A Phase I Study of Granulocyte-Macrophage-Colony Stimulating Factor/Interleukin-3 Fusion Protein (PIXY321) following Ifosfamide, Carboplatin, and Etoposide Therapy for Children with Recurrent or Refractory Solid Tumors

A Report of the Children's Cancer Group

Mitchell S. Cairo, M.D.[1]
Mark D. Krailo, Ph.D.[2]
Joel A. Weinthal, M.D.[3]
Rita Secola, R.N., M.S.N.[4]
Sharon Bergeron, R.N., B.S.N.[5]
Carmella van de Ven, M.A.[1]
Bruce R. Blazar, M.D.[6]
Leslie Garrison, M.D.[7]
Gregory H. Reaman, M.D.[8]

[1] Georgetown University Medical Center, Washington, DC.

[2] University of Southern California School of Medicine, Los Angeles, California.

[3] Texas Oncology, P.A., Dallas, Texas.

[4] Children's Hospital, Los Angeles, California.

[5] Children's Hospital of Orange County, Orange, California.

[6] University of Minnesota Medical Center, Minneapolis, Minnesota.

[7] Immunex Corporation, Seattle, Washington.

[8] Children's National Medical Center, Washington, DC.

Presented in part at the Annual Meeting of the American Society of Clinical Oncology, Los Angeles, California, May 1995.

Supported by grants from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services, and by a grant from Immunex.

BACKGROUND. This Phase I trial was developed to determine the safety, biologic activity, and effects on hematopoietic recovery of PIXY321 following ifosfamide, carboplatin, and etoposide chemotherapy for children with recurrent or refractory solid tumors.

METHODS. Children (age $<$ 22 years at diagnosis) received ifosfamide 1800 mg/m^2/day $\times$ 5 days, carboplatin 400 mg/m^2/day $\times$ 2 days, and etoposide 100 mg/m^2/day $\times$ 5 days, followed by daily subcutaneous administration of PIXY321. Dose-limiting toxicity was defined as Grade IV toxicity related to PIXY321. Pharmacokinetic and endogenous cytokine production studies were conducted during Course 1, and peripheral blood (PB) progenitor cell and receptor expression studies were conducted during Course 1 when the white blood cell count recovered to $\geq$1000/mm^3.

RESULTS. Twenty-four children received ifosfamide, carboplatin, and etoposide chemotherapy plus PIXY321, the latter at doses of 500 μg/m^2/day (n = 3), 750 μg/m^2/day (n = 6), 1000 μg/m^2/day (n = 9), or 500 μg/m^2/twice a day (n =6). PIXY321 was well tolerated, with only 1 dose-limiting toxicity (chills, occurring at a dose of 750 μg/m^2/day). The maximum tolerated dose was not reached in this study. The median days to absolute neutrophil count recovery ($\geq$1000/mm^3) and platelet recovery ($>$100,000/mm^3) during Course 1 following PIXY321 (1000 μg/m^2/day) were 22 days (range, 5–33 days) and 20 days (range, 5–31 days), respectively. There was a 2500, 5000, 3000, and 390% increase in PB granulocyte-macrophage colony-forming units, erythrocyte blast-forming units, granulocyte erythrocyte macrophage and megakaryocyte colony-forming units, and CD34+ cells, respectively.

Principal Children's Cancer Group participants in this study (with their National Cancer Institute grants in parentheses) were as follows: W. Archie Bleyer, M.D., Anita Khayat, Ph.D., Harland Sather, Ph.D., Mark Krailo, Ph.D., Jonathan Buckley, M.B.B.S., Ph.D., Daniel Stram, Ph.D., and Richard Sposto, Ph.D., Group Operations Center, Arcadia, CA (CA 13539); Mitchell S. Cairo, M.D., Children's Hospital of Orange County, Orange, CA (CA 69274); Ronald Chard, M.D., Children's Hospital & Medical Center, Seattle, WA (CA 10382); Gregory Reaman, M.D., Children's National Medical Center, Washington, DC (CA 03888); Jorge Ortega, M.D., Children's Hospital of Los Angeles, Los Angeles, CA (CA 02649); Joseph Mirro, M.D., Children's Hospital of Pittsburgh, Pittsburgh, PA (CA 36015); Anna Meadows, M.D., Children's Hospital of Philadelphia, Philadelphia, PA (CA 11796); Robert Wells, M.D., Children's Hospital Medical Center, Cincinnati, OH (CA 26126); Jerry Finklestein, M.D., Harbor/UCLA & Miller Children's Medical Center, Torrance/Long Beach, CA (CA 14560); and Gerald Gilchrist, M.D., Mayo Clinic and Foundation, Rochester, MN (CA 28882).

