A Phase I Clinical, Pharmacologic, and Biologic Study of Thrombopoietin and Granulocyte Colony-Stimulating Factor in Children Receiving Ifosfamide, Carboplatin, and Etoposide Chemotherapy for Recurrent or Refractory Solid Tumors: A Children’s Oncology Group Experience

Anne L. Angiolillo, Virginia Davenport, Mary Ann Bonilla, Carmella van de Ven, Janet Ayello, Olga Militano, Langdon L. Miller, Mark Krailo, Gregory Reaman, and Mitchell S. Cairo

Abstract

Purpose: Ifosfamide, carboplatin, and etoposide (ICE) are associated with grade III/IV dose-limiting thrombocytopenia. The Children’s Oncology Group conducted a phase I dose escalation, pharmacokinetic, and biological study of recombinant human thrombopoietin (rhTPO) after ICE in children with recurrent/refractory solid tumors (CCG-09717) to assess the toxicity and maximum tolerated dose of rhTPO administered at 1.2, 2.4, or 3.6 µg/kg per dose.

Experimental Design: Children received ifosfamide 1,800 mg/m² on days 0 to 4, carboplatin 400 mg/m² on days 0 to 1, and etoposide 100 mg/m² on days 0 to 4. rhTPO was administered i.v. on days +4, +6, +8, +10, and +12 at 1.2, 2.4, or 3.6 µg/kg per dose.

Results: rhTPO was well tolerated and maximum tolerated dose was not reached. Median time to platelet recovery ≥100,000/µL of rhTPO at 1.2, 2.4, and 3.6 µg/kg/d was 24 days (22-24d), 25 days (23-29d), and 22 days (16-37d), respectively. Patients required a median of 2 days of platelet transfusions (0-7 days). Mean (± SD) rhTPO maximum serum concentrations were 63.3 ± 9.7 and 89.3 ± 16.7 ng/mL and terminal half-lives were 47 ± 13 and 64 ± 42 hours after 2.4 and 3.6 µg/kg/d, respectively. There was a significant increase in colony-forming unit megakaryocyte upon WBC count recovery.

Conclusions: rhTPO was well tolerated. Time to hematologic recovery and median number of platelet transfusions seem to be improved compared with historical controls receiving ICE + granulocyte colony-stimulating factor (CCG-0894).

Approximately one third of children with malignant solid tumors experience either refractory or recurrent disease requiring retrieval therapy. We have previously shown that ifosfamide, carboplatin, and etoposide (ICE) combination chemotherapy is an excellent retrieval regimen in these patients and is associated with an overall response rate (complete response + partial response) of 51% (1). Grade III/IV thrombocytopenia, however, remains the major dose-limiting toxicity (DLT) for dose intensification of ICE chemotherapy in these children (1). Children with recurrent/refractory solid tumors and receiving ICE + granulocyte colony-stimulating factor (G-CSF) had a 92% incidence of grade III/IV thrombocytopenia and required a median of six platelet transfusions during the first cycle of ICE chemotherapy; only 30% of patients recovered their platelet count by day 21 to start cycle 2 (1).

Regulation of megakaryocytopoiesis is controlled in part by several hematopoietic cytokines including interleukin (IL)-1, IL-3, IL-6, IL-11, granulocyte-macrophage colony-stimulating factor, erythropoietin, fibroblast growth factor, stem cell factor, leukemia inhibitory factor, and thrombopoietin (2, 3). Thrombopoietin, the most potent cytokine that regulates platelet production, is a 60- to 70-kDa polypeptide of 322 amino acids (4–7). Thrombopoietin has both megakaryocyte colony-stimulating activity and maturation activity (4) and is encoded by a single-copy gene on human chromosome 3q27-28 (7). Thrombopoietin is produced primarily in the liver and kidney and is expressed in muscle stromal cells, bone marrow, and spleen (8, 9). We (M.S.C.) have previously showed an inverse relationship of circulating thrombopoietin serum levels and...
Thrombopoietin in Children Receiving ICE

Thrombopoietin has been shown to enhance platelet recovery in adults with cancer after myelosuppressive chemotherapy and/or radiotherapy (16–18). In addition, in a phase I/II clinical trial, 12 adults with sarcoma received a single i.v. dose of thrombopoietin without chemotherapy. Platelet counts increased markedly in a dose-dependent fashion (14). Most importantly, we (M.S.C.) have shown in a carboxplatin-myelosuppressed murine model that thrombopoietin after carboxplatin effectively improved platelet nadir and platelet recovery (15).

