A Phase I Trial of Recombinant Human Thrombopoietin in Patients With Delayed Platelet Recovery After Hematopoietic Stem Cell Transplantation

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ABSTRACT

Delayed platelet recovery is a significant complication after both autologous and allogeneic hematopoietic stem cell transplantation (HSCT). A multicenter, phase I dose-escalation study of recombinant human thrombopoietin (rhTPO) was conducted to assess its safety and to obtain preliminary data on its efficacy in patients with persistent severe thrombocytopenia ($<20,000/\mu L$) >35 days after HSCT. Thirty-eight patients, 37 of whom were evaluable, were enrolled in the study from April 1996 through January 1997. rhTPO was administered at doses of 0.6, 1.2, and 2.4 $\mu g/kg$ as a single dose (group A) or in multiple doses every 3 days for a total of 5 doses (group B). No significant adverse effects were observed. Ten patients had recovery of platelet counts during the 28-day study period; 3 of these 10 had an increase in marrow megakaryocyte content 7 days after completing treatment with rhTPO. When all baseline marrows were compared with samples after rhTPO treatment, there was no difference in marrow megakaryocyte content ($P = 0.49$). This study design could not answer the question of whether the recoveries of platelet counts observed in some patients were spontaneous or influenced by rhTPO treatment; nonetheless, the authors found no correlation between the dose of rhTPO and the recovery of platelet counts. Increases in serum TPO levels were dose-dependent and remained significantly elevated for up to 72 hours after treatment. To evaluate response, further studies of treatment strategies with rhTPO in patients with delayed platelet recovery are required.

KEY WORDS

Megakaryocytes • Thrombocytopenia • Marrow transplantation

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) after myeloablative chemoradiotherapy is an effective therapy for a variety of disorders of hematopoiesis including hematologic malignancies [1]. Thrombocytopenia is a significant compli-cation that must be effectively managed after HSCT to prevent severe bleeding. Thrombocytopenia occurring after HSCT may be isolated and prolonged [2-4]. Variables associated with the rate of recovery of platelet counts after both autologous and allogeneic HSCT include: (1) source of stem cells, (2) CD34+ cell count, (3) platelet count before autologous HSCT, (4) graft-versus-host disease (GVHD), (5) infection, (6) veno-occlusive disease (VOD); and (7) the type of hematopoietic or malignant disease present at transplant

[2,4]. Median times to recovery of platelet counts after transplantation with autologous marrow, peripheral blood stem cells (PBSC), and allogeneic marrow and PBSC were 40, 13, 27, and 16 days, respectively [2]. At 8 weeks after transplantation, 20% and 24% of autologous and allogeneic marrow recipients and 5% and 19% of autologous and allogeneic PBSC recipients, respectively, were still platelet transfusion–dependent. After an initial recovery of platelet counts, a second decrease to <20,000/μL may occur in 5% to 28% of patients [4]. Effective strategies to increase platelet counts in thrombocytopenic patients after transplantation may reduce bleeding complications and decrease the need for platelet transfusions.

Thrombopoietin is the ligand for c-mpl and is the key cytokine regulating megakaryocytopoiesis [5-11]. It is largely produced in the liver, but by other organs as well [12,13]. After binding to c-mpl, which is expressed on platelets and megakaryocytes, thrombopoietin is internalized and metabolized, resulting in the elimination of the cytokine [14]. Thrombopoietin serum levels are regulated by platelet and megakaryocyte mass [15]. After HSCT, serum thrombopoietin levels inversely correlate with platelet count [16,17]. In animal models of myelosuppression, the administration of recombinant thrombopoietin reduces severity and duration of thrombocytopenia [18-26]. After administration of recombinant human thrombopoietin (rhTPO) to patients with advanced cancer, before chemotherapy treatment, both platelet counts and megakaryocyte marrow content increase substantially [27,28]. Administering rhTPO to patients after chemotherapy has also resulted in a higher nadir and a shorter time to recovery of platelet count [29,30]. Based on the demonstration of safety and efficacy in preclinical and early clinical studies, a phase I study was conducted to evaluate the safety and tolerance level of rhTPO as treatment for delayed platelet recovery in patients after HSCT. Secondary objectives included evaluating its efficacy and obtaining pharmacokinetic data.