The authors thank Sally Anderson, Linda Rahl, and Renee Dulak for their assistance in the preparation of the manuscript and Irina Vishnevsky and Elvira Magianto for their assistance in data management and analysis.

Address for reprints: Mitchell S. Cairo, M.D., Children's Cancer Group, P.O. Box 60012, Arcadia, CA 91066–6012.

Received December 26, 1997; revision received March 17, 1998; accepted March 17, 1998.

CONCLUSIONS. In summary, this pediatric Phase I trial demonstrated that PIXY321 was well tolerated by children and resulted in platelet recovery a median of 20 days after ICE chemotherapy and an increase in the number of PB progenitor cells above baseline. However, based on recent negative results with PIXY321 in randomized Phase II/III trials involving adult subjects, PIXY321 is not currently available for future trials involving children. ***Cancer*** **1998;83:1449–60.**

KEYWORDS: PIXY321; ICE (ifosfamide, carboplatin, and etoposide); children; hematologic recovery.

The use of ifosfamide, carboplatin, and etoposide (ICE), either singly or in combination, has been successful in the treatment of children with recurrent or refractory solid tumors.[1–7] Combination chemotherapy such as ICE, however, is associated with a significant degree of myelosuppression. Thrombocytopenia continues to remain a significant limitation in the use of combination chemotherapy despite the recent use of several myeloid hematopoietic growth factors, such as granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF), to help decrease the duration and increase the nadir of neutropenia and to facilitate dose intensity. Neither G-CSF nor GM-CSF has been demonstrated to reduce the duration of thrombocytopenia, the nadir of thrombocytopenia, or the frequency of platelet transfusions following dose-intense combination chemotherapy.[8,9]

Anovel cytokine, GM-CSF/interleukin (IL)-3 fusion protein (PIXY321), is a recently described hematopoietic growth factor that was generated by constructing a plasmid containing the coding regions for the cDNAs of human GM-CSF and IL-3.[10,11] The sequences were connected by a synthetic linker and the plasmid was expressed in a yeast culture system. The human GM-CSF was altered by changing ASN-27 to ASP-27 and THR-39 to GLU-39, resulting in a human GM-CSF analog (ASP^{27}, GLU^{39}). In addition, human IL-3 was changed from ASN-15 to ASP-15 and from ASN-70 to ASP-70, resulting in a human IL-3 analog (ASP^{15}, ASP^{70}). PIXY321 has been demonstrated to have a 10- to 20-fold increase in binding affinity to several myeloid leukemia cell lines and is significantly more potent than either GM-CSF or IL-3 alone or in combination in inducing in vitro erythrocyte-blast forming unit (BFU-E), granulocyte-macrophage-colony forming unit (CFU-GM), and granulocyte erythrocyte macrophage and megakaryocyte-colony forming unit (CFU-GEMM) colony formation.[10] PIXY321 has also been demonstrated in nonhuman primates to enhance both neutrophil and platelet recovery following total body irradiation.[12] A Phase I trial of PIXY321 following myelosuppressive chemotherapy (cyclophosphamide, doxorubicin, and dacarbazine) (DTIC) in adult patients with sarcoma had originally suggested that this new cytokine may improve both neutrophil and platelet recovery.[13]

The safety, biologic activity, and effect on hematopoietic recovery of PIXY321 following myelosuppressive chemotherapy in children is unknown. In the current study, we report the results of a Phase I dose-escalation trial (CCG-0924) of PIXY321 following ICE chemotherapy in children with recurrent or refractory solid tumors.