Clinical trials in humans have shown thrombopoietin to enhance platelet recovery in adult patients with cancer after myelosuppressive chemotherapy and/or radiotherapy (16–18). In addition, in a phase I/II clinical trial, 12 adults with sarcoma received a single i.v. dose of thrombopoietin without chemotherapy. Platelet counts increased markedly in a dose-dependent manner and no major side effects were observed. Platelet response was accompanied by a dose-related increase in bone marrow colony-forming unit (CFU) megakaryocytes and expansion and mobilization of myeloid, erythroid, and megakaryocytic progenitor cells (16). Vadhan-Raj et al. recently reported the results of a clinical trial in adults with sarcoma and showed the importance of timing recombinant human thrombopoietin (rhTPO) administration before and after chemotherapy for optimal effect on platelet nadir and recovery (19).

The Children’s Oncology Group conducted a study (CCG-09717) of rhTPO plus G-CSF in children receiving ICE chemotherapy for recurrent or refractory solid tumors. This dose-escalation phase I trial investigated pharmacokinetics and toxicities associated with administering rhTPO after ICE. Additional aims included evaluating the depth and duration of neutropenia and thrombocytopenia, evaluating the number of days of platelet transfusion events during ICE chemotherapy with rhTPO plus G-CSF, and quantifying peripheral blood CFU megakaryocyte before and after ICE with rhTPO plus G-CSF.

Materials and Methods

Patient eligibility. CCG-09717 was opened for patient accrual in November 1998 and was closed in November 2002. Patients with recurrent or refractory solid tumors who were ≥1 year and ≤21 years of age were eligible for study entry. All patients (excluding those with brain stem tumor) were required to have histologic verification of malignancy and radiologic or histologic evidence of recurrence at initial diagnosis. Disease categories were sarcoma (soft tissue and bone) and kidney, brain, and other solid tumors (gonadal, germ cell, malignant melanoma, retinoblastoma, liver, and miscellaneous tumor). Patients must have recovered fully from toxic effects of any prior therapy and received no colony-stimulating factors for 10 days. Patients who had received craniospinal irradiation (>3,600 cGy) or radiation therapy (including total body irradiation) to >50% of bone marrow space were ineligible. Patients who had received the exact combination and dosage of ICE within the last 3 months were also ineligible. 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.

Chemotherapy administration. Patients received ifosfamide 1,800 mg/m² on days 0 to 4, carboplatin 1,000 mg/m² on days 0 to 1, and etoposide 100 mg/m² on days 0 to 4. 2-Mercaptosulfonic acid and i.v. hydration were administered during each of the 5 days of ifosfamide. Chemotherapy was repeated every 21 days and the absolute neutrophil count (ANC) was ≥1,000/µL and platelet count was ≥100,000/µL. G-CSF was discontinued at least 2 days before subsequent chemotherapy. Chemotherapy dose modifications for renal impairment and hematuria were protocol directed. There was no dose modification for bone marrow suppression. Surgical tumor resection and radiation therapy for palliation and/or to obtain a partial or complete response of local lesions was to be considered after the patient had completed two courses of chemotherapy and response to ICE was assessed.

Colon-stimulating factor administration. RhTPO was provided and distributed by Pharmacia & Upjohn, Inc. (Peapack, NJ), in 3-mL vials containing 2 mL of 0.1 mg/mL rhTPO in buffer solution. All patients received rhTPO according to an assigned cohort level. Three dose levels were used: 1.2, 2.4, and 3.6 µg/kg per dose. Patients received rhTPO i.v. on days +4, +6, +8, +10, and +12 (five doses total). There was no rhTPO dose modification in this study. Filgrastim (recombinant human met G-CSF) was injected s.c. at 5 µg/kg/d for all rhTPO dose levels beginning 24 hours after chemotherapy ended. G-CSF was continued until the postnadir ANC was ≥1,000/µL for 2 consecutive days, or 28 days maximum. RhTPO could be discontinued if a patient developed documented DLT (excluding hematologic) related to rhTPO, evidence of grade III/IV thrombosis, thrombocytopenia thought to be related to neutralizing rhTPO antibody formation, or platelet count ≥1,000,000/µL after platelet nadir.

