A Phase I Clinical, Pharmacological, and Biological Trial of Interleukin 6 Plus Granulocyte-Colony Stimulating Factor after Ifosfamide, Carboplatin, and Etoposide in Children with Recurrent/Refractory Solid Tumors: Enhanced Hematological Responses but a High Incidence of Grade III/IV Constitutional Toxicities

Francisco Bracho, Mark D. Krailo, Violet Shen, Sharon Bergeron, Virginia Davenport, Wen Liu-Mares, Bruce R. Blazar, Angela Panoskaltsis-Mortari, Carmella van de Ven, Rita Secola, Matthew M. Ames, Joel M. Reid, Gregory H. Reaman, and Mitchell S. Cairo

INTRODUCTION

IL-6 is a pleiotropic cytokine originally described as IFN-β2 (1–4) with effects on immunoregulation, induction of acute phase protein production, hematopoiesis, and megakaryocytopoiesis (1, 4). Specifically, IL-6 acts synergistically with IL-3 to stimulate multilineage hematopoietic blast colony formation (5) and induces megakaryocyte CFUs from murine bone marrow cells (6–8). Preclinical in vivo studies have demonstrated that IL-6 increases platelet numbers in healthy rodents and primates (9–15). In experimentally irradiated animals, IL-6 accelerated hematopoietic recovery (6, 10, 15, 16). In animals with chemotherapy-induced myelosuppression, IL-6 enhanced receptor expression studies were performed during course one. Nineteen patients were evaluable for toxicity and received IL-6 at doses of 2.5 (n = 8), 3.75 (n = 5), or 5.0 (n = 6) μg/kg/day. Dose-limiting constitutional toxicity occurred in two of six patients at 5.0 μg/kg/day, two of five patients at 3.75 μg/kg/day, and two of eight patients at 2.5 μg/kg/day. The maximum tolerated dose (MTD) exceeded the lowest dose tested. Because of lack of drug availability, an MTD was not established. The maximum concentration of IL-6 (2.5 μg/kg/day) was 0.799 ± 1.055 ng/ml (mean ± SD). During the first course, the median time to absolute neutrophil count ≥1,000/mm³ and platelets ≥100,000/mm³ was estimated at 19 and 23 days, respectively. Peripheral blood progenitor cells expressing receptors to IL-3, IL-6, and G-CSF increased significantly over baseline (P < 0.05). After the first dose of IL-6, IFN-γ levels were abnormal in 13 patients, and IL-1β levels were abnormal in 10 patients. IL-6 has a high incidence of constitutional toxicity and a lower MTD in children compared with adults. In vivo use of IL-6 in children after chemotherapy remains limited. However, IL-6 may be more optimally investigated in children under ex vivo conditions.

ABSTRACT

A Phase I trial was conducted to determine the safety, biological activity, and hematopoietic recovery by the combination of interleukin 6 (IL-6) and granulocyte-colony stimulating factor (G-CSF) after myelosuppressive chemotherapy in children. Patients <22 years of age at diagnosis with either recurrent or refractory solid tumors received ifosfamide 1,800 mg/m²/day × 5 days, carboplatin 400 mg/m²/day × 2 days, and etoposide 100 mg/m²/day × 5 days, followed by daily s.c. G-CSF (5 μg/kg/day) and IL-6 (2.5, 3.75, or 5.0 μg/kg/day). Pharmacokinetic, proinflammatory mediator levels, hematopoietic colony assays, and cytokine

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2 To whom requests for reprints should be addressed, at Children’s Cancer Group, P.O. Box 60012, Arcadia, CA 91066-6012. Phone: (626) 447-0064; Fax: (626) 445-4334.

The abbreviations used are: IL, interleukin; rhIL, recombinant human IL; CFU, colony-forming unit; CCG, Children’s Cancer Group; ICE, ifosfamide, carboplatin, and etoposide; CSF, colony-stimulating factor; G-CSF, granulocyte-CSF; GM-CSF, granulocyte/macrophage-CSF; ANC, absolute neutrophil count; NCI, National Cancer Institute; DLT, dose-limiting toxicity; CBC, complete blood count; AUC, area under the curve; MNC, mononuclear cell; PE, phycoerythrin; MTD, maximum tolerated dose; PBPC, peripheral blood progenitor cell; TNF, tumor necrosis factor.

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platelet recovery and prevented significant thrombocytopenia (17, 18). In several adult human Phase II/II trials (19–24), IL-6 at doses of 0.3 to 30 μg/kg/day increased platelet count before and after chemotherapy, accelerated platelet recovery after chemotherapy, and allowed an increase in chemotherapy dose intensity. Toxicities related to IL-6 in these patients included constitutional symptoms such as fever, chills, and myalgia, as well as dose-limiting organ toxicity.