METHODS

Patient Eligibility

This protocol (CCG-0924) was opened for patient entry in July 1993 and was closed to patient entry in April 1995. Patients with refractory or recurrent solid tumors who were age <22 years at diagnosis were eligible for study entry. All patients were required to have histologic verification of malignancy at the time of initial diagnosis. Patients with bone marrow involvement by tumor, patients who had received craniospinal irradiation ($\geq$3600 centigray) or radiation therapy to $>$50% of their bone marrow space, or patients who had previously received total body irradiation were ineligible for study entry. All patients were required to have recovered from previous colony stimulating factor (CSF) therapy and 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. The patient or legal guardian must have signed a documented informed consent indicating awareness of the investigational nature and risks of this study. Patients were required to have had an adequate performance status as defined by $\geq$60 on a Lansky scale (age 1–16 years) or Karnofsky scale (age $>$16 years). Patients were required to be a minimum of age 12 months at the time of study entry. Adequate bone marrow function at time of study entry was defined as an absolute neutrophil count (ANC) of $\geq 1000/mm^3$ and a platelet count of $\geq 100{,}000/mm^3$.

Chemotherapy Administration

Ifosfamide was administered at 1800 mg/m^2/day x 5 days, carboplatin 400 mg/m^2/day $\times$ 2 days, and etoposide 100 mg/m^2/day $\times$ 5 days. Mesna and intravenous hydration were administered during each of the 5 days of ifosfamide. Chemotherapy was repeated when hematologic recovery was achieved (ANC $\geq$1000/mm^3 and platelet count $\geq$100,000/mm^3). Repeated courses of chemotherapy were planned every 21 days. The patient was to have PIXY321 discontinued for at least 2 days prior to subsequent chemotherapy. No modification was made for ICE administration in Courses 1 and 2. In Courses 3–6, however, ICE chemotherapy was reduced by 25% if, in the previous course, hematologic recovery failed to occur by Day 21. There was no modification in ICE administration for renal toxicity during Courses 1 and 2. After Course 2, if the creatinine clearance or glomerular filtration rate (GFR) decreased to 50–74% of normal, the ifosfamide and carboplatin doses were reduced by 25%. If the creatinine clearance or GFR decreased to 25–49% of normal, the ifosfamide and carboplatin doses were reduced by 50%. Finally, if after Course 2 there was evidence of Fanconi's syndrome, i.e., renal tubular acidosis, proteinuria, hypophosphatemia, decreased serum CO_2, and increased chloride, the ifosfamide was discontinued. Patients were not to undergo surgery until they had completed four courses of chemotherapy. In addition, no patients received radiotherapy while receiving protocol therapy.

Colony Stimulating Factor Administration

There were 4 dose levels employed in this study: 500 μg/m^2/day, 750 μg/m^2/day, 1000 μg/m^2/day, and 500 μg/m^2 twice a day. PIXY321 was administered by subcutaneous injection. It was kindly supplied by Immunex (Seattle, WA) and was distributed through the National Cancer Institute. PIXY321 was continued from Day 5 until Day 18 of each course unless the ANC was $\geq$20,000/mm^3 or the platelet count was $\geq$900,000/mm^3 for 2 days between Days 13 and 18, at which time PIXY321 was discontinued for the remainder of the course. Patients failing to achieve an ANC $\geq$1000/mm^3 by Day 18 continued at the assigned dose level of PIXY321 until the ANC reached $\geq$1000/mm^3. Chemotherapy was resumed a minimum of 2 days after discontinuation of PIXY321, provided that the ANC was $\geq$1000/mm^3 and platelet count $\geq$100,000/mm^3. PIXY321 was continued at the assigned dose level until a maximum of Day 25 of any course. Patients failing to achieve the desired ANC and platelet count (i.e., 1000/mm^3 and 100,000/mm^3, respectively) by Day 28 during Courses 3 through 6 were considered off protocol therapy and in follow-up. For patients who experienced Grade IV dose-limiting toxicity (DLT) secondary to PIXY321, administration of PIXY321 was permanently discontinued. DLT was defined as Grade IV toxicity related to PIXY321 using National Cancer Institute common toxicity criteria: pain that persisted beyond 2 doses and that was not controlled by narcotic analgesia, or shaking chills that were unrelated to infection during PIXY321, persisted beyond the first 2 injections, and were not controlled by medication.

Dose Escalation

Patients were entered in cohorts of three; all three patients in each cohort were assigned the same dose. If none of the three patients demonstrated DLT, the dose level was escalated in the next cohort. If two or more demonstrated DLT, the dose was considered intolerable and was reduced to the previous dose level in the next cohort. If one of three patients demonstrated DLT, the next cohort was treated at the same dose level. If none of those three demonstrated DLT, the dose was escalated in the next cohort; otherwise, the dose was considered intolerable and reduced in the next cohort. Six patients were to be treated at the maximum tolerated dose (MTD). No more than one patient could demonstrate DLT at the MTD. Intrapatient dose escalation was not done in this study. Only the toxicity evaluation made during the first course of therapy was used in the determination of the MTD.

