocol was reviewed and approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale of the Centre Léon Bérard).

At the time of inclusion, all patients underwent physical examination including pulse and blood pressure; full blood count with reticulocytes; electrolytes, liver function tests; prothrombin time, partial thromboplastin time (PTT), fibrinogen, ESR, C-reactive protein; urine analysis; immunoglobulin (A, E, G, M) levels; plasma cytokine levels (TNF-α (tumour necrosis factor-α), IL-1b, IL-2, IL-2 receptor, erythropoietin, IL-6); fluorescence activated cell sorter (FACS) analysis of circulating mononuclear cells; measurement of any clearly identifiable primary or metastatic lesion either by X-rays, computer tomography (CT), magnetic resonance imaging (MRI), MIBG, scintigraphy, or other appropriate imaging techniques.

Before treatment, patients also underwent bone marrow aspiration with morphology, cytology and ploidy of megakaryocytes. Bone marrow biopsy was not mandatory at inclusion. During treatment, physical examination and all the above blood tests and urine analysis were repeated on days 8, 15, 22 and 29, except for blood count and reticulocytes performed twice weekly. Evaluation of the tumour response was performed on days 15 and 29, with the same imaging technique used at entry. Bone marrow aspiration was repeated on day 15 and included morphology, cytology and ploidy of megakaryocytes.

*Response criteria*

Extent of disease was evaluated less than two weeks before inclusion in the trial, at the end of the course of IL-6, and two weeks later by clinical examination and imaging studies when indicated. Complete remission was defined as the complete disappearance of all lesions, persisting for at least 4 weeks from its documentation. Partial remission was defined as a decrease in all known lesions by 50% or more lasting for at least 4 weeks from documentation. Progressive disease was defined as an increase of 25% or more in any existing lesion and/or the appearance of a new lesion. Stable disease was defined as a non-significant change in measurable lesion (e.g. decrease of less than 50% or increase of less than 25%).

*Pharmacokinetic study*

Blood samples were collected for pharmacokinetic study in 3 patients at three different dose levels (1, 2.5 and 5 μg/kg). Venous blood samples (2.5 ml) were obtained from an indwelling Silastic central line prior to rhIL-6 administration, and at the following post infusion times: 0100, 0300, 0500, 1000, 1500 and 2400 hours. Blood samples were placed into glass tubes containing EDTA. Cytokine levels were measured using enzyme-linked immunosorbent assay (ELISA) kits. IL-6 ELISA kits were obtained from Genzyme (TEBU, Le Perray-en-Yvelines, France; IT-6 ELISA: sensitivity 70 pg/ml, Genzyme). Erythropoietin

(EPO) and tumour necrosis factor (TNF) were measured using radioimmunoassay (RIA) kits (IRMA TNF, Medgenix, Fleurus, Belguim; sensitivity 5 pg/ml; EPO RIA, Sorin Biomedica, Stillwater, Minnesota, U.S.A.). Every determination was repeated at least twice in separate experiments. Differences in cytokine concentrations between each assay do not exceed 10%. All these assays are highly specific and do not cross-react with other cytokines.

Other patients without pharmacokinetic studies were followed weekly, with a measurement of all cytokines on days 1, 8 and 15.

## RESULTS

Patient characteristics and prior therapies are listed in Table 1. All patients were previously treated by one or two multi-agent chemotherapy protocols. Among 4 patients with neuroblastoma, 3 previously received high-dose chemotherapy followed by bone marrow transplantation (including one with total body irradiation). One patient with rhabdomyosarcoma also received high-dose chemotherapy followed by bone marrow rescue. Two patients with medulloblastoma and 1 with anaplastic ependymoma were previously treated by craniospinal irradiation. All patients fulfilled the eligibility criteria. Lansky scale [23] ranged from 90 to 100%. All had measurable disease and either progressive (10 children) or refractory disease (2 children). Two children had bone marrow involvement at the time of study entry: one had minimal metastatic disease (1–2% malignant cells), whereas the second had a more pronounced involvement (50–80%).