Recombinant human thrombopoietin dose escalation. The rhTPO dose level was assigned at enrollment and there was no intrapatient dose escalation. DLT was defined as any grade IV nonhematologic toxicity that was definitely, probably, or possibly related to rhTPO. Dose escalation was not considered until at least three evaluable patients had been entered at the current dose level. If none experienced DLT, the dose was escalated to the next higher level in the three subsequent patients. If one of the three patients experienced DLT at the current dose, up to three more were accrued at the same level. If none of these three additional patients experienced DLT, then the dose was escalated in subsequent patients. If one or more of those three additional patients experienced DLT, the maximum tolerated dose (MTD) would be deemed exceeded and three more patients would be treated at the next lower dose (unless six patients had already been treated at that prior dose).

The MTD was the dose level at which fewer than one third of patients experienced DLT. No intrasubject dose escalation was permitted. Only the toxicity evaluation made during the first course of therapy was used to determine MTD.

Hematologic recovery. Platelet recovery was defined as when an unsupported platelet count remained above 100,000/µL for 1 day in the absence of a prior platelet transfusion within the previous 72 hours. Complete blood count (CBC), differential, and platelet counts were obtained on days 0, 4, 6, 7, then every other day on days 8 to 18 and day 21, and daily when either the platelet count was <20,000/µL, ANC <500/µL, ANC >10,000/µL, or platelet count >100,000/µL during each course.

Recombinant human thrombopoietin pharmacokinetics. Blood samples were drawn for pharmacokinetic analysis during the first course of therapy for patients receiving 2.4 and 3.6 µg/kg rhTPO doses only. Serum rhTPO concentrations were assessed at the following time points: day 4—baseline predose, 10 minutes, then 3, 6, 12, and
immobilized antibody bound any thrombopoietin present. After the samples. Standards and samples were pipetted into the wells and the wells of a microtiter plate and used to extract thrombopoietin from thrombopoietin. The first was a capture antibody that was precoated to ELISA. The assay used a combination of two monoclonal antibodies to RhTPO serum concentrations were determined using a quantitative method following rhTPO and G-CSF after ICE chemotherapy (all thrombopoietin) were tabulated and antibody titers were summarized by dose level and were compared at all dose levels. Incidence of anti-rhTPO antibodies was tabulated and antibody titers were summarized by dose level and time since day –1 of treatment phase. From the fitted equation, the pharmacokinetic parameters were calculated as follows: area under the serum concentration versus time curve after the first dose (AUC0-∞), maximum serum rhTPO concentrations and areas under the concentration-time curve for day +4 doses were examined for dose proportionality. In addition, maximum (10 minutes, Cmax) and minimum (predose, Cmin) serum rhTPO concentrations for days +6, +8, +10, and +12 were summarized by dose group and examined across the dosing period to assess accumulation. A two-compartment open model with i.v. bolus input and first-order output was fitted to serum rhTPO data obtained after the two dose levels using weighted (1/Tobs) nonlinear regression analysis (WinNonlin, Version 1.5, Scientific Consulting, Inc., Apex, North Carolina). Choice of the model and of the weighting function was done based on the residual analysis and of the coefficients of variation of the estimated parameters. Pharmacokinetic parameters were compared at all dose levels. Incidence of anti-rhTPO antibodies was tabulated and antibody titers were summarized by dose level and time since day –1 of treatment phase. From the fitted equation, the pharmacokinetic parameters were calculated as follows: area under the serum concentration versus time curve after the first dose (AUC0-∞) = Dose/Vss/K00, CL = Dose/ AUC0-∞, Vss = CL × MRT where CL is the serum clearance, Vss is the volume of distribution at steady state, and MRT is the mean residence time.