MATERIALS AND METHODS
Patients

From April 1996 through January 1997, 38 patients were enrolled in this study, which involved 9 institutions within the United States. After autologous or allogeneic HSCT, patients ≥16 years of age were eligible for the study if platelet recovery had not occurred by day 35 or, if after initial platelet recovery, there was a failure to maintain a platelet count ≥20,000/μL without a platelet transfusion for 7 or more days before the start of treatment. Platelet recovery after transplantation was defined as the maintenance of a platelet count ≥20,000/μL without a platelet transfusion for at least 7 days. The study was approved by the institutional review board at each center and explained in detail to each patient, who in turn provided written informed consent.

Patients were not eligible for therapy if they had any of the following: (1) inadequate renal function (creatinine >2.0 mg/dL) or hepatic function (bilirubin >1.5 mg/dL; 1.5-2.5 mg/dL was allowed if serum glutamic-oxalocetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) values were <2.5 times normal and

the prothombin time was within normal limits); (2) previous history of platelet disorder or bleeding diathesis; (3) history of any clinically significant thromboembolic event (excluding clotting of a central line); (4) active VOD, or a history of clinically significant VOD (defined as bilirubin ≥6.0 mg/dL with painful hepatomegaly after transplantation); (5) evidence of progressive neoplastic disease; (6) serious bleeding requiring >2 units of packed red blood cells or causing a ≥10% decrease in hematocrit in 1 day within 7 days of enrolling in the study; (7) uncontrolled serious active infection within the previous 7 days; (8) any experimental drug use within 28 days of the study; (9) progressive GVHD; or (10) recorded temperature >38.5°C (101°F) within 72 hours of the start of the study. Other hematopoietic growth factors, aside from granulocyte colony-stimulating factor (G-CSF), were not allowed during any of the treatment cycles of this study. Platelet transfusions were administered as clinically indicated for bleeding. Prophylactic platelet transfusions were not administered routinely for platelet counts ≥20,000/μL. Routine platelet transfusions, if required, were administered after the morning platelet count and pharmacokinetic studies. A platelet transfusion event was defined as a pool of 6 units of random-donor platelets prepared from donated whole blood units or single-donor platelets collected by platelet pheresis.

Study Design

The study was a multicenter phase I nonrandomized open-label dose-escalation trial of single (group A) or multiple (group B) intravenous doses of rhTPO administered to patients who had delayed platelet recovery after either autologous or allogeneic HSCT. The trial was designed to include 6 patients in each of 6 dose groups. Two additional patients were included in group A at the dose level of 0.6 μg/kg. Because 1 patient had died on day 13 of the study and to maintain study numbers, another patient joined group A. The second patient was enrolled because of simultaneous registration from 2 clinical sites for the last available slot in the cohort. The last patient slot of group A at the dose level of 2.4 μg/kg was not filled, but that patient was instead enrolled in group B at the dose level of 0.6 μg/kg. Patients were stratified at each dose group according to the type of transplant procedure undergone so that at least 1 patient at each dose level had received an allogeneic transplant and 1 had received an autologous transplant.

Dose levels were assigned in ascending order at 0.6, 1.2, and 2.4 μg/kg based on actual body weight. Patients in group A at all dose levels received rhTPO as a single intravenous injection on the first day (day 0) of the study period. Patients in group B received rhTPO every 3 days for a total of 5 intravenous injections. The study period consisted of the 28 days following the initial rhTPO administration.

Patients could be offered additional treatment with rhTPO during an optional extension period (OEP) if, after the study period, patients had not recovered acceptable platelet counts. The OEP was an additional 28-day cycle after the study period during which the patient could receive rhTPO at the same dose and frequency as during the study or at the highest dose that had been safely tolerated to that point in the trial. To be eligible for the OEP, patients must

have safely tolerated rhTPO administration during the original 28-day study period, satisfied all eligibility criteria for entry into the study, had negative tests for neutralizing TPO-specific antibodies, and been free of all evidence of dose-limiting toxicity.