All children received a single 14-day course of IL-6 and were assessable for response and toxicity.

*Toxicities (Table 1)*

All 12 patients developed fever up to WHO grade II–III. Fever occurred early within 1–3 h following the first IL-6 injection and resolved before the following injection. The maximum body temperature ranged from 38.5 to 41°C. Fever was not dose related: the average maximum temperature was 39.4°C, 39.7°C, 39.1°C and 39.9°C for the four dose levels of 1, 2.5, 5 and 10 μg/kg, respectively. Chills occurred during the onset of fever in most patients. These symptoms were minimised once patients were given paracetamol as a prevention, but no tachyphylaxis occurred during continued administration. Progressive fatigue was reported by 7 children and by all parents. Fatigue lasted until completion of the treatment and required 1–2 weeks to resolve. Four patients complained of nausea or vomiting, which required anti-emetic therapy in one child. All children experienced loss of appetite during the treatment, but only one had a significant weight loss of 5%. Two children developed infections requiring therapy. One had a *Streptococcus pneumoniae* pneumonia with positive blood cultures that occurred on day 3 of IL-6 and resolved with antibiotics. The second developed a *Staphylococcus epidermidis* catheter-related infection during the follow-up period, that was treated with antibiotics and required catheter removal. Finally, no dose-limiting toxicity was encountered at any dose level.

*Biochemical effects (Table 2)*

Serum creatinine levels remained stable throughout the study. Serum levels of urea showed a slight but non-significant decrease during IL-6 treatment. Total serum immunoglobin levels increased during treatment (mean ± SEM; day 1, 10.68 ± 0.9 g/l; day 15, 12.21 ± 1.1 g/l, $P = 0.005$). Levels returned to pretreatment values two weeks after cessation of IL-6 administration. These changes in immunoglobulin levels predominantly involved IgA (mean ± SEM; day 1, 1.32 ± 0.2 g/l; day 15, 2.07 ± 0.35 g/l, $P = 0.001$) and IgM (mean ± SEM; day 1, 0.85 ± 0.1 g/l; day 15, 1.18 ± 0.1 g/l, $P = 0.009$). No significant changes were noted for serum IgG and IgE. Serum levels of albumin decreased significantly during treatment (mean ± SE; day 1, 43.9 ± 1.4 g/l; day 15, 39.3 ± 1.0 g/l, $P = 0.007$), whereas the total serum protein level varied in the opposite direction (mean ± SEM; day 1, 59.9 ± 2.1 g/l; day 15, 65.8 ± 2.5 g/l, $P = 0.02$).

Transient asymptomatic and grade I WHO increases in aspartate transaminase (AST) (5 patients), gamma glutamyl transpeptidase (γGT) and bilirubin (1 patient each) were recorded and returned to baseline values within 1–2 weeks. No significant changes in serum electrolytes were observed.

*Haematological effects (Tables 2 and 3)*

Patients demonstrated a statistically significant increase in platelet counts over the pretreatment baseline (Table 3). The platelet counts showed an initial decrease from a base-

*Table 1. Patients' characteristics*

| Patient no. | Sex/age (years) | Diagnosis | Previous therapy | Dose of IL-6 (μg/kg/d) | Side-effects observed during IL-6 |
|---|---|---|---|---|---|
| 1 | M/6 | osteosarcoma | chemo-XRT | 1 | F(39.4) |
| 2 | F/4 | medulloblastoma | surg-chemo-CS XRT | 1 | F(38.7) |
| 3 | F/11 | neuroblastoma | surg-chemo-HDC-TBI | 1 | F(40.1), pneumonia |
| 4 | M/15 | ependymoma | surg-chemo-CS XRT | 2.5 | F(38.5),Fg,V,WL |
| 5 | F/8 | neuroblastoma | surg-chemo-HDC-XRT | 2.5 | F(41) |
| 6 | F/13 | osteosarcoma | surg-chemo | 2.5 | F(39.5), |
| 7 | M/4 | rhabdomyosarcoma | chemo-HDC | 5 | F(39.5),Fg |
| 8 | M/3 | neuroblastoma | surg-chemo-HDC | 5 | F(39.5),Fg,V |
| 9 | M/14 | glioma | surg-chemo-XRT | 5 | F(38.2),Fg |
| 10 | F/5 | neuroblastoma | surg-chemo | 10 | F(40.2),Fg |
| 11 | F/9 | medulloblastoma | surg-chemo-CS XRT | 10 | F(39.6),Fg,N |
| 12 | M/13 | Ewing's sarcoma | chemo-XRT | 10 | F(39.3),Fg,V,infection |