Recently, Bouffet et al. (25) reported the results of a Phase I study of Escherichia coli-derived IL-6 administered s.c. without adjuvant chemotherapy to 12 children with relapsed solid tumors. Platelet counts rose significantly after 1 week of IL-6 therapy. IL-6 was tolerated without major organ toxicity at 10 μg/kg/day; however, side effects included fever, chills, fatigue in most patients, and anemia that required transfusion in 3 patients. They recommended that further Phase II/III trials with IL-6 in children should incorporate doses of 5–10 μg/kg/day to evaluate the efficacy of IL-6 in reducing thrombocytopenia after cytotoxic chemotherapy.

Given the potential of IL-6 to ameliorate chemotherapeutically induced thrombocytopenia and the frequent incidence of constitutional symptoms associated with previous human IL-6 clinical trials, the CCG conducted a Phase I trial of IL-6 to determine safety and tolerability after myelosuppressive chemotherapy before conducting a definitive Phase II/III trial. The ICE chemotherapy regimen has been demonstrated to be associated with an excellent response rate in children with recurrent or refractory solid tumors (51%; Ref. 26). However, delayed platelet recovery has been the major limiting factor to dose intensification of ICE chemotherapy in children with recurrent or refractory solid tumors, and serious hematopoietic toxicity occurs despite support with double the dose of G-CSF (10 versus 5 μg/kg/day; Ref. 26).

Given the pleiotropic effects of IL-6, this CCG study was designed to quantify the frequency and severity of constitutional toxicities related to IL-6 in children, as well as to study the biological correlates of constitutional symptoms and thrombocytopenia. The objectives of the study were to: (a) determine the safe and maximum tolerated dose of IL-6 in children when administered simultaneously with G-CSF after ICE chemotherapy; (b) evaluate the effects of IL-6 and G-CSF on hematopoietic recovery after ICE chemotherapy; (c) determine the pharmacokinetics of IL-6 in children; (d) determine the effects of IL-6 on circulating serum proinflammatory mediators in children; and (e) determine the effects of IL-6 and G-CSF after ICE chemotherapy on circulating subsets of hematopoietic progenitor cells in children.

**PATIENTS AND METHODS**

**Patient Eligibility.** This protocol (CCG-0931) was opened for patient entry in May 1994 and was closed to patient entry in December 1996 because of lack of drug availability. Patients with refractory or recurrent solid tumors who were <22 years of age at diagnosis were eligible for study entry. All patients were required to have histological verification of malignancy at the time of initial diagnosis and radiological and/or histological evidence of recurrence. Patients with bone marrow involvement by tumor, patients who had received craniospinal irradiation (≥3600 cGy) or radiation therapy to >50% of their bone marrow space, or patients having received previously total body irradiation were ineligible for study entry. Patients with a diagnosis of coagulopathy, thrombotic disorder, autoimmune disease, asthma requiring ongoing therapy, history of HIV, hepatitis B infection, or lymphoma were also excluded. All patients must have recovered from previous CSF therapy and have been off all CSFs for more than 10 days. All patients were required to have adequate bone marrow, liver, renal, and cardiac function at the time of study entry. Adequate bone marrow function at the time of study entry was defined as an ANC ≥1000/mm3 and platelet count ≥100,000/mm3. The patient or legal guardian must have signed a documented informed consent approved by the institutional review board indicating awareness of the investigational nature and risks of this study. Patients must have had an adequate performance status of 60 on a Lansky scale (age, 1–16 years) or Karnofsky scale (age, >16 years). Patients were required to be a minimum of 12 months of age at the time of study entry.

**Chemotherapy Administration.** Eligible patients received ifosfamide 1.800 mg/m2 on days 0–4, carboplatin 400 mg/m2 on days 0–1, and etoposide 100 mg/m2 on days 0–4. 2-Mercaptoethane sulfonic acid and i.v. hydration were administered during each of the 5 days of ifosfamide. Five doses of 2-mercaptoethane sulfonic acid (360 mg/m2) were given: first dose during ifosfamide, second as a 3-h infusion, and then as a 15- to 30-min infusion every 3 h. Chemotherapy was repeated every 21 days or later when hematological recovery was achieved (ANC ≥1,000/mm3 and platelet count ≥100,000/mm3). IL-6 and G-CSF were discontinued at least 2 days prior to subsequent chemotherapy. After course one, ICE chemotherapy could be reduced by 25% if, in the previous course, hematological recovery failed to occur by day 21. The protocol directed surgical tumor resection was to be considered only after the patient had completed four courses of chemotherapy and response to ICE was assessed. Additionally, no patients received radiotherapy while receiving protocol therapy.