After treatment with rhTPO, recovery of platelets was defined as the ability to achieve and maintain a platelet count $\geq 20,000/\mu L$ without a platelet transfusion for at least 7 consecutive days within the 28-day study period. A secondary endpoint was recovery of platelet counts to $\geq 50,000/\mu L$, maintained without platelet transfusion for 7 days.

Appropriate to the trial design and objectives, the analyses were descriptive in nature.

Dose, Route, and Regimen of rhTPO

rhTPO (Genentech, San Francisco, CA) is a polypeptide derived from a genetically engineered mammalian cell line containing the human gene for thrombopoietin. It was provided as a sterile liquid ready for parenteral administration in a preservative-free buffer solution. rhTPO was shipped on wet ice and stored at temperatures of 2-8°C (35-46°F). Endotoxin levels were less than 0.4E U/mL according to limulus amebocyte lysate clot formation assay.

Study Assessments

All screening evaluations were performed before initial administration of the study drug and consisted of a medical history and physical examination, chest X-ray, electrocardiogram, complete blood count (CBC) with differential and platelet count, serum chemistry, electrolyte profile, urinalysis, serologic studies (to detect TPO-specific antibodies), and a bone marrow biopsy and aspirate. The serum chemistry and electrolyte profile included sodium, potassium, chloride, bicarbonate, total bilirubin, alkaline phosphatase, SGPT, SGOT, blood urea nitrogen (BUN), creatinine, calcium, phosphorous, uric acid, lactate dehydrogenase, and total protein.

During the 28-day study period, a weekly interim history and a symptom-directed physical examination were undertaken for each patient. CBC and differential with platelet count was completed during the first 5 days of treatment and then at least twice weekly or as clinically indicated. Weekly serum chemistry, electrolyte profiles, and assays for TPO-specific antibodies were also recorded. Patients were also subject to a urinalysis 28 days after the first rhTPO infusion and a bone marrow biopsy and aspirate 7 days after the final rhTPO administration. Evaluations during the OEP followed the same schedule as that during the original study period. After completing the 28-day study period or the OEP, patients were followed for an additional 28 days to assess continuing or new adverse events. Patients were evaluated every 2 weeks (days 14 and 28) during this follow-up period.

Marrow Assessment

Bone marrow aspirates and biopsies were acquired before and 1 week after rhTPO treatment. All marrow samples were assessed by the clinical pathologist at the site where the patient had undergone transplantation. Three levels of megakayocyte marrow content were established based on the clinical reports: absent, decreased, or normal. In addition, the marrow samples collected for the study were evaluated by an independent study pathologist. To describe the observations of the pathologist, megakayocytes were quantitated. Because of the limitation of the samples submitted, however, the assessment was only semiquantitative in some samples. The quantitation of megakaryocytes was based solely on tissue sections made from either marrow biopsies or particle preparations. All slides were given numerical codes to mask the source and date of each specimen. Megakaryocytes were quantitated by counting the total number in the section and dividing by the total number of optical fields counted. Biopsies were counted with $10\times$ optical fields, and particles with $16\times$ optical fields. Identification of a megakaryocyte in question was resolved by using higher-powered instruments. The field diameter of the $10\times$ was 1.8 mm and that of the $16\times$ was 1.12 mm, using a Leitz Laborlux (E. Leitz, Stuttgart, Germany) with $10\times$ eyepieces and an 18° field of view. The biopsies varied somewhat in width, most filling 70% to 90% of the total field. No attempt was made to adjust for these slight differences in the width of various biopsies. Determinations of the megakaryocytes in particle sections were more semiquantitative, because the sections did not contain contiguous marrow particles. The microscopist visually grouped contiguous clusters until they approximated the volume of the $16\times$ field. To assess the effect of treatment, the results of baseline marrows were pooled and compared with marrow samples collected after treatment with rhTPO regardless of dose.