M, male; F, female; XRT, radiation therapy; CS, cranio-spinal; HDC, high-dose chemotherapy; chemo, chemotherapy; surg, surgery; F, fever (maximum temperature, °C); Fg, fatigue; V, vomiting; WL, significant weight loss; N, nausea.

*Table 2. Biological and haematological changes during and after IL-6*

| | Units | D1 | D8 | D15 | D22 | D29 | *P* value (*t*-test) |
|---|---|---|---|---|---|---|---|
| Protein | g/l | 59.9 ± 2.1 | 62 ± 2.8 | 65.8 ± 2.5 | 62 ± 3.1 | 68.2 ± 3.7 | 0.02 |
| Albumin | g/l | 43.9 ± 1.4 | 41.2 ± 0.9 | 39.3 ± 1 | 42.9 ± 1.2 | 43.7 ± 1 | 0.007 |
| IgM | g/l | 0.85 ± 0.1 | | 1.18 ± 0.1 | | 1.21 ± 0.24 | 0.009 |
| IgA | g/l | 1.32 ± 0.2 | | 2.07 ± 0.35 | | 1.61 ± 0.22 | 0.001 |
| CRP | mg/l | 8.5 ± 2.3 | 115 ± 20 | 98 ± 19 | 9 ± 2 | 8.6 ± 3 | <0.001 |
| ESR | mm/h | 25 ± 6 | 94 ± 9 | 98 ± 10 | 46 ± 10 | 27 ± 5 | <0.001 |
| Fibrinogen | g/l | 3.5 ± 0.2 | 6.5 ± 0.6 | 6.3 ± 0.5 | 3.4 ± 0.3 | 3.5 ± 0.3 | 0.001 |
| Haemoglobin | g/dl | 11.7 ± 0.6 | 9.9 ± 0.5* | 9.7 ± 0.6* | 11 ± 0.6* | 11.4 ± 0.7* | 0.004 |
| Reticulocytes | G/l | 148 ± 46 | 79 ± 23 | 88 ± 28 | 149 ± 40 | 158 ± 56 | NS |

±relates to the standard error of the mean; *9 patients only (3 patients having been transfused); NS, non-significant.

line mean of $205 \times 10^9$/l to $188 \times 10^9$/l at day 3. The increase then became significant at day 8 (mean value: $290 \times 10^9$/l, $P = 0.034$) and achieved a maximum at day 15 (mean value: $373 \times 10^9$/l, $P = 0.019$). The increase from baseline remained significant at day 22, seven days after the end of IL-6 administration (mean value: $324 \times 10^9$/l) and the platelet count then returned to initial values at day 28. There was a relationship between the dose of IL-6 and the increase in platelet count. At day 15 patients treated at the 1 and 2.5 μg levels showed an increase from a baseline mean of $201 \times 10^9$/l to $252 \times 10^9$/l ($P = 0.43$), while patients treated at the 5 and 10 μg levels showed an increase from $209 \times 10^9$/l to $493 \times 10^9$/l ($P = 0.055$). Comparison between the bone marrow specimens obtained at days 1 and 15 did not reveal an increase in the number of megakaryocytes. Ploidy of bone marrow megakaryocytes assessed by flow cytometric analysis showed a non-significant change in ploidy distribution by increasing the percentage of 64N cells from 1.9 before treatment to 5% at day 15.