Evaluation

All patients were identified to the Children's Cancer Group (CCG) registrar within 72 hours of starting chemotherapy. At the time of registration, the dose level of PIXY321 was assigned to each patient as described above. Chemotherapy and PIXY321 were continued past Course 2 until disease progression, failure to achieve an ANC $\geq$1000/mm^3 and platelet count $\geq$100,000/mm^3 by Day 28, Grade IV renal toxicity resulting from ifosfamide, or a maximum of 6 courses of therapy. Complete blood count (CBC), differential, and platelet counts were obtained on Days 0, 4, 6, 8, 10, 12, 14, 16, 18, and 21 during the first 2 courses of therapy. In subsequent courses, CBC, differential, and platelet counts were obtained on Days 0, 4, 11, 18, and 21. Complete neutrophil recovery was defined as an ANC of $\geq$1000/mm^3 and complete platelet recovery (to a platelet count of $\geq$100,000/mm^3) a minimum of 2 days after discontinuation of CSFs.

Hematologic Recovery, Transfusions, and Infections

Time to hematologic recovery was calculated for each course administered during protocol therapy according to the following algorithm. Each patient who

started a course of chemotherapy was evaluated for each of three measures of hematologic recovery: 1) recovery of ANC (RANC); 2) recovery of platelet count (RPC); and 3) hematologic recovery (RH). RANC was considered to be the time from the start of the course until the first date the ANC reached 1000/mm^3 after the nadir of chemotherapy or the date the next course of chemotherapy was delivered, whichever came first. Such an observation was considered to be complete. If a patient was removed from protocol therapy prior to the recovery of ANC to 1000/mm^3, the RANC was extended to 1 day beyond the longest complete observation and the patient was considered censored for the RANC at that date. RPC was considered to be the time from the start of the course until the first date the platelet count reached 100,000/mm^3 after the nadir of chemotherapy or the date the next course of chemotherapy was delivered, whichever came first. Such an observation was considered to be complete. If a patient was removed from protocol therapy prior to recovery of platelet count to 100,000/mm^3, the RPC was extended to 1 day beyond the longest complete observation and the patient was considered censored for RPC at that date. RH was considered to be the time from the start of the course until the first date when both the ANC reached 1000/mm^3 and the platelet count reached 100,000/mm^3 after the nadir of chemotherapy or the date the next course of chemotherapy was delivered, whichever came first. Such an observation was considered to be complete. If a patient was removed from protocol therapy prior to concomitant recovery of ANC to 1000/mm^3 and platelet count to 100,000/mm^3, the RH was extended to 1 day beyond the longest complete observation and the patient was considered censored for the RH at that date. Finally, the median number of transfusions, days of fever, days of infection requiring hospitalization, and days of fever and neutropenia were determined.

Peripheral Blood Progenitor Cells

During the first course of PIXY321 administration, peripheral blood progenitor cells were determined at a central laboratory before therapy and when the white blood cell count (WBC) had recovered to $>$1000/mm^3. Mononuclear cells (MNC) were isolated from the blood samples by density gradient separation with Ficoll Hypaque. An aliquot of this MNC fraction (10^4/mL) was added to Iscove's modified Dulbecco's medium/0.9% methylcellulose (StemCell Technologies, Inc., Vancouver, British Columbia, Canada) supplemented with 2 U/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 per well and incubated in a 5% CO_2 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 MNC isolated from peripheral blood, as described above, were stained with fluorochrome-conjugated monoclonal antibodies for cytokine receptor and CD34$^+$ expression. CD34$^+$ cell populations were assessed using the human progenitor cell antigen (HPCA) antibody conjugated to phycoerythrin (PE) (Becton Dickinson, Mountain View, CA). Receptor expression was measured using the following antibodies: IL-3/PE (R & D Systems, Minneapolis, MN), IL-6/PE (R & D Systems), GM-CSF/PE (R & D Systems), and stem cell factor (SCF)/PE (R & D Systems). Analysis was performed on a FACStar™ flow cytometer (Becton Dickinson) with gating on the leukocyte populations of interest using the Simultest Leukogate antibody cocktail. Isotype controls were included for each staining experiment.