Peripheral blood progenitor cells. Peripheral blood progenitor responses and subsets of progenitors were determined at the following time points. Peripheral blood was drawn at day 0 of course 1 before ICE chemotherapy and every other day until ANC is ≥1,000/µL after nadir. Briefly, for CFU megakaryocyte progenitor determination, light-density, peripheral blood mononuclear cells 10⁷/µL were suspended in Iscove’s media and added to collagen/media solution containing IL-3, IL-6, and rhTPO (StemCell Technologies, Vancouver, British Columbia, Canada). Cells were then dispensed to a chambered slide and incubated at 37°C, 5% CO₂, and >95% humidity. After 10 to 12 days, slides were dehydrated, fixed, and stained with GPIIb/IIIa monoclonal antibody, and colonies counted. Statistical analysis. Any patient who completed the scheduled rhTPO administration was considered in the MTD of thrombopoietin. Patients were evaluated for time to hematologic recovery for each

Table 1. Patient characteristics (evaluable patient)

<table>
<thead>
<tr>
<th>Median age at study entry (y)</th>
<th>6.5 (2-17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>5/7</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>3</td>
</tr>
<tr>
<td>Primitive neuroectodermal tumor</td>
<td>2</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>2</td>
</tr>
<tr>
<td>Ewings</td>
<td>1</td>
</tr>
<tr>
<td>Glioma</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Desmoplastic small cell</td>
<td>1</td>
</tr>
<tr>
<td>Dose levels (µg/kg)</td>
<td></td>
</tr>
<tr>
<td>Dose level 1 (1.2)</td>
<td>3</td>
</tr>
<tr>
<td>Dose level 2 (2.4)</td>
<td>3</td>
</tr>
<tr>
<td>Dose level 3 (3.6 µg/kg)</td>
<td>6</td>
</tr>
</tbody>
</table>

24 hours postdose; days 6, 8, and 10—5 minutes before and 10 minutes after rhTPO administration; day 12—predose, 10 minutes, then 24, 48, and 72 hours postdose. Patients also were evaluated for presence of anti-rhTPO antibodies on day –5 of each course until removed from protocol therapy. An additional antibody level was obtained if platelet count did not recover by day +35. Blood samples were collected into plain redtop tubes and allowed to clot at room temperature. The samples were then centrifuged and the separated serum stored frozen between –20°C and –70°C.

RhTPO serum concentrations were determined using a quantitative ELISA. The assay used a combination of two monoclonal antibodies to thrombopoietin. The first was a capture antibody that was precipitated to the wells of a microtiter plate and used to extract thrombopoietin from the samples. Standards and samples were pipetted into the wells and the immobilized antibody bound any thrombopoietin present. After washing away unbound substances, a second enzyme-linked monoclonal antibody specific for bound thrombopoietin was added to the wells.

After a wash to remove unbound antibody enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of thrombopoietin bound in the initial step. The color development was stopped and the intensity of the color was measured.

RhTPO concentrations were determined using a calibration curve with a working range of 31.2 to 2,000 pg/mL. The between-run precision was 5.9% coefficient of variation, and the analytic recovery ranged from 83% to 103% for rhTPO added to serum. Serum samples for rhTPO antibodies were screened by ELISA based on full-length thrombopoietin, C-mpl receptors, or both. Reactive sera were tested using a bioassay based on inhibition of the thrombopoietin-dependent cell line. Neutralizing antibodies were defined as those that were inhibitory on bioassay and associated with clinically significant thrombocytopenia.

For all subjects, average serum rhTPO concentration-time curves were plotted for 2.4 and 3.6 µg/kg dose groups. Across subjects and doses, maximum serum rhTPO concentrations and areas under the concentration-time curve for day +4 doses were examined for dose proportionality. In addition, maximum (10 minutes, Cmax) and minimum (predose, Cmin) serum rhTPO concentrations for days +6, +8, +10, and +12 were summarized by dose group and examined across the dosing period to assess accumulation. A two-compartment open model with i.v. bolus input and first-order output was fitted to serum rhTPO data obtained after the two dose levels using weighted (1/Tobs) nonlinear regression analysis (WinNonlin, Version 1.5, Scientific Consulting, Inc., Apex, North Carolina). Choice of the model and of the weighting function was done based on the residual analysis and of the coefficients of variation of the estimated parameters. Pharmacokinetic parameters were compared at all dose levels. Incidence of anti-rhTPO antibodies was tabulated and antibody titers were summarized by dose level and time since day –1 of treatment phase. From the fitted equation, the pharmacokinetic parameters were calculated as follows: area under the serum concentration versus time curve after the first dose (AUC0-∞) = Dose/Vss/K00, CL = Dose/ AUC0-∞, Vss = CL × MRT where CL is the serum clearance, Vss is the volume of distribution at steady state, and MRT is the mean residence time.