All patients had a decrease in haemoglobin that required blood cell transfusions in three. Anaemia could not be explained by repeated blood samples before and during treatment. A significant decrease in haemoglobin level occurred within the 3 first days of treatment (day 1, 11.7 ± 0.6 g/dl; day 3, 10.6 ± 2.3 g/dl, $P < 0.004$). Haemoglobin level decreased further, reaching a nadir between day 8 (9.9 g/dl) and day 14 (9.7 g/dl). Haemoglobin levels normalised at day 29, 2 weeks after the completion of treatment. Similar changes were found for red blood cell (RBC) counts and venous hematocrit. Concomitantly, the circulating reticulocyte count decreased during the treatment period and returned to baseline values during the follow-up period. Erythropoietin levels showed a slight but non-significant increase during treatment in the whole cohort of patients (mean ± SE; day 1, 12.6 ± 2.4 pg/ml; day 8, 14 ± 1.8 pg/ml; day 15, 13.8 ± 2.1 pg/ml).

No consistent changes in leukocyte, neutrophil and lymphocyte counts were observed. Monocytes increased transiently at day 3 from a mean baseline value of $0.6 \times 10^9$/l to $0.9 \times 10^9$/l ($P = 0.005$) and then returned to baseline values. A phenotypic and functional analysis of peripheral lymphocytes was performed along with the course of IL-6 therapy. At the onset, all patients demonstrated modifications of lymphocyte distribution, including an excess of CD8-positive T cells and decreased percentage of natural killer (NK) cells. At completion of IL-6 therapy, the percentage of both CD8 T lymphocytes and NK cells showed a decrease ($P = 0.057$ and 0.04, respectively). There was no significant modification of the other components. The NK and CD8 cells changes remained stable 2 weeks after completion of IL-6 therapy.

*Effect on acute-phase proteins (Table 2)*

In response to the IL-6 treatment, serum levels of positively regulated acute-phase proteins increased. C-reactive protein levels increased significantly during the first week of treatment (mean ± SEM; day 1, 8.5 ± 2.3 mg/l; day 8, 115 ± 20 mg/l, $P < 0.001$) and remained stable during the

*Table 3. Changes in peripheral blood platelet count (×10⁹/L) in 12 patients treated with IL-6 at 1, 2.5, 5 and 10 μg/kg/day for 14 days*

| Patient number | Dose of IL-6 (μg/kg) | D1 | D3 | D8 (variation/D1 %) | D15 (variation/D1 %) | D22 | D29 |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 314 | 334 | 338 (+8) | 393 (+25) | 382 | ND |
| 2 | 1 | 160 | 153 | 150 (−6) | 231 (+44) | 225 | 184 |
| 3 | 1 | 100 | 90 | 85 (−15) | 95 (−5) | 95 | 69 |
| 4 | 2.5 | 112 | 77 | 117 (+14) | 127 (+13) | 104 | 145 |
| 5 | 2.5 | 228 | ND | 345 (+51) | 354 (+55) | 341 | 197 |
| 6 | 2.5 | 296 | 263 | 290 (−2) | 316 (+7) | 309 | 259 |
| 7 | 5 | 111 | 120 | 121 (+9) | 110 (−1) | 140 | 160 |
| 8 | 5 | 165 | 146 | 188 (+14) | 172 (+4) | 178 | 185 |
| 9 | 5 | 206 | 166 | 319 (+55) | 613 (+198) | 503 | 253 |
| 10 | 10 | 204 | 202 | 360 (+76) | 440 (+116) | 305 | 157 |
| 11 | 10 | 225 | ND | 618 (+175) | 867 (+285) | 669 | 380 |
| 2 | 10 | 343 | 325 | 544 (+59) | 759 (+121) | 610 | ND |
| Mean | | 205 | 188 | 290 | 373 | 322 | 199 |

ND, not done.