Thrombopoietin Assay

Serum samples were collected 5 minutes before and 10 minutes after each rhTPO administration and 24 hours after the final rhTPO administration. Samples were frozen at –70°C for shipping to a central laboratory (Genentech). Serum concentrations of rhTPO were determined using a c-mpl–based enzyme-linked immunosorbent assay (ELISA) with a detection limit of 0.078 ng/mL [17,31].

Thrombopoietin Antibody Assay

Screening for antibodies to TPO was performed on serum samples drawn twice before start of the study and weekly during the study. Sera were rigorously screened using ELISA assays based on full-length and truncated TPO; sera that were reactive in the screen were tested in a functional assay based on blocking c-mpl receptor binding and a bioassay based on the inhibition of a TPO-dependent cell line [32]. Neutralizing antibodies were defined as being inhibitory in the bioassay and associated with clinically significant thrombocytopenia.

RESULTS

Patients and Treatments

Demographics and transplant characteristics of the 38 patients enrolled in the study are summarized in Table 1. Thirty-three of the 38 patients failed to recover platelet counts $\geq 20,000/\mu L$ at any time after transplantation. Five

Table 1. *Patient Characteristics (n=38)**

Male/female	25/13
Median age (range)	48.1 y (18.6-68.2)
ECOG performance status 0/1/2	5/27/6
Median days since transplant (range)	64.5 (40-129)
Diagnoses	
Leukemia	16
Acute myeloid leukemia	8
Chronic myelogenous leukemia	5
Acute lymphocytic leukemia	2
Acute undifferentiated leukemia	1
Non-Hodgkin's lymphoma	11
Myelodysplastic syndrome	5
Breast cancer	4
Hodgkin's disease	1
Aplastic anemia	1
Stem cell source	
Allogeneic marrow	
Matched unrelated	12
Matched related	4
Mismatched related	1
Allogeneic PBSC	
Matched related	5
Autologous PBSC	11
Autologous PBSC + marrow	3
Autologous marrow	2
Patient history at baseline	
Graft-versus-host disease	11
Cytomegalovirus status	
Active viremia	5
History of infection	3
Veno-occlusive disease	3
Absolute neutrophil count (day 0)	
≤500/mL	4
≤1500/mL	7

*ECOG indicates Eastern Cooperative Oncology Group; PBSC, peripheral blood stem cells.

Table 2. *Adverse Events During Observation Periods of the Study*

Event	Number of Patients
Infection/sepsis	23
Bacterial sepsis	5
Viral infection	10
Fungal infection	4
Bacterial pneumonia	4
Fever	10
Graft-versus-host disease	7
Disease relapse	3

that were possibly related to rhTPO administration were less serious and are listed in Table 3. No thrombotic episodes occurred during the observation period. Twenty-two bleeding events were reported during the study period, but were of limited clinical significance. With the exception of 1 patient with gastrointestinal hemorrhage, none required support with red blood cell transfusions (Table 4).

Two patients died while participating in the study, 1 during the study period and the second during follow-up. A third patient's death was reported after the follow-up period. All 3 deaths were considered to be secondary to complications related to the transplant and included parainfluenza virus pneumonia, disseminated Aspergillus, and Aspergillus pneumonia/central nervous system infection.

No significant trends in the occurrence, extent, or distribution of abnormal values were observed for serum SGOT, SGPT, total bilirubin, creatinine, BUN, or other serum chemistries. There were no changes in urinalysis findings. Hematologic toxicity related to the administration of rhTPO was not observed.

Platelet Recovery

A total of 10 patients achieved a platelet count ≥20,000/μL and were independent of platelet transfusions during the 28-day study period (Table 5). Median platelet

patients who had undergone allogeneic transplantation initially achieved a recovery of platelet counts ≥50,000 and were independent of platelet transfusions but then experienced a subsequent sustained decrease in platelet counts to <20,000/μL and became platelet transfusion–dependent (secondary failure of platelet recovery).