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Statistical analysis. Any patient who completed the scheduled rhTPO administration was considered in the MTD of thrombopoietin. Patients were evaluated for time to hematologic recovery for each

Table 2. Hematologic recovery during cycle 1 ICE chemotherapy

<table>
<thead>
<tr>
<th>rhTPO dose levels (µg/kg)</th>
<th>1.2</th>
<th>2.4</th>
<th>3.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet ≥100,000/µL</td>
<td>24 (22-24)</td>
<td>25 (23-29)</td>
<td>22 (16-37)</td>
</tr>
<tr>
<td>ANC ≥1,000/µL</td>
<td>20 (19-24)</td>
<td>20 (18-21)</td>
<td>19.5 (17-33)</td>
</tr>
</tbody>
</table>

NOTE: Values are median number and range of days (in parentheses).
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 in which the ANC exceeded 1,000/µL. Any
CBC done in the interval from the start of chemotherapy to day 9 of
the course was not considered 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 above 1,000/µL (‘‘recovered date’’) was identified. Next, the
date closest to the recovered date at which the patient’s ANC was
<1,000/µL (‘‘last below date’’) was identified. Patients removed from
therapy because of disease progression before recovery of ANC
were censored for recovery of ANC at the time of 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 the recovery of ANC to at least 1,000/
\(A_L\) for rhTPO dose levels are illustrated in Table 2
and Fig. 1. Patients received a median of 2 days (0-7 day) of
platelet transfusions.

The median number and range of days to ANC recovery
\(\geq 1,000/\mu L\) for rhTPO dose levels 1.2, 2.4, and 3.6 µg/kg are
illustrated in Table 2 and Fig. 2.

RhTPO pharmacokinetics. The pharmacokinetics of rhTPO
were studied in nine patients who received rhTPO at the
2.4 (\(n = 3\)) and 3.6 µg/kg dose (\(n = 6\)). Mean rhTPO
pharmacokinetic parameters are summarized in Table 3. Detectable serum concentrations of thrombopoietin were
observed in all patients before the first rhTPO dose. The mean
baseline thrombopoietin concentration was 1.22 ng/mL (range,
0.3-2.2 ng/mL). Mean baseline thrombopoietin concentration
for each dosing level was 1.63 ± 0.52 and 1.02 ± 0.69 ng/mL for
patients receiving 2.4 and 3.6 µg/kg, respectively (Table 3).
Because these levels were very low, no correction was applied in
the analysis. Primary pharmacokinetic parameters following day
4 rhTPO administration are described in Table 4. Concentration
versus time curves after single and repeated rhTPO adminis-
trations are presented in Fig. 3. The high variability of the half-
life (Table 3) after the 3.6-µg/kg dose was due to a single patient
outlier with a terminal half-life of 149 hours. The volume of
distribution values at steady state were 66 ± 9 and 94 ± 34 mL/
kg at 2.4 and 3.6 µg/kg dose levels, respectively. The peak rhTPO
concentrations were achieved 10 minutes after drug adminis-
tration at each dose level. A dose-dependent increase in mean
\(C_{max}\) and \(AUC_{0-\infty}\) values was noted between two dose groups
(Table 3). Significant accumulation of rhTPO was not observed,
although modest increases in trough concentrations were noted
with repeated dosing (Fig. 3).

Peripheral blood colony-forming unit megakaryocyte progenitor
cells. Megakaryocyte progenitors (CFU megakaryocytes) were
evaluated at baseline and when the WBC had recovered to

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Table 3. Pharmacokinetic results following first-dose rhTPO in children after ICE chemotherapy

<table>
<thead>
<tr>
<th>Dose level (µg/kg)</th>
<th>No. patients</th>
<th>(C_{min}) (ng/mL)</th>
<th>(C_{max}) (ng/mL)</th>
<th>(AUC_{0-\infty}) (ng.h/mL)</th>
<th>CL (mL/h/kg)</th>
<th>Half-life (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>3</td>
<td>1.63 ± 0.51</td>
<td>63.3 ± 9.7</td>
<td>2.350 ± 630</td>
<td>1.08 ± 0.33</td>
<td>47.4 ± 13.4</td>
</tr>
<tr>
<td>3.6</td>
<td>6</td>
<td>1.02 ± 0.69</td>
<td>89.3 ± 15.7</td>
<td>3.066 ± 846</td>
<td>1.24 ± 0.30</td>
<td>64.4 ± 41.7</td>
</tr>
</tbody>
</table>

Abbreviations: \(C_{min}\), minimum concentration (baseline); \(C_{max}\), maximum mean concentration; \(AUC_{0-\infty}\), area under the concentration \(\times\) time curve extrapolated to infinity; CL, systemic clearance.
≥1,000/µL after the nadir was determined and there was a significant (P < 0.001) increase of peripheral blood CFU megakaryocyte formation (Fig. 4).