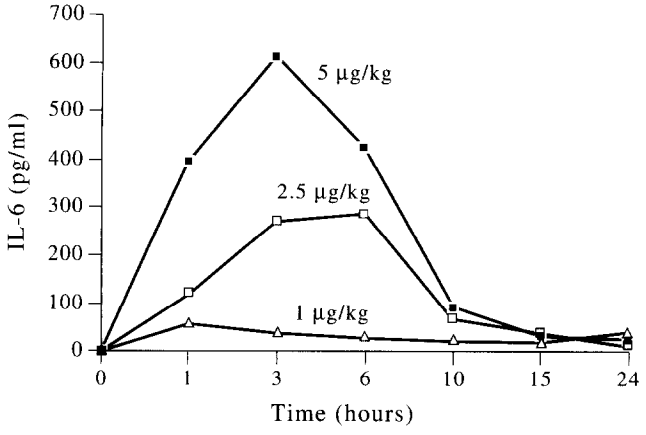


**Figure 1. Pharmacokinetic profile of serum IL-6 measurement for patients 1, 4 and 7 over a 24 hour period following three different doses (initial subcutaneous dose of 1, 2.5 and 5 μg/kg, respectively).**

second week of treatment (mean ± SEM; day 15, 98 ± 19 mg/l). They decreased dramatically after treatment and returned to baseline values at day 22. Mean fibrinogen levels increased from a baseline of 3.6 g/l to 6.5 g/l at day 8, 6.3 g/l at day 15 and normalised at day 22 (3.4 g/l). ESR showed the same profile, with a mean baseline value of 25–94 mm at day 8 and 98 mm at day 15. ESR decreased over a 2 week period to baseline values. TNF-α serum levels did not significantly vary throughout the treatment period (mean ± SEM; day 1, 12.6 ± 2.4 pg/ml; day 8, 14 ± 1.8 pg/ml; day 15, 13.8 ± 2.1 pg/ml).

*Pharmacokinetics*

Baseline levels of IL-6 were assessed in all patients. They ranged from 2.3 to 29.6 pg/ml (median 5.3 pg/ml). Patients 1, 4 and 7 underwent pharmacokinetic studies following the first dose of IL-6. Data are presented in Figure 1. The peak level was noted at 1 h and for the 1 μg/kg dose, 6 h for the 2.5 μg/kg dose and 3 h for the 5 μg/kg dose. Serum IL-6 levels in the range of 300 pg/ml were detectable at 6 h in patients 4 and 7. There are not enough data for a two compartmental analysis, but the half-life in a one compartmental model ranges from 2.4 to 5.7 h, suggesting large inter-individual variations. The area under the curve for the 3 patients ranged from 0.027 to 0.095 μg/ml.mn per 1 μg per kg dose.

*Antitumour response*

None of the 12 children treated with IL-6 showed an objective response. Six patients had stable disease and 6 experienced tumour progression. Two patients with evidence of progressive disease were withdrawn from the follow-up period and received chemotherapy within 8–9 days after treatment completion.

## DISCUSSION

IL-6 has been assessed in phase I and II studies in adult patients [13–22]. These studies have shown that IL-6 is a potent thrombopoietic factor at doses that are clinically well tolerated in adults. However, there was no available information for the *in vivo* use of IL-6 in children. This phase I study demonstrates that IL-6 administration is feasible in children. IL-6 significantly increases platelet counts and toxicity up to 10 μg/kg/day is not a limiting factor. Side-effects previously reported in adults include headache, fever, chills and myalgia commonly described as a flu-like syndrome [13, 14, 16, 19, 22]. Dose-limiting toxicity has been observed at 30 μg/kg/day, and includes cardiac dysrhythmia and severe hepatotoxicity [13]. In our experience, using a lower yet effective dose for platelet increase, most of the side-effects, in particular fever, were easily overcome or minimised with the use of paracetamol. However, fatigue, the most common toxicity reported, was not decreased by paracetamol. Since quality of life of their child is a major concern for parents, both daily subcutaneous injections and fatigue could be limiting factors for further development or dose escalation of IL-6 in this setting. However, it should be noted that IL-6 was much better tolerated than IL2 previously assessed in our institution [24]. In particular, hypotension and vascular leak observed with IL2 and other cytokines have not been reported with IL-6.