Thirty-six of the 38 patients completed the initial 28-day study after start of treatment; during the study, 1 patient died and a second elected to discontinue participation. Thirty-three of these 36 completed the follow-up period; 1 patient died before completion of the follow-up period and 2 patients elected to withdraw. Eleven patients had repeat rhTPO treatments in the OEP: 8 patients had only 1 OEP treatment, 2 had 2, and 1 had 5.

Adverse Events

Sixteen patients experienced serious adverse events. Overall, no pattern of adverse events was associated with the administration of rhTPO. The adverse events recorded were consistent with those seen in an immunosuppressed transplant patient population with thrombocytopenia and, in some cases, neutropenia (Table 2). Other adverse events

Table 3. *rhTPO-related Adverse Events During the Study Period*

Event	Number of Patients
Headache	3
Arthralgia	1
Bone pain	1
Epistaxis	1
Fatigue	1
Fever	1
Lightheadedness	1
Petechiae	1
TPO-specific antibodies*	0

*Antibody detected in one patient during pretreatment screening evaluation.

Event	Number of Patients
Ecchymoses/hematoma	7
Gastrointestinal bleeding	5
Requiring RBC transfusion	1
Hematuria	4
Epistaxis	3
Vaginal bleeding	2

RBC indicates red blood cell.

count at the end of the 28-day cycle in patients with platelet recovery was 28,000/μL. (range 25,000-134,000). Platelet counts showed a gradual sustained increase through the 28-day period in all recovered patients. No responding patient experienced a decline in platelet counts during follow-up relative to those observed at the end of the 28 days. Three of the 10 patients with platelet recovery were classified as having secondary failure of platelet recovery at study entry. Only 2 of the 10 patients exhibited a recovery to >50,000/μL. The platelet recoveries occurred without association to rhTPO dose level. No dose–response effect was observed between TPO and the frequency of either platelet or red blood cell transfusion events during the study (Table 6). Of the 11 patients enrolled in the OEP, all but 1 had the additional treatments at the same rhTPO dose that had been received during the original study. One patient who entered into 5 sequential OEPs had an escalated dose of rhTPO as the study progressed. In total, 2 patients had recovery of platelet counts during these additional rhTPO treatments. In 1 patient there was recovery of platelet counts to 31,000/μL by day 29 of the first OEP; in the second patient there was recovery of platelet counts to 50,000/μL by day 28 of a second OEP.

Assessment of Marrow Megakaryocyte Content

Of the 37 patients evaluable on the study, 32 had baseline marrow samples of sufficient quantity and quality and were evaluated semiquantitatively for megakaryocyte content by pathologists at the study site. Eight had absent, 22 had decreased, and 2 had normal megakaryocyte content. Twenty-seven patients had adequate marrow samples 1 week

after rhTPO treatment for comparison with the baseline sample. Six patients increased by 1 level of megakaryocyte marrow content from their baseline evaluation. Only 2 of these 6 patients also had evidence of clinical improvement with the recovery of platelet counts by day 28.

Slides from the marrow samples taken from 36 patients were available for assessment by the independent study pathologist. According to independent analyses, 3 had normocellular marrows (>70% cellularity) and all but 1 had a decrease in megakaryocyte content before transplantation. An association between an increase in megakaryocyte content and rhTPO dose was not observed. The mean number of megakaryocytes per microscopic field in the pooled baseline marrow samples was 0.95 ± 1.18 compared with 1.54 ± 2.24 after rhTPO treatment ($P = 0.49$, Wilcoxon's rank-sum). Three patients had an increase in megakaryocyte content associated with recovery of platelet counts (Table 5).

rhTPO Pharmacokinetics

In the presence of a normal marrow and platelet count, TPO levels are usually <0.078 ng/mL, the limit of detection for the assay used in this study (Y.G. Meng, unpublished observations and Meng et al. [17]). All patients in this study had elevated TPO levels before receiving the first dose of rhTPO. The mean baseline TPO level for evaluable patients was 1.38 ng/mL (SD $\pm$ 1.17). There was a dose–response increase in the peak serum TPO levels in groups A and B (Figure 1). In group B, days 3, 6, 9, and 12 trough levels in the 1.2 and 2.4 μg/kg dose groups were similar and increased >10-fold over baseline values. The peak levels of serum TPO achieved were greatest in the 2.4 μg/kg dose group and increased >50-fold over baseline values.