**Recombinant human thrombopoietin toxicity and antibody formation.** There were no grade II to IV toxicities probably or definitely related to rhTPO administration. Furthermore, there was no evidence of DLT attributable to rhTPO at any of the three rhTPO levels. There were no episodes of toxic-related deaths related to rhTPO or ICE administration. Antibody formation to rhTPO did not occur in any patient. A thrombopoietin MTD was not reached.

**Discussion**

In an attempt to improve the incidence of grade III/IV thrombocytopenia, reduce platelet transfusions and/or improve the percentage of patients recovering their platelet counts by day 21, we investigated the addition of IL-6 to ICE + G-CSF in this same population. We previously reported in this same journal that IL-6 was quite toxic and intolerable and, furthermore, failed to improve the results of ICE + G-CSF without IL-6 (21).

This phase I trial evaluated the safety of rhTPO after ICE chemotherapy in pediatric patients with recurrent/refractory solid tumors. In this study, thrombopoietin was well tolerated in all patients and there was no evidence of DLT or antibody formation at any dose level. The MTD of rhTPO after ICE in children has yet to be determined. In the adult experience with thrombopoietin, thrombocytosis (platelet count ≥1 x 10^9/µL) and/or thrombosis has been seen. These toxicities, however, were not observed in the pediatric trial.

We have conducted a series of four trials of ICE chemotherapy in children with recurrent/refractory solid tumors with different hematopoietic cytokine combinations in an attempt to enhance hematologic recovery and/or reduce the need for platelet transfusions (1). In CCG-0894, we administered identical doses of ICE and post-ICE G-CSF (5 versus 10 µg/kg/d), resulting in a median of 21 (14-42) days to ANC recovery ≥1,000/µL, a median of 27 (15-65) days to platelet recovery ≥100,000/µL, and a median of 6 days of platelet transfusions (1). In comparison, in the present study, the median time to ANC recovery ≥1,000/µL in cycle 1 after ICE followed by rhTPO (3.6 µg/kg) and G-CSF was 19.5 (17-33) days. Median time to platelet recovery ≥100,000/µL in cycle 1 was 22 (16-37) days. The median number of days of platelet transfusions during cycle 1 was 2 days (0-7). Hematologic recovery time for ANC, platelet counts, and number of transfusions required improved for those patients receiving ICE followed by rhTPO plus G-CSF compared with ICE + G-CSF (1).

### Table 4. Compartamental pharmacokinetic parameters of rhTPO after day 4 i.v. administration of 2.4- and 3.6-µg/kg doses to children after ICE chemotherapy

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose 2.4 µg/kg</th>
<th>Dose 3.6 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_{10} (h^{-1})</td>
<td>K_{12} (h^{-1})</td>
</tr>
<tr>
<td>1</td>
<td>0.026</td>
<td>0.247</td>
</tr>
<tr>
<td>2</td>
<td>0.035</td>
<td>0.112</td>
</tr>
<tr>
<td>3</td>
<td>0.024</td>
<td>0.030</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.028 (0.006)</td>
<td>0.130 (0.110)</td>
</tr>
</tbody>
</table>

Abbreviations: K_{10}, elimination rate constant; K_{12}, transfer rate constant from central compartment 1 to compartment 2; K_{21}, transfer rate constant from compartment 2 to compartment 1; V_c, volume of distribution to central compartment.

### Table 3.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Patient no.</td>
</tr>
<tr>
<td>-------------</td>
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<tr>
<td></td>
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<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
</tbody>
</table>

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This phase I trial evaluated the safety of rhTPO after ICE chemotherapy in pediatric patients with recurrent/refractory solid tumors. In this study, thrombopoietin was well tolerated in all patients and there was no evidence of DLT or antibody formation at any dose level. The MTD of rhTPO after ICE in children has yet to be determined. In the adult experience with thrombopoietin, thrombocytosis (platelet count ≥1 x 10^9/µL) and/or thrombosis has been seen. These toxicities, however, were not observed in the pediatric trial.