**CSF Administration.** Recombinant human *E. coli*-derived IL-6 (SDZ ILS 969), kindly provided by Novartis Pharmaceuticals, was distributed by the NCI. SDZ ILS 969 is supplied as a sterile lyophilizate in vials containing 150 mg of recombinant human IL-6 (r-hIL-6) per vial. The formulated lyophilizate contains ≥90% pure r-hIL-6 and <0.6 EU/vial of bacterial endotoxin. The biological specific activity was 6.1 × 107 units/mg as determined by hybridoma B13.29 cell line proliferation assay. There were three dose levels used in this study, 2.5, 3.75, and 5.0 μg/kg/day. Recombinant human met G-CSF (r-metHuG-CSF) was also provided by the NCI. The dose of G-CSF was 5 μg/kg/day for all dose levels of IL-6 and for all courses. IL-6 and G-CSF were administered as two separate daily s.c. injections beginning 24 h after the end of chemotherapy. G-CSF was continued until the post nadir ANC was ≥1,000/mm3. IL-6 was administered until the platelet count was ≥100,000/mm3 for 2 consecutive days or a maximum of 35 days. In course one only, the first dose of IL-6 preceded the first dose of G-CSF by 12 h to allow for pharmacokinetic sampling. Protocol therapy was terminated for a patient at the time of documented disease progress, regardless of the course during which this progression was noted. Patients failing to achieve the desired ANC and platelet count (*i.e.*,...
IL-6 Dose Escalation. All patients were identified to the CCG registrar within 72 h of starting chemotherapy, and the dose level of IL-6 was assigned at that time. Chemotherapy and IL-6 were continued past course 2 unless either disease progression, failure to achieve an ANC ≥1,000/mm³, or platelet count ≥100,000/mm³ by day 35, grade IV renal toxicity resulting from ifosfamide, or a maximum of eight courses of therapy.

Dose escalation was defined as either grade IV toxicity or recurrent grade III toxicity related to IL-6 using the NCI Common Toxicity Criteria (version 1) or pain which persisted beyond the time of injection to the last detectable serum concentration during the first incubation. Unbound antibody-enzyme reagent was removed from the wells by washing, followed by addition of substrate solution to the wells. The color that developed was directly proportional to the amount of IL-6 bound in the initial step. After color development was stopped, the intensity of the color was measured at a wavelength of 450 nm.

Each sample (standard, quality control, and unknown) was analyzed in triplicate, and the individual ELISA response values, expressed as absorbance, were used to construct the standard curve. The standard curve was created from the mean absorbance of each triplicate via the SOFTmax 4-parameter program. The concentration estimate was interpolated from the individual standard curves, using the mean response of each triplicate analysis. Samples that had concentration estimates above the upper limit of quantification (200 pg/ml) in the primary analysis were diluted in pooled normal human serum to bring the concentration within the dynamic range of the assay and reanalyzed.

Estimates of the pharmacokinetic parameters were obtained by noncompartmental analysis using GraphPad Prism (GraphPad Software, Inc.; version 2.00). The terminal elimination rate constant (kₜₑ) was calculated by linear least squares regression of the linear terminal elimination phase of the graph of natural logarithm of the serum concentration versus time. AUC₀–₁₂ was determined by trapezoidal approximation from the time of injection to the last detectable serum concentration (C₁₂₅₀) with residual area after C₁₂₅₀, calculated by AUC₁₂₅₀ = C₁₂₅₀/kₜₑ. The elimination half-life was calculated by t₁/₂ = 0.693/kₜₑ.

Peripheral Blood Progenitor Cells. During the first course of IL-6 administration, peripheral blood progenitor cells were measured at the CCG Hematopoiesis Resource Laboratory at Children’s Hospital of Orange County before therapy and when the WBCs recovered to >1,000/mm³. MNCs were isolated from the blood samples by density gradient separation with Ficoll-Hypeaque. An aliquot of this MNC fraction (10⁴ cells/ml) was added to Iscove’s modified Dulbecco’s medium/0.9% methylcellulose (StemCell Technologies, Inc., Vancouver, British Columbia, Canada) supplemented with 2 units/ml of erythropoietin (Epogen; Amgen, Thousand Oaks, CA). Colony formation was induced by stimulation with phytohemagglutinin human leukocyte conditioned medium (StemCell Technologies, Inc.). The cells were plated in 24-well plates (Costar, Cambridge, MA) at 500 cells/well and incubated in a 5% CO₂ humidified incubator at 37°C. Colonies were scored after 14–21 days, and clusters of 25+ cells were considered colonies.