IL-6 was found to increase significantly the platelet count in 9 patients in this study, with an apparent dose-dependent effect. The platelet count was found to increase after an initial decrease at day 3. This transient decrease might be explained by haemodilution [26]. The increase in circulating platelets ranged from 0.04–2.85-fold according to the dose of IL-6 and inter-individual variability. It is important to note that most of the patients were previously heavily treated, and that cumulative bone marrow toxicity induced by chemotherapy and radiotherapy may have altered the effects of IL-6. Whether the effect of IL-6 in less pretreated or in newly diagnosed patients could lead to a more significant rise in platelet count remains unclear. Based on the present experience and previous studies in adult patients, a dose between 5 and 10 μg/kg/day for future paediatric studies should be appropriate.

The rise in platelet count does not differ from previous reports in adult patients. The mechanism of this increase has been initially assessed in healthy monkeys [26]. Following IL-6 administration, the ploidy of megakaryocytes demonstrates a shift towards higher values, resulting in enhanced platelet production. This shift in ploidy, though less significant, has also been observed in this study.

Several reports have focused on IL-6-induced anaemia [27, 28]. Due to an increase in transfusion requirements, a study of IL-6 in patient with aplastic anaemia was stopped [29]. In the present study, anaemia was found to be normocytic, without any sign of haemolysis and bone marrow examination did not reveal significant changes in the number or the appearance of erythroid precursors. Changes in erythropoietin levels have been observed by some authors [4], but are unlikely to explain the early developing anaemia. An inflammatory process secondary to IL-6 therapy could explain both anaemia and low reticulocyte count. For some authors, anaemia caused by IL-6 could be related to an increase in plasma volume [28]. These changes in plasma volume could contribute to the decrease in serum albumin levels, and the initial reduction in the platelet count. Since IL-6 does not affect the red blood cell volume, the benefit of blood transfusions is questionable and guidelines for transfusion requirements should be reassessed in phase II or III studies.

IL-6 has been originally identified as a factor inducing immunoglobin production in Epstein–Barr virus transformed B-cell lines [30]. It also induces cytotoxic T-cell differen-

tiation and T cell growth [31]. In our study, the total number of lymphocytes was not affected during treatment, but IL-6 induced a slight decrease in both NKH1 and CD8 cells. Baseline immunological assessment demonstrated alterations in most of the patients at the time of study entry with an excess of CD8-positive T cells. The impaired immune status might explain the paradoxical effect of IL-6 on T-cells in this report. IL-6 administration was found to modulate immunoglobulin production by B-cells *in vivo*: serum IgA and IgM levels significantly increased during treatment, whereas IgG and IgE levels remained stable. Although the rise of immunoglobulin was not observed in adult studies, these results indicate that the production of IgA and IgM is enhanced by exogenous IL-6 administration in children.

In response to the IL-6 treatment, serum levels of positively regulated acute-phase proteins rapidly increased. Changes in fibrinogen, CRP and ESR were consistent with previous reports in adults [32]. Similarly, correlation between CRP and IL-6 levels *in vivo* have been reported in pathological situations [1, 33]. In our study, the range of response was approximately the same for the four dose levels, indicating that doses as low as 1 μg/kg day of IL-6 induce the maximal stimulation of CRP and fibrinogen production. Similar observations were made for the fever induced by IL-6 [34].

Several *in vitro* and animal studies have suggested that IL-6 might potentially benefit cancer patients by stimulating host defence mechanisms against tumours [31, 33]. None of the patients had an objective response in the present study, possibly because of an impaired baseline immunological status and subsequently a poor immunological response to cytokine. However, it must also be noted that the anti-tumour activity of IL-6 has not been reproducibly demonstrated *in vivo* in man and that recently, tumour progression apparently induced by exogenous IL-6 administration has been reported [35].

The 2.4–5.7 h half-life make a twice-daily injection more suitable than a single daily injection. This is in contrast with previous reports in adult patients [13]. However, in the phase I study conducted by Weber and associates [13], recommendations for daily administration were based on significant residual levels of IL-6 following higher doses (up to 30 μg/kg) that cannot be recommended in clinical trials. Further studies in children could assess IL-6 given intravenously.