We have conducted a series of four trials of ICE chemotherapy in children with recurrent/refractory solid tumors with different hematopoietic cytokine combinations in an attempt to enhance hematologic recovery and/or reduce the need for platelet transfusions (1). In CCG-0894, we administered identical doses of ICE and post-ICE G-CSF (5 versus 10 µg/kg/d), resulting in a median of 21 (14-42) days to ANC recovery ≥1,000/µL, a median of 27 (15-65) days to platelet recovery ≥100,000/µL, and a median of 6 days of platelet transfusions (1). In comparison, in the present study, the median time to ANC recovery ≥1,000/µL in cycle 1 after ICE followed by rhTPO (3.6 µg/kg) and G-CSF was 19.5 (17-33) days. Median time to platelet recovery ≥100,000/µL in cycle 1 was 22 (16-37) days. The median number of days of platelet transfusions during cycle 1 was 2 days (0-7). Hematologic recovery time for ANC, platelet counts, and number of transfusions required improved for those patients receiving ICE followed by rhTPO plus G-CSF compared with ICE + G-CSF (1).
We recently studied ICE with IL-6 plus G-CSF (CCG-0931) in children with recurrent/refractory solid tumors, to determine safety, biological activity, and hematopoietic recovery effects (21). IL-6 is a pleiotropic cytokine that affects megakaryocyteopoiesis (22, 23). 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/d. The MTD exceeded the lowest dose tested and DLTs were principally severe constitutional symptoms. All patients receiving IL-6 with G-CSF developed grade IV thrombocytopenia and required a median of five platelet transfusions during course 1, which seems to be a higher number compared with our current thrombopoietin study (21).

The pharmacokinetics of rhTPO administered i.v. postchemotherapy in children were characterized by low clearance and limited distribution into total body water. Wolff et al. showed, in an adult population receiving a similar rhTPO dose (2.4 μg/kg), Cmax (39.6 ± 20.2 ng/mL), clearance (3.22 ± 1.5 mL/h/kg), T1/2 (37.1 ± 7.3 hours), and AUC0-∞ (844 ± 319 ng/mL; ref 24). Comparing these results with children at 2.4 μg/kg, it seems that rhTPO has a higher T1/2 (47.4 ± 13.4 hours), Cmax (63.3 ± 9.7 ng/mL, and AUC (2,350 ± 630 ng.h/mL) and is cleared less rapidly in children than adults. Thrombopoietin protein is synthesized at a constant rate and is metabolized and cleared by the binding to C-mpl receptor on platelets and megakaryocytes (10). The higher number of cells expressing C-mpl, the lower the thrombopoietin serum level. These results suggest that children with thrombocytopenia receiving ICE for recurrent/refractory solid tumors may have fewer megakaryocytes or platelets expressing C-mpl than adults with thrombocytopenia after ablative therapy and stem cell transplantation (24).

The increase in peripheral blood CFU megakaryocyte formation after myelosuppressive chemotherapy seen in our study is in agreement with results reported in several phase I studies with thrombopoietic growth factors. Murray et al. reported small increases in CFU megakaryocyte frequency and a >5-fold increase in CFU granulocyte, erythroid, monocye, megakaryocyte (CFU-GEMM) frequency in the peripheral blood of adult sarcoma patients after administering rhTPO before chemotherapy (25). A phase I study of PIXY321 (IL-3/ granulocyte macrophage colony-stimulating factor fusion protein) in adult sarcoma patients before chemotherapy reported a significant increase in CFU-GEMM (26). Similarly, we reported a significant increase in CFU-GEMM compared with baseline with PIXY321 after ICE in children with recurrent/refractory solid tumors (27). Further, CFU-GEMM colony formation significantly increased over baseline in our study of children with refractory/recurrent solid tumors treated with ICE followed by IL-6 with G-CSF (21).

In summary, the administration of rhTPO is well tolerated in children with recurrent/refractory solid tumors. No DLT or antibody formation was noted at any rhTPO dosing level and the MTD in children after myelosuppressive chemotherapy has yet to be determined. A randomized study would be required to determine if ICE + thrombopoietin + G-CSF versus ICE + G-CSF in children with recurrent/refractory solid tumors significantly enhances time to platelet recovery and/or reduces the need for platelet transfusions.

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