Peripheral Progenitor Cell Receptor Expression. Aliquots of MNCs isolated from peripheral blood, as described above, were stained with fluorochrome conjugated monoclonal antibodies for cytokine receptor, CD41⁺ and CD34⁺ expression. CD34⁺ cell populations were assessed using the human progenitor cell antigen antibody conjugated to PE (Becton Dick-
inson, Mountain View, CA). Receptor expression was measured using the following antibodies: IL-3/PE (R&D Systems), IL-6/PE (R&D Systems), GM-CSF/PE (R&D Systems), and stem cell factor/PE (R&D Systems). Analysis was performed on a FACStar flow cytometer (Becton Dickinson) with gating on the cell factor/PE (R&D Systems), GM-CSF/PE (R&D Systems), and stem cell factor/PE (R&D Systems), IL-3/PE (R&D Systems), IL-1α, IL-1β, TNF-α, and IFN-γ from samples drawn on day 5 of the first course only, before the first dose of IL-6, and at 1, 2, 4, 6, 8, 10, and 12 h after s.c. administration of IL-6. The G-CSF dose was held until all samples were drawn. When possible, investigators were to submit a serum specimen for analysis of the above-mentioned mediators prior to the start of ICE chemotherapy. Isotype controls were included for each staining experiment.

Induction of Endogenous Inflammatory Mediators. Serum was to be analyzed for concentrations of IL-1α, IL-1β, TNF-α, and IFN-γ from samples drawn on day 5 of the first course only, before the first dose of IL-6, and at 1, 2, 4, 6, 8, 10, and 12 h after s.c. administration of IL-6. The G-CSF dose was held until all samples were drawn. When possible, investigators were to submit a serum specimen for analysis of the above-mentioned mediators prior to the start of ICE chemotherapy. Aliquots of patient serum and plasma from different time points were shipped on dry ice overnight to the CCG Cytokine Reference Laboratory at the University of Minnesota Hospital and stored at −70°C. Levels of IL-1α, IL-1β, TNF-α, and IFN-γ were determined by sandwich ELISA using commercially available kits (R&D Systems, Minneapolis, MN) according to manufacturers’ instructions for either the serum or plasma, depending on the optimal matrix empirically determined by the reference lab. The concentrations for each proinflammatory mediator, in pg/ml, were interpolated from standard curves generated for each assay performed. Values were categorized as abnormally elevated if they were above the reference range for 95% of apparently healthy individuals.

Statistical Analysis. All eligible patients were evaluated for delivery of therapy. Any patient who completed at least 1 week of IL-6 therapy or who was removed from protocol therapy because of DLT was considered in the evaluation of the MTD.

Patients were evaluated for time to hematological recovery for each course of therapy delivered. Any patient who was evaluable for determination of the MTD and had submitted serial peripheral blood counts was considered a candidate for this analysis. The time to ANC recovery was calculated as the number of days from the start of the course to the first CBC where the ANC exceeded 1,000/mm³. Any CBC done in the interval from the start of chemotherapy to day 9 of the course was not considered in this analysis, because peripheral blood counts dropped precipitously for all patients during this time frame. From among the remaining reported CBCs, the date the patient’s CBC was first >1,000/mm³ (“recovered date”) was identified. Next, the date closest to the recovered date at which the patient’s ANC was <1,000/mm³ (“last below date”) was identified. Patients removed from therapy because of disease progression prior to recovery of ANC to at least 1,000/mm³ were right censored for recovery of ANC at the time of the last reported CBC. Because of patterns of patient care, the “last below” and “recovered dates” were separated by an average of 2 days (range, 1–7). The survivor function for recovery of ANC to at least 1,000/mm³, therefore, was estimated using the interval censoring methodology of Turnbull (27). A similar procedure was followed to estimate the time to recovery of platelets to 100,000/mm³.

Results of the progenitor and receptor studies are expressed as mean ± SE. The equality of the mean biological measures between baseline and posttherapy time points was assessed using Student’s t test. The fold change was calculated by dividing the mean posttherapy value by the mean pretherapy value.

RESULTS

Patient Characteristics. Between May 1994 and December 1996, 25 patients, ages 23 months through 21 years (median age, 14 years) with recurrent or refractory solid tumors were enrolled on CCG-0931 (Table 1). One patient did not receive any protocol therapy, and five additional patients started chemotherapy but did not receive IL-6. These six patients are not considered further. Among the 19 patients considered evaluable for toxicity, two patients were removed from protocol therapy prior to day 21. One was removed on day 14 because of sepsis and multiorgan failure, and one was removed on day 18 because of fever, chills, and bruising at injection sites. Nine patients completed only one course of therapy, four patients completed two courses of therapy, and six completed three or more courses of therapy.