The increasingly aggressive use of chemotherapy in childhood has led to major improvement in survival. However, chemotherapy-induced thrombocytopenia has resulted in an increase in the need for platelet concentrates to prevent haemorrhagic complications. Further development and licensing of IL-6 is still uncertain. In this study, we demonstrate that IL-6 can increase thrombocyte counts in children with cancer. The capacity of this cytokine to prevent or limit thrombopenia induced by cytotoxic chemotherapy should be tested in paediatric phase II or III studies with a daily dose of IL-6 of 5–10 μg/kg/day in one or two injections.


1. Kishimito T. The biology of interleukin 6. *Blood* 1989, **74**, 1–10.
2. Chen L, Mory Y, Zilberstein A, *et al.* Growth inhibition of human breast carcinoma and leukemia/lymphoma cell lines by recominant interferon-2. *Proc Natl Acad Sci USA* 1988, **85**, 8037–8041.
3. Rennick D, Jackson J, Yang G, *et al.* Interleukin-6 interacts with interleukin-4 and other hematopoietic growth factors to selectively enhance the growth of megakaryocytic, erythroid, myeloid, and multipotential progenitor cells. *Blood* 1989, **73**, 1828–1835.
4. Patchen ML, MacVittie TJ, Williams JL, *et al.* Administration of interleukin-6 stimulates multilineage hematopoiesis and accelerates recovery from ratiation-induced hematopoietic depression. *Blood* 1991, **77**, 472–480.
5. Stahl CP, Zucker-Franklin D, Evatt BL, *et al.* Effects of human interleukin-6 on megakaryocyte development and thrombocytopoiesis in primates. *Blood* 1991, **78**, 1467–1475.
6. Lotem J, Shabo Y, Sachs L. Regulation of megakaryocyte development by interleukin-6. *Blood* 1989, **74**, 1545–1551.
7. Hill RJ, Warren MK, Stenberg P, *et al.* Stimulation of megakaryocytopoiesis in mice by human recombinant interleukin-6. *Blood* 1991, **77**, 2–8.
8. Stahl CP, Zucker-Franklin D, Evatt BL, *et al.* Effects of human interleukin-6 on megakaryocyte development and thrombocytopoiesis in primates. *Blood* 1991, **78**, 1467–1475.
9. Geissler K, Valent P, Bettelheim P, *et al. In vivo* synergism of recombinant human interleukin-3 and recombinant human interleukin-6 on thrombopoiesis in primates. *Blood* 1992, **79**, 1155–1160.
10. Young RI, Howard Scarffe J. Interleukin-6 in thrombopoiesis. *Interferons and Cytokines* 1995, **26**, 9–12.
11. Blay JY, Rossi JF, Wijdenes J, *et al.* Role of IL-6 in paraneoplastic thrombocytosis. *Blood* 1993, **82**, 2261–2262.
12. Emilie D, Wijdenes J, Gisselbrecht C, *et al.* Administration of an anti-interleukin 6 monoclonal antibody to patients with acquired immunodeficiency syndrome and lymphoma. *Blood* 1994, **84**, 2472–2479.
13. Weber J, Yang JC, Topalian SL, *et al.* Phase I trial of subcutaneous interleukin-6 in patients with advanced malignancies. *J Clin Oncol* 1993, **11**, 499–506.
14. Van Gameren MM, Willemse PHB, Mulder NH, *et al.* Effects of recombinant human interleukin-6 in cancer patients: a phase I-II study. *Blood* 1994, **84**, 1434–1441.
15. Hokland PR, Mikkelsen P, Segel E. IL-6 might relieve long-standing thrombocytopenia in autotransplanted AML patients. *Br J Haematol* 1994, **87**, 876–877.
16. DHondt V, Humblet Y, Guillaume T, *et al.* Thrombopoietic effects and toxicity of interleukin-6 in patients with ovarian cancer before and after chemotherapy. A multicentric placebo-controlled, randomized phase Ib study. *Blood* 1995, **85**, 2347–2353.
17. Gordon MS, Nemunaitis J, Hoffman R, *et al.* A phase I trial of recombinant human interleukin-6 in patients with myelodysplastic syndromes and thrombocytopenia. *Blood* 1995, **85**, 3066–3076.
18. Schrezenmeier H, Marsh JCW, Stromeyer P, *et al.* A phase I/II trial of recombinant human interleukin-6 in patients with aplastic anaemia. *Br J Haematol* 1995, **90**, 283–292.
19. Lazarus HM, Winton EF, Williams SF, *et al.* Phase I multicenter trial of interleukin 6 therapy after autologous bone marrow transplantation in advanced breast cancer. *Bone Marrow Transplantation* 1995, **15**, 935–942.
20. Weiss GR, Margolin KA, Sznol M, *et al.* A phase II study of the continuous intravenous infusion of interleukin-6 for metastatic renal cell carcinoma. *J Immunother* 1995, **18**, 52–56.
21. Estey E, Andreeff M. Phase II study of interleukin-6 in patients with smoldering relapse of acute myelogenous leukemia. *Leukemia* 1995, **9**, 1440–1443.
22. Veldhuis GJ, Willemse PHB, Sleijfer DT, *et al.* Toxicity and efficacy of escalating dosages of recombinant human interleukin-6 after chemotherapy in patients with breast cancer or non-small-cell lung cancer. *J Clin Oncol* 1995, **13**, 2585–2593.
23. Lansky SB, List MA, Lansky LL, *et al.* The measurement of performance in childhood cancer patients. *Cancer* 1987, **60**, 1651–1656.
24. Negrier S, Michon J, Floret D, *et al.* Interleukin-2 and lymphokine activated killer in 15 children with advanced metastatic neuroblastoma. *J Clin Oncol* 1991, **9**, 1363–1370.