TPO-Specific Antibody Assessment After Therapy

No neutralizing TPO-specific antibodies were observed. One subject had antibodies specific to the full-length TPO molecule detected in the screening ELISA before receiving rhTPO. This patient did not have an increase in antibody titer after receiving rhTPO and there were no clinical sequelae.

DISCUSSION

The reasons for prolonged thrombocytopenia after transplantation are multifactorial, and it is likely that the

Table 5. *Platelet Recovery by rhTPO Dose Group During the Study Period*

	Group A Dose (μg/kg)			Group B Dose (μg/kg)			
	0.6	1.2	2.4	0.6	1.2	2.4	Total
Recovery of platelet counts (n)	8	6	5	7	6	6	38
≥20,000/ μL	2	1	2	2	2	1	10
50,000/ μL	0	0	2	0	0	0	2
Increase in megakaryocyte content of marrow							
Evaluable marrow studies (n)	6	5	4	6	4	5	30
Megakaryocyte increase associated with platelet recovery	0	0	1	1	1	0	3

Table 6. *Transfusion Events During the Study Period*

Dose Group (μg/kg)	Number of Patients	Median Number of Platelet Transfusion Events/Patient (Range)	Median Numberof Red Blood Cell Transfusion Events/Patient (Range)
Group A			
0.6	8	9 (2-20)	3 (0-11)
1.2	6	3 (1-16)	1 (0-8)
2.4	5	7 (2-12)	4 (2-5)
Group B			
0.6	7	9 (1-14)	2 (2-5)
1.2	6	8 (0-13)	3 (2-5)
2.4	6	4 (0-13)	3 (0-8)

same mechanisms contribute to both primary and secondary failures (platelet counts initially recover, but then later decrease). Thrombocytopenia may occur after transplantation because platelet production from the marrow is reduced as a result of poor engraftment or the presence of inhibitory factors [33-37]. All but 3 patients in this study had hypocellular marrows and all patients had decreased numbers of marrow megakaryocytes at study entry, confirming a deficit in the capacity for platelet production. Transplant-related complications including infections, VOD of the liver, autoimmune/alloimmune thrombocytopenia, GVHD, and hemolytic-uremic syndrome may also contribute to low platelet counts because of increased consumption [2,4,38-44]. In the presence of a deficit in the capacity for platelet production, disorders that cause even low-grade platelet consumption may result in severe thrombocytopenia. Platelet survival was not assessed in this study, so it is unknown to what extent increased consumption contributed to the severity of the thrombocytopenia. The 3 patients with baseline marrow cellularity >70% may have had a significant component of increased consumption that contributed to the development of severe thrombocytopenia. Even so, if the platelet production from the marrow was to some degree inadequate, stimulation of megakaryocytopoiesis with thrombopoietin might be expected to increase platelet counts [45].

Preclinical studies in mice were the first to show that the administration of rhTPO or megakaryocyte growth and differentiation factor (MGDF) (a 163-amino acid sequence identical to the N-terminal portion of the native thrombopoietin) after (1) nonmyeloablative chemotherapy or total-body irradiation (TBI) or (2) myeloablation and transplantation could reduce both the time to recovery and the nadir of the platelet counts [18-22,46]. Recovery of platelet counts after sublethal TBI (500 cGy) or single-dose chemotherapy in nonhuman primates was accelerated with the administration of rhTPO or MGDF [23,26,47,48]. Treatment with rhTPO and MGDF also stimulated the recovery of other lineages, including red blood cells and neutrophils [49-51]. Thrombopoietin was less effective in this model than in other models for decreasing the time to platelet recovery after marrow or blood stem cell transplantation in nonhuman primates or

dogs, although early administration of high-dose TPO may be more effective (R.A.N., unpublished observations) [52-54].