Dose Selection and Toxicity Related to IL-6. Toxicity related to IL-6 was frequent, and constitutional toxicities be-
came dose limiting. There were no noted cardiac, neurological, or hepatic toxicities attributable to IL-6. Table 2 summarizes toxicities associated with IL-6. Recurrent or refractory (grade III/IV) chills were particularly frequent, occurring in 10 of 19 evaluable patients including 4 of 6 patients at the highest dose level of 5.0 μg/kg/day.

The initial cohort of 3 patients received IL-6 at 2.5 μg/kg/day, and the next cohort received IL-6 at 5.0 μg/kg/day. Among the 6 patients who received IL-6 at 5.0 μg/kg/day, one child experienced dose-limiting grade III chills, and another experienced dose-limiting grade IV bone pain and myalgia. An intermediate de-escalation dose-limiting grade III chills, and another experienced dose-limiting grade IV hypotension and grade IV bone pain. Dose de-escalation was not continued because of lack of available rhIL-6. Therefore, no MTD could be established, but it is estimated to be <2.5 μg/kg/day.

**IL-6 Pharmacokinetics.** The pharmacokinetics of IL-6 were studied in 16 patients who received doses of 2.5, 3.75, or 5.0 μg/kg/day. Pharmacokinetic parameters are summarized in Table 3. A profile of the mean serum IL-6 concentration-time data for patients who received 2.5 μg/kg/day is illustrated in Fig. 1. At 2.5 μg/kg/day, peak serum concentration of IL-6 was observed 2.8 h after administration, and the elimination half-life was 3 h. The equivalent values for all doses combined were 4 and 2.8 h, respectively. Four patients had an apparent plateau concentration of IL-6, and their calculated elimination half-life and AUC0–∞ could not be determined. The mean AUC0–12 values appeared to increase as the dose was increased, but the substantial interpatient variability found for AUC at each dose level and the limited dose response did not permit evaluation of the linearity of the pharmacokinetics.

**Hematological Recovery, Transfusions, and Infections.** Two patients did not complete the full course of chemotherapy and IL-6/G-CSF therapy, and therefore 17 patients were evaluable for hematological recovery. During course one, the median number of days to ANC recovery ≥1,000/mm³ was 19 (Fig. 2), and the median number of days to platelet recovery ≥100,000/mm³ was 23 (Fig. 3). All patients developed grade IV thrombocytopenia. Evaluation of all courses revealed similar data for ANC recovery (median, 18 days) but delayed platelet recovery (median, 26 days). Patients received a median of five platelet transfusions during course one. During course one, two patients had fulminant infections, two others had infections with chills, rigors, and/or fever, and six had presumed infections with fever. The remaining patients had no evidence of infection.

**Subsets of Peripheral Blood Progenitor Cells.** Specimens were received on 12 children and subsets of PBPCs at baseline and when the WBCs had recovered to ≥1,000/mm³ after the nadirs were determined. The results are summarized in Fig. 4. Flow cytometric analysis of PBPCs (Fig. 4) demonstrated a significant (P < 0.05) increase above baseline when the WBCs were ≥1,000/mm³ in the percentage of cells expressing receptors to IL-3 (93 ± 3.5% versus 23.02 ± 10.11%), GM-CSF (93.67 ± 1.3% versus 22.77 ± 22.3%), and IL-6 (97.62 ± 0.12% versus 29.67 ± 13.9%; mean ± SEM). There was >25-fold increase in the subset of cells expressing CD41^+ (27.03 ± 15.8% versus 1.03 ± 0.62%, P = 0.055; Fig. 4) and a 4-fold increase in the CD34^+ population (0.63 ± 0.59% versus 0.15 ± 0.08%, P = 0.48). There was no significant change in the percentage of PBPCs expressing c-kit^+ (data not shown). Progenitor hematopoietic colony assays demonstrated a 2.8-fold increase in CFU-GM and a 5.8-fold increase in granulocytic erythroid megakaryocytic monocytic colony formation over baseline.