Induction of Endogenous Cytokines

On Day 5 of the first course only, following the first dose of PIXY321, endogenous cytokine levels were determined. Aliquots of patient sera (1.5 mL) and plasma (1.0 mL) from different time points (0 hour, 30 minute, 1 hour, 2 hours, 4 hours, and 6 hours post PIXY321 injection) were shipped on dry ice (overnight) to the Cytokine Reference Laboratory of the University of Minnesota Hospital and stored at −70°C. Levels of IL-1α, IL-1β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and G-CSF were determined by sandwich enzyme-linked immunosorbent assay using commercially available kits according to manufacturers' instructions for either the serum or plasma, depending on the optimal matrix empirically determined by the reference lab (plasma IL-1α, serum G-CSF, R & D Systems; serum IL-1β, R & D Systems; serum TNF-α, Boehringer Mannheim, Indianapolis, IN; plasma IFN-γ, Endogen, Cambridge, MA). The concentrations for each cytokine, in pg/mL, were interpolated from standard curves generated for each assay performed.

Pharmacokinetics

On Day 5 of the first course administered to patients who received 500 or 1000 μg/m^2/day, plasma and serum were obtained prior to and at 5 minutes, 30 minutes, and 1, 2, 4, 6, 8, and 12 hours after the first dose of PIXY321. Microtiter plates with 96 wells each were coated with anti-IL-3 monoclonal antibodies, and serum samples were diluted and titrated in dupli-

TABLE 1
Demographic Characteristics of Evaluable Patients

Characteristics		Dose level			
		500 $\mu g/m^2$/day	750 $\mu g/m^2$/day	1000 $\mu g/m^2$/day	500 $\mu g/m^2$/bid
Race	White	2	3	5	6
	Black	1	1	0	0
	Other	0	2	4	0
Gender	Male	0	3	6	5
	Female	3	3	3	1
Tumor type	Brain	0	0	3	0
	Osteosarcoma	1	2	1	0
	Ewing's sarcoma	0	1	0	2
	Other tumor	2	3	5	4
Age (yrs) at study entry	<11	3	3	6	2
	≥11	0	3	3	4
Age (yrs) at initial diagnosis	<11	3	3	7	2
	≥11	0	3	2	4

bid: twice a day.

cate with control PIXY321 samples prediluted on each plate. The plates were incubated for 1 hour and then washed. Sheep anti-GM-CSF polyclonal serum was added to the wells and incubated for 1 hour. Peroxidase-labeled antisheep immunoglobulin (Ig)G was added for 1 hour, then peroxidase substrate was added for 10 minutes and optimal densities were determined. The standard curve was fitted to a four-parameter model and sample concentrations were determined. The limit of detection of assay was 125 pg/mL of PIXY321.

Statistical Methods

The interpolation strategy yields conservative estimates of the median time to hematologic reconstitution. This method was chosen because the mechanism that caused the patient's exact recovery time to be unobserved, namely, termination of protocol therapy, was related to disease progression and probably the individual's capacity for hematologic recovery. An analysis of individuals censored at the time of termination of protocol therapy, if recovery was not observed by that point, did not appreciably change any of the estimates of the median values of RANC, RPC, or RH.

Results of the progenitor and receptor studies are expressed as mean values ± the standard error of the mean (SEM). The SEMs were calculated from the three replicates from baseline and from posttherapy samples. The equality of mean biologic measures between baseline and posttherapy time points was assessed using the paired Student's *t* test, with each patient contributing a pair of observations for the analysis.

Survival distributions for RANC, RPC, and RH were calculated by the Kaplan-Meier method.[14] Estimates of the median times to RANC, RPC, and RH were derived from the survival curves.

RESULTS

Twenty-five patients were enrolled in CCG-0924. One patient could not be evaluated because the patient was removed from protocol therapy after the last dose of ifosfamide but before any PIXY321 was administered. Three patients received 500 $\mu g/m^2$/day, 6 received 750 $\mu g/m^2$/day, 9 received 1000 $\mu g/m^2$/day, and 6 received 500 $\mu g/m^2$ twice a day. Twenty-four patients were evaluable for toxicity and hematologic response. The demographic characteristics of the evaluable patients are depicted in Table 1.