The first human studies of rhTPO were carried out with cancer patients who were scheduled to receive chemotherapy. To evaluate its effects on otherwise intact marrow, rhTPO was administered before chemotherapy. Platelet counts increased 61% to 213% over baseline and the rhTPO half-life in the serum was 20-30 hours [27]. After chemotherapy, rhTPO and pegylated-MGDF stimulated recovery to baseline platelet counts more rapidly than placebo or historical controls [29,30,55]. Some of the chemotherapy regimens used in these studies did not induce severe thrombocytopenia in the placebo controls. MGDF reduces the duration of severe thrombocytopenia and the need for platelet transfusions after autologous marrow transplantation but is less effective after blood stem cell transplantation [56,57]. This study is the first report of the treatment with rhTPO of a marrow failure syndrome associated with prolonged severe thrombocytopenia.

The administration of rhTPO to patients in other settings has generally been well tolerated. Adverse effects observed in this study were primarily considered transplant-related rather than associated with rhTPO. A small number of thrombotic events have been described in other clinical studies, but it remains unclear if there is an association with rhTPO [29,30]. Antibodies to rhTPO did not develop in this study, although a non-neutralizing antibody that bound TPO was identified in the serum of 1 patient before the start of treatment with rhTPO. In preclinical studies, the development of neutralizing antibodies to rhTPO was associated with a transient decrease of platelet counts which persisted for weeks to months [58,59]. In these preclinical studies, platelet survival was unchanged, indicating that the decreased platelet counts were secondary to decreased production. The use of rhTPO in other species may increase the risk for the development of neutralizing antibodies. In more than 400 subjects treated in clinical studies of rhTPO, no subject has developed neutralizing antibodies (Genentech, data on file).

There was no apparent dose-response effect noted on platelet counts or marrow megakaryocyte content, although

Serum TPO Levels, Group A
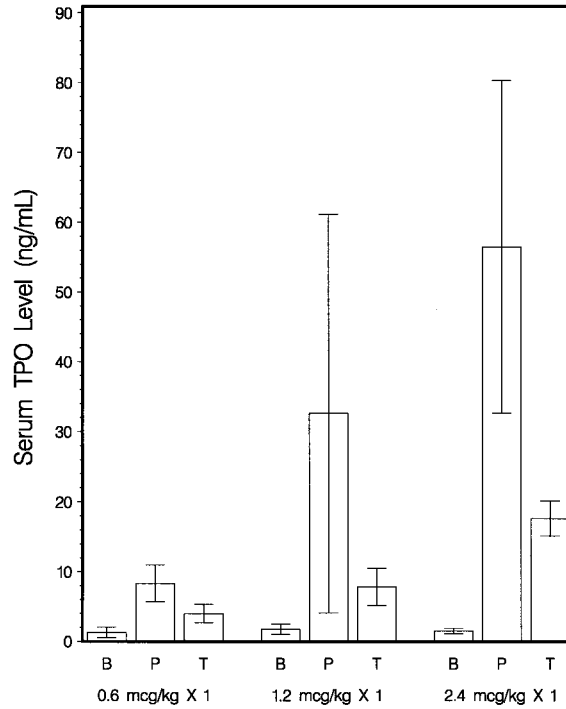
Serum TPO Levels, Group B
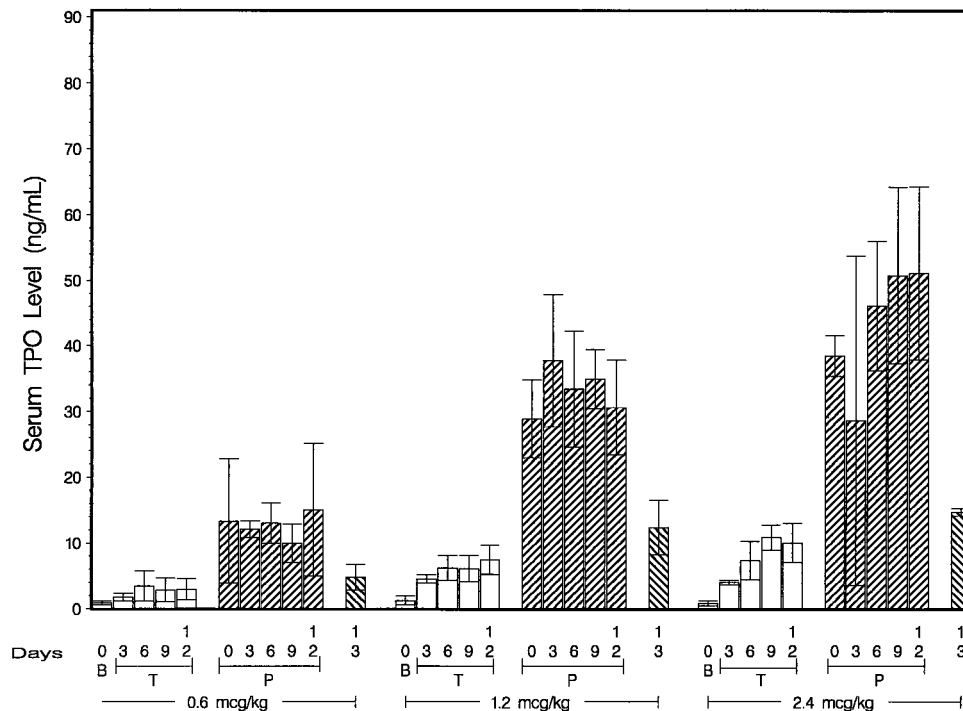