**Induction of Proinflammatory Mediators.** Cytokine levels for IL-1α, IL-1β, TNF-α, and IFN-γ were completed on 18 patients. Levels were drawn on day 5 before the first dose of IL-6 and at 1, 2, 4, 6, 8, 10, and 12 h after s.c. administration. The G-CSF dose was held until all samples were drawn. Serum IFN-γ was elevated in 13 of 18 patients after IL-6 administration, and 7 of these patients had elevated levels at hour 0. Serum IL-1β was elevated in 10 of 18 patients, 1 of whom had an elevated level at hour 0. Serum IL-1α was elevated in 2 patients, 1 of whom had an elevated level at hour 0. One patient had an elevated TNF-α level only after IL-6.

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**Table 3.** Pharmacokinetic parameters of IL-6 in children

<table>
<thead>
<tr>
<th>Dose</th>
<th>Cmax (ng/ml)</th>
<th>Tmax (h)</th>
<th>t1/2 (h)a</th>
<th>AUC0–12</th>
<th>AUC0–∞ a</th>
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</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.799 ± 0.155</td>
<td>2.8 ± 1.3</td>
<td>3.01 ± 1.09</td>
<td>3.828 ± 4.616</td>
<td>4.351 ± 5.077</td>
</tr>
<tr>
<td>3.75</td>
<td>0.933 ± 0.769</td>
<td>6.4 ± 3.3</td>
<td>2.86 ± 1.87</td>
<td>5.559 ± 3.611</td>
<td>5.272 ± 4.088</td>
</tr>
<tr>
<td>5.0</td>
<td>1.131 ± 0.425</td>
<td>3.2 ± 1.8</td>
<td>2.56 ± 0.57</td>
<td>6.052 ± 2.374</td>
<td>7.181 ± 1.837</td>
</tr>
</tbody>
</table>

a Four patients with plateau concentration of IL-6 not included in analysis. All values are mean ± standard deviation.
DISCUSSION

Thrombocytopenia remains a major DLT of myelosuppressive multiagent chemotherapy. IL-6 has been demonstrated in animal and adult human studies to shorten the duration of thrombocytopenia after myelosuppressive chemotherapy (19, 23, 24). *E. coli*-derived rhIL-6 also has been demonstrated not to be associated with any significant organ toxicity in children at doses of 5–10 μg/kg/day when given without prior chemotherapy (25). The CCG conducted a Phase I trial of IL-6 in children with recurrent/refractory tumors to determine a safe and tolerated dose to use in a Phase III randomized trial to determine whether IL-6 could accelerate platelet recovery and reduce the incidence of grade III/IV thrombocytopenia in children receiving ICE chemotherapy. All dose levels tested exceeded the MTD, and DLTs were principally constitutional “flu-like” symptoms. With few exceptions (28), *E. coli*-derived IL-6 (10 μg/kg/day) has been tolerated after chemotherapy in adults with only modest constitutional toxicity (19, 23, 24, 29). At higher doses of IL-6 (20 μg/kg/day), Veldhuis et al. (24) demonstrated DLT characterized by headache, myalgia, fever, chills, and nausea in adult patients with solid tumors. Organ toxicities at doses of IL-6 higher than 20 μg/kg/day have included cardiac arrhythmias, neurological deficits (confusion, hallucinations, and hemiplegia), and severe hepatotoxicity (23, 24, 29).

Bouffet et al. (25) studied the use of *E. coli*-derived IL-6 given s.c. in children with recurrent/refractory solid tumors. Children were given IL-6 without chemotherapy to determine the toxicity, thrombopoietic, and antitumor effects of IL-6 (25).

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**Fig. 2** Probability of ANC recovery ≥1,000/mm³ in course one of ICE followed by IL-6 + G-CSF by Kaplan-Meier statistics (*n* = 17).

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**Fig. 3** Probability of platelet count recovery ≥100,000/mm³ in course one of ICE followed by IL-6 + G-CSF by Kaplan Meier statistics (*n* = 17).
In that study, IL-6 was escalated from 1.0 to 10 μg/kg/day in 12 children without organ toxicity. Fever was the only form of grade III/IV toxicity, with most patients having fever and chills requiring prophylactic paracetamol (acetaminophen) therapy. Additionally, fatigue was a universal complaint at doses >2.5 μg/kg/day. These constitutional toxicities were not evaluated as dose-limiting. 10 μg/kg/day was considered tolerable, and the MTD was not reached in that study. The authors further recommended evaluating IL-6 at a dose between 5 and 10 μg/kg/day in children after myelosuppressive chemotherapy. In our study, 2.5 μg/kg/day after ICE chemotherapy was intolerable because of severe constitutional toxicities. However, no prophylactic treatment was given for fevers or chills.