All patients had completed protocol therapy and required data submission to address the study objectives at the time the analysis was done. The number of courses with toxicity related to treatment with PIXY321 by dose level is shown in Table 2. These side effects were generally mild. There was one dose-limiting toxicity (chills) at the dose of 750 $\mu g/m^2$/day in the first 3 patients, but the subsequent 3 patients treated at that dose did not show any evidence of dose-limiting toxicity. The toxicities associated with PIXY321 were manageable, and the MTD was not determined in this study.

Hematologic Recovery

The median days to ANC recovery (≥1000/mm^3) during Course 1 following PIXY321 (1000 $\mu g/m^2$/day) was 22 days (range, 5–33 days). The median days to ANC

TABLE 2
Toxicities Associated with PIXY321

Toxicity Observed		Dose level ($\mu g/m^2$) 500 qd (10)[a]	750 qd (17)	1000 qd (16)	500 bid (13)
Fever	Grade I	0	1	1	2
	Grade II	1	0	2	1
	Grade IV	0	0	0	0
Chills	Mild	2	1	3	2
	Persistent	1	1	0	0
Myalgia	Mild	0	1	0	4
	Moderate–nonnarcotic tx	0	1	1	1
	Moderate–narcotic tx	0	0	0	3
Bone pain	Mild	0	0	1	3
	Moderate–nonnarcotic tx	0	1	1	0
	Intractable—req. narcotics	0	0	0	2
Local reaction	Grade I	0	0	2	1
	Grade II	0	1	1	3

qd: daily; bid: twice a day; tx: toxicity.
[a] No. of courses evaluated for toxicity.

TABLE 3
Hematologic Recovery for Evaluable Patients Receiving ICE and PIXY321 by Dose Level

Characteristic	All courses dose level[a,b]				Course 1 only dose level[a,b]			
	500	750	1000	500 bid	500	750	1000	500 bid
No evaluable	3	6	9	6	3	6	9	6
Median days to ANC >1,000/mm^3	24 (7–33)	24 (7–42)	18 (5–42)	21 (7–75)	23 (22–33)	24 (17–33)	22 (5–33)	21 (17–33)
Median days[3] to platelet count >100,000/mm^3	26 (11–32)	23 (12–32)	20 (5–31)	22 (5–75)	21 (16–31)	20 (16–31)	20 (5–31)	21 (12–31)

ICE: ifosfamide, carboplatin, and etoposide therapy; ANC: absolute neutrophil count; bid: twice a day.
[a] Doses are expressed in $\mu g/m^2$/day.
[b] Figures in brackets represent the range.
[c] Median determined from the Kaplan–Meier estimate of the survivor function.

recovery during all courses combined at each dose level are shown in Table 3. The time to recovery of ANC in Course 1 and all courses is depicted in Figures 1A and 1B, respectively. Seventy-five percent of patients who received PIXY321 (1000 $\mu g/m^2$/day) recovered their ANC $\geq$1000/mm^3 by 23 days in Course 1. The median days to platelet recovery ($\geq$100,000/mm^3) during Course 1 following PIXY321 (1000 $\mu g/m^2$/day) was 20 days (range, 5–31 days). The median days to platelet recovery during all courses combined at each dose level are shown in Table 3. The time to platelet recovery in Course 1 and all courses combined is depicted in Figures 2A and 2B, respectively. Seventy-five percent of patients who received PIXY321 (1000 $\mu g/m^2$/day) recovered their platelet count $\geq$100,000/mm^3 by 23 days in Course 1. The time to recovery of both ANC $\geq$1000/mm^3 and platelet count $\geq$100,000/mm^3 for Course 1 and all courses combined is given in Figures 3A and 3B, respectively. PIXY321 was never discontinued for maximal elevation of blood counts.