25. Bouffet E, Michon J, Frappaz D, *et al.* Pilot study of immunotherapy using Interleukin 2 in children with solid tumors or lymphomas. *Int J Pediatr Hematol/Oncol* 1995, **2**, 489–493.
26. Asano S, Okano A, Ozawa K, *et al. In vivo* effects of recombinant human interleukin-6 in primates: stimulated production of platelets. *Blood* 1990, 75, 1602–1605.
27. Nieken J, Mulder NH, Buter J, *et al.* Recombinant human interleukin-6 induces a rapid and reversible anemia in cancer patients. *Blood* 1995, **86**, 900–905.
28. Atkins MB, Kappler K, Mier JW, Isaacs RE, Berkman EM. Interleukin-6-associated anemia: determination of the underlying mechanism. *Blood* 1995, **86**, 1288–1291.
29. Marsh JCW, Socie G, Schrezenmeier H, *et al.* Haemopoietic growth factors in aplastic anaemia: a cautionary note. *Lancet* 1994, **344**, 172–173.
30. Muraguchi A, Kishimoto T, Miki Y, *et al.* T-cell replacing factor (TRF)-induced IgG secretion in human B blastoid cell line and demonstration of acceptors to TRF. *J Immunol* 1981, **127**, 412–416.
31. Okada M, Kilara M, Kishimoto S, *et al.* IL-6/BSF-2 functions as a killer helper factor in the *in vitro* induction of cytotoxic T cells. *J Immunol* 1988, **143**, 1543–1549.
32. Banks RE, Forbes MA, Storr M, *et al.* The acute phase protein response in patients receiving subcutaneous IL-6. *Clin Exp Immunol* 1995, **102**, 217–223.
33. Lotz M. Interleukin-6. *Cancer Invest* 1993, **11**, 732–742.
34. Gauldie J, Richard C, Harnish D, *et al.* Interferon β2/BSF-2 shares identity with monocyte derived hepatocyte stimulatory factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci USA* 1987, **84**, 7251–7256.
35. Ravoet C, De Grève J, Vandewoude K, *et al.* Tumor stimulating effects of recombinant human IL-6. *Lancet* 1994, **344**, 1576–1577.