When compared with historical studies with G-CSF alone after ICE chemotherapy, children with recurrent/refractory solid tumors appear to have enhanced platelet recovery during the first course with IL-6 + G-CSF versus G-CSF alone. In a previous CCG study (CCG-0894), 118 children were followed for hematological recovery after ICE chemotherapy at two doses of G-CSF, 5 and 10 μg/kg/day (26). Median time to platelet recovery in the current study to ≥100,000/mm³ was 23 days at dose levels exceeding the MTD of IL-6. In contrast, patients treated on CCG-0894 had a median time to platelet recovery of 27 days (26). However, the median number of platelet transfusions in course one was five and appears similar to a median of six in the CCG-0894 G-CSF trial (26).

Several factors may possibly contribute to the decreased MTD in children receiving IL-6 after chemotherapy compared with adults or children who have not received chemotherapy. The addition of prior myelosuppressive chemotherapy in our pediatric trial may have facilitated the development of constitutional side effects of IL-6. In support of this theory, Lazarus et al. (28) noted an excessive amount of toxicity of E. coli-derived IL-6 at 3.0 μg/kg/day in adult patients after high-dose chemo-

therapy and autologous bone marrow transplantation. They suggested that the increase in dose intensity during preparative regimens prior to transplantation may predispose patients to increased IL-6-related toxicities (28). Of note, one-third of children in the present study had elevated IFN-γ levels after ICE chemotherapy prior to IL-6 administration. All of these patients had a subsequent rise in IFN-γ levels coincident with or tailing after the peak IL-6 levels.

The pharmacokinetics of s.c. administered IL-6 in children demonstrate high peak serum levels that were reached in 4 h with an elimination half-life of ~2.2 h. Bouffet et al. (25) measured serum IL-6 levels in one child each at three dose levels (1.0, 2.5, and 5.0 μg/kg/day) and found levels within the lower range of our current study. Comparing the same dose of IL-6 (2.5 μg/kg/day) in children and adults, it appears that children in our study have a higher $C_{\text{max}}$ (0.799 ± 1.055 ng/ml; mean ± SD) than reported previously in adults (highest $C_{\text{max}}$, 254 pg/ml; Refs. 29 and 30). The more rapid absorption of IL-6 may contribute to the higher incidence of grade III/IV constitutional toxicities at lower doses in children compared with adults.

In our study, IL-6 increased the level of circulating proinflammatory mediators IL-1β and IFN-γ in many children. These mediators may be partly responsible for the increase in constitutional toxicities secondary to IL-6. Samples were collected after the first dose of IL-6 but before the first dose of G-CSF; therefore, exogenous G-CSF probably did not contribute to the elevation of proinflammatory mediators. Conversely, in several adult studies without chemotherapy, the level of proinflammatory mediators did not increase after administering E. coli or yeast-derived IL-6 (23, 31). Some studies have demonstrated an increase after IL-6 in TNF-α mRNA (32) or IFN-γ mRNA (4) without a corresponding increase in serum levels (32) after IL-6. Only one study (33) demonstrated an increase in serum TNF-α in two of eight patients. Thus, the difference in tolerance to IL-6 may relate directly to a difference in induction of proinflammatory mediators in children compared with adults. However, it should be noted that there was no statistical correlation with abnormal mediator levels and grade III/IV constitutional toxicity, although the study was not designed to evaluate proinflammatory mediator levels at the time of constitutional toxicities.

The increased severity of constitutional toxicity without organ toxicity at lower doses of IL-6 in children versus adults underlines the need to conduct separate Phase I trials of biological agents and immunomodulators in children. In preclinical animal studies, IL-6 was thought to be safe and without severe constitutional toxicities. Adult IL-6 Phase I studies demonstrated substantial low grade but controllable inflammatory effects of IL-6 (34). Conversely, agents considered intolerable in adults may be well tolerated in children. Similar doses of PIXY321 are tolerated in children with less frequent constitutional side effects than in adults (35, 36). Recently, IL-11 has been shown by Kirov et al. (37) to be well-tolerated in children at 150% the adult MTD (75 μg/kg/day versus 50 μg/kg/day). Furthermore, the pharmacokinetics of IL-11 are different in children versus adults; children eliminate IL-11 more rapidly than adults (37).
An increase of subsets of cells expressing CD34+ and CD41+ after therapy compared with baseline (day 0) was demonstrated in this study. The number of CFU-GM colonies also increased compared with baseline in this small sample population. IL-6 + G-CSF appears to enhance hematopoietic recovery in vivo by stimulation of both committed and uncommitted subsets of progenitor cells. Chemotherapy alone, G-CSF alone, or the combination thereof have been demonstrated to enhance mobilization of PBPCs (38–41). Preclinical studies have demonstrated both additive and synergistic effects of IL-6 and G-CSF in enhancing PBPC mobilization (42, 43). In one human study of 27 adults with adenocarcinoma, combined IL-6 and G-CSF were demonstrated to dramatically increase PBPCs 36-fold, whereas IL-6 alone generated an 8-fold rise in PBPCs (44).