Figure 1. *Serum TPO levels. rhTPO was administered at doses of 0.6 μg/kg, 1.2 μg/kg, and 2.4 μg/kg as a single dose (Group A) or in multiple doses every 3 days for a total of 5 doses (Group B). Baseline (B), peak (P) and trough (T) mean serum TPO levels were obtained for both Groups A and B. Baseline serum TPO levels were measured just before administration of rhTPO dose. Peak serum TPO levels were obtained 10 minutes after the bolus infusion of the rhTPO dose. In Group A, all trough levels were done at 24 hours after treatment. In Group B, trough levels were obtained immediately before the next dose (at 72 hours) except for the serum TPO level on day 13, which was 24 hours after the last dose. Shown are the mean ± standard deviation values for each dose.*

this study was not designed to evaluate such a response. The dose range that was selected had previously been demonstrated to be biologically active [27,28]. Scheduling of doses in group B was based on the prolonged half-life of rhTPO as a result of the decreased platelet count in the blood and megakaryocyte mass in the marrow. TPO levels achieved were dose-related and sustained for up to 3 days, significantly above the already elevated baseline levels at study entry. The significance of serum TPO levels in relation to clinical response is uncertain. However, it was ascertained that the doses studied with a 3-day dosing schedule were safe and sufficient to maintain substantially increased trough serum levels of TPO. In normal mice, 50% of all c-mpl receptors were saturated with rhTPO at a dose of 6.4 µg/kg [60]. The dosing of rhTPO required to saturate c-mpl in humans who are thrombocytopenic has not been reported. The number of responses in the lower dose groups was similar to that observed in the higher dose groups. In patients who recovered platelet counts during the 28-day study period, the start of recovery was usually observed after 14 days of rhTPO treatment. Because spontaneous recovery of platelet counts would be expected in some patients, it is uncertain in the absence of a dose-response relationship whether responses occurred due to the rhTPO.

In summary, rhTPO could be administered safely to patients with severe prolonged thrombocytopenia after HSCT. At the rhTPO doses and schedules studied, no dose-response relationship was observed among those patients who recovered platelet counts during the 28-day study period. Further studies to evaluate rhTPO dosing and schedules or a combination of cell therapy and cytokines are required in patients with delayed platelet recovery.

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