Seven patients did not recover ANC of $\geq$1000/mm^3 and platelet count of $\geq$100,000/mm^3 by Day 28 during 1 of the first 2 courses. Two patients demonstrated recovery of counts during Course 1 but did not receive a second course of chemotherapy. Three patients were removed from therapy because of progressive disease and did not have sufficient data submitted to evaluate whether blood counts recovered before Day 28 of Course 1. Fourteen patients received at most two courses of therapy. Of the 10 patients who received 3 or more courses of therapy, 1 patient did not recover ANC of $\geq$1000/mm^3 and platelet count of

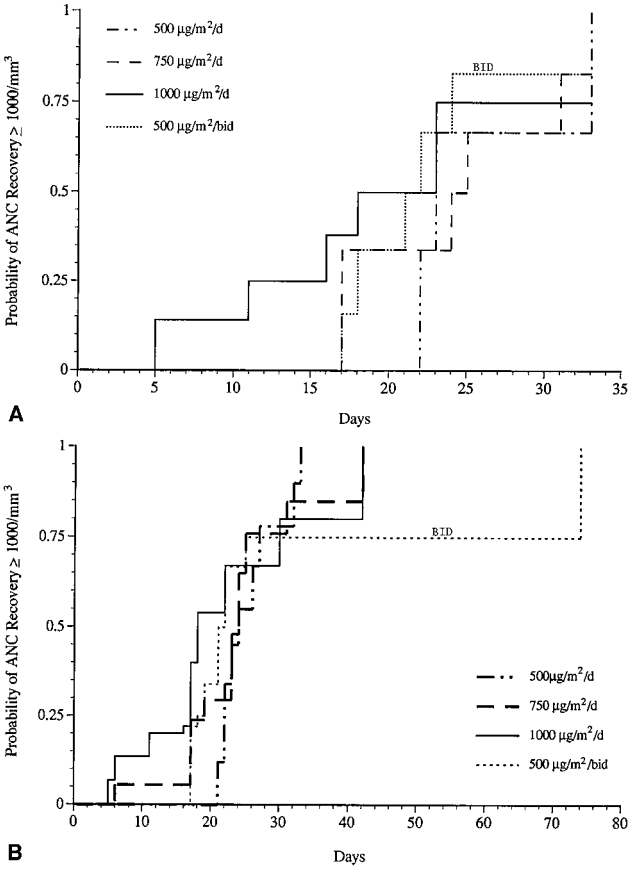

FIGURE 1. (A) Kaplan-Meier estimate of the probability of absolute neutrophil count (ANC) recovery to $\geq$1000/mm^3 in Course 1 following PIXY321. (B) Kaplan-Meier estimate of the probability of ANC recovery to $\geq$1000/mm^3 in all courses following PIXY321.

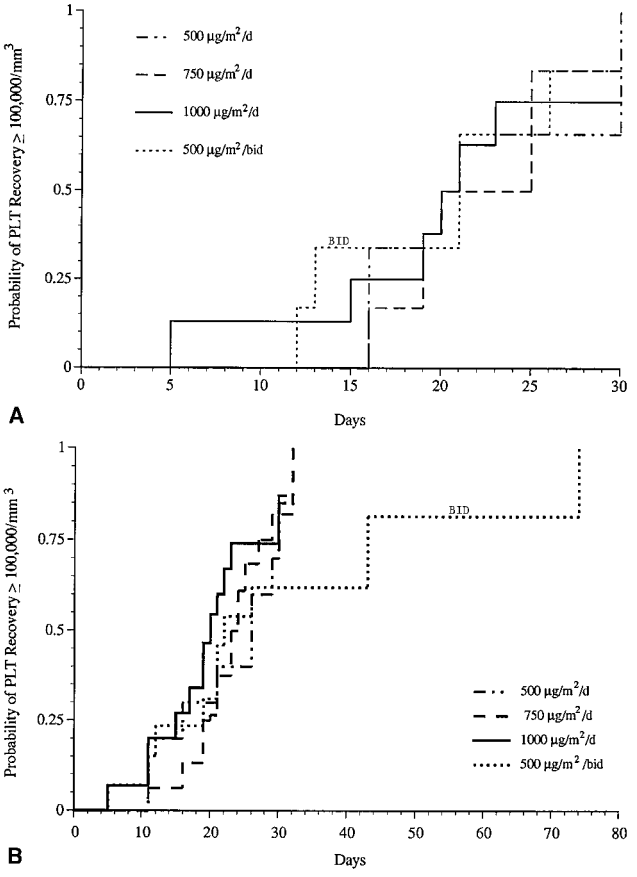

FIGURE 2. (A) Kaplan-Meier estimate of the probability of platelet recovery to $\geq$100,000/mm^3 in Course 1 following PIXY321. (B) Kaplan-Meier estimate of the probability of platelet recovery to $\geq$100,000/mm^3 in all courses following PIXY321.