In the present study, we have demonstrated that IL-6 + G-CSF significantly increases the subpopulation of PBMC expressing receptors to IL-3, IL-6, and GM-CSF and may increase hematopoietic CFUs compared with baseline values. IL-6 + G-CSF could potentiate the hematopoietic effects of each of these endogenous hematopoietic growth factors by increasing growth factor receptor expression. Unfortunately, we do not have data with G-CSF alone to determine the true contribution of the addition of IL-6. Perhaps a short, ostensibly more tolerable course of IL-6 can “prime” the hematopoietic system for the action of other hematopoietic growth factors to enhance hematological recovery after myelosuppression. Sequential administration of IL-6 followed by G-CSF has been shown to increase the platelet count and the number of bone marrow hematopoietic progenitors in neonatal rats over and above that produced by G-CSF alone (45).

In summary, IL-6 and G-CSF may decrease the time to platelet recovery after myelosuppressive chemotherapy (ICE) and G-CSF alone; however, the excessive toxicity of IL-6 after chemotherapy in children may preclude extensive use of IL-6 in vivo. Unlike Bouffet et al. (25), this study suggests that the modest potential decrease in time to platelet recovery does not warrant further in vivo Phase II/III studies of IL-6 after myelosuppressive chemotherapy in children. The increase in constitutional toxicities of IL-6 in children as compared with adults may be related to the increased absorption of IL-6 and/or the in vivo increase of circulating proinflammatory mediators. Currently, other thrombopoietic agents may have more clinical applicability with wider therapeutic indices. IL-11 has been approved for chemotherapy-induced severe thrombocytopenia. Other thrombopoietic proteins, including thrombopoietin, are in Phase III clinical trials in adults, and CCG has opened a Phase I/II trial of ICE chemotherapy, followed by recombinant human thrombopoietin and G-CSF (CCG-09717). Future investigations of IL-6 to expand and/or activate stem/immune cells ex vivo may be more promising.

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APPENDIX
Participating principal investigators—Children’s Cancer Group

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<tr>
<th>Institution</th>
<th>Investigators</th>
<th>Grant no.</th>
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<tbody>
<tr>
<td>Group Operations Center</td>
<td>W. Archie Bleyer, MD</td>
<td>CA 13539</td>
</tr>
<tr>
<td>Arcadia, CA</td>
<td>Anita Khayat, PhD</td>
<td></td>
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<tr>
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<td>Harland Sather, PhD</td>
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<td></td>
<td>Mark Kralo, PhD</td>
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<td></td>
<td>Jonathan Buckley, MBBS, PhD</td>
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<td>Daniel Stram, PhD</td>
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<td></td>
<td>Richard Sposto, PhD</td>
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</tr>
<tr>
<td>University of California Medical Center</td>
<td>Katherine Matthay, MD</td>
<td>CA 17829</td>
</tr>
<tr>
<td>San Francisco, CA</td>
<td>Diane Puccetti, MD</td>
<td>CA 05436</td>
</tr>
<tr>
<td>University of Wisconsin Hospital</td>
<td>Gregory Reaman, MD</td>
<td>CA 03888</td>
</tr>
<tr>
<td>Madison, WI</td>
<td>Frederick Ruymann, MD</td>
<td>CA 03750</td>
</tr>
<tr>
<td>Children’s National Medical Center</td>
<td>A. Kim Ritchey, MD</td>
<td>CA 36015</td>
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<tr>
<td>Washington, DC</td>
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<tr>
<td>Children’s Hospital of Columbus</td>
<td>James Whitlock, MD</td>
<td>CA 26270</td>
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<td>Columbus, OH</td>
<td>Joseph Neglia, MD</td>
<td>CA 07306</td>
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<td>Children’s Hospital of Pittsburgh</td>
<td>Beverly Lange, MD</td>
<td>CA 11796</td>
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<td>Robert Wells, MD</td>
<td>CA 26126</td>
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<td>Nashville, TN</td>
<td>Joseph Neglia, MD</td>
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<tr>
<td>University of Minnesota Health Sciences Center</td>
<td>Beverly Lange, MD</td>
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<td>Minneapolis, MN</td>
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<td>Children’s Hospital of Philadelphia</td>
<td>Carola Arndt, MD</td>
<td>CA 28882</td>
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<tr>
<td>Philadelphia, PA</td>
<td>Richard Drachtman, MD</td>
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