$\geq$100,000/mm^3 by Day 28 of the last course of therapy, and 1 patient did recover counts by Day 28. Eight patients were removed from therapy because of progressive disease before Day 28 of their last course of therapy and prior to count recovery.

Transfusions

The median number of days requiring red cell transfusions, stratified by dose level in Course 1 and all courses, is depicted in Table 4. Patients who received 1000 μg/m^2/day of PIXY321 had a median of 1 day of red cell transfusions for all courses. The median number of days required for platelet transfusions, stratified by dose level for Course 1 and all courses, is depicted in Table 4. The median number of days requiring platelet transfusions following 1000 μg/m^2/day of PIXY321 for all courses was 1.5 days.

Days in Hospital

The median number of days of fever or infection requiring hospitalization, stratified by dose level, is depicted in Table 4. The median number of days of fever or infection requiring hospitalization following 1000 μg/m^2/day of PIXY321 for all courses was 7.5 days.

Fever and Neutropenia

The median number of days of fever and neutropenia (38.5°C and ANC $<$500/mm^3) is stratified by dose level and depicted in Table 4. The median number of days of fever and neutropenia following 1000 μg/m^2/day of PIXY321 for all courses was 4 days.

Peripheral Blood Progenitor Colony Formation/receptor Expression

The number of total CFUs, including BFU-E, CFU-GM, and CFU-GEMM after 1000 μg/m^2/day, is depicted in Table 5. The total number of CFU/10^4 MNC following 1000 μg/m^2/day of PIXY321 was 131.7 $\pm$ 41.1 (a 1317% increase). There was a significant increase in CFU-GM, BFU-E, and CFU-GEMM compared with baseline values ($P < 0.01$) (Table 5). There was also a significant increase

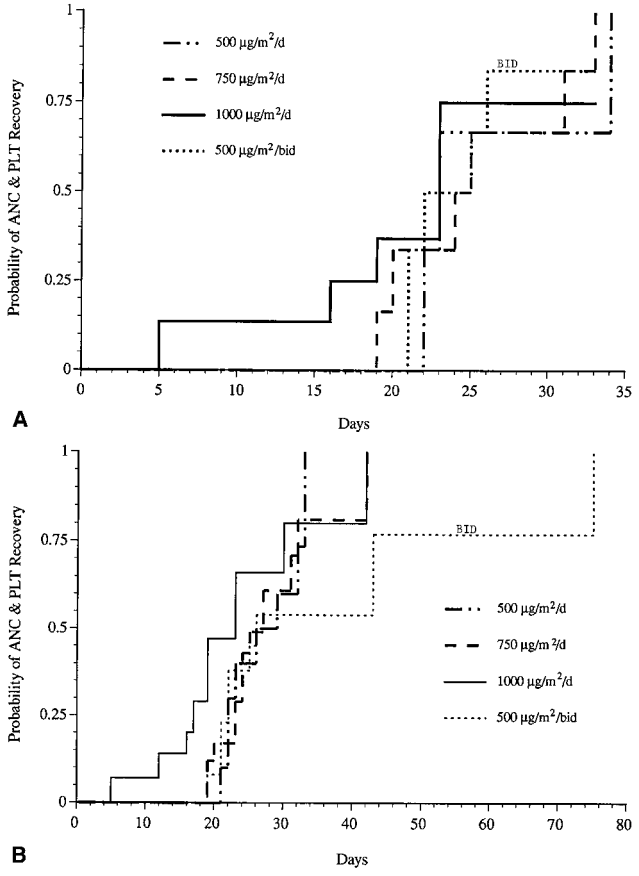

FIGURE 3. (A) Kaplan-Meier estimate of the probability of recovery of both absolute neutrophil count (ANC) to $\geq$1000/mm^3 and platelet count to $\geq$100,000/mm^3 in Course 1 following PIXY321. (B) Kaplan-Meier estimate of the probability of recovery of both ANC to $\geq$1000/mm^3 and platelet count to $\geq$100,000/mm^3 in all courses following PIXY321.

in CD34$^+$ and c-*kit* expression ($P < 0.01$ and $P < 0.05$, respectively) as well as a significant increase in IL-3, IL-6, and GM-CSF receptor expression ($P < 0.01$) (Table 6). There was no evidence of a dose response, although the small number of patients studied precluded a definitive analysis of a dose response to PIXY321.

Cytokine Induction

To determine whether PIXY321 had secondary effects on the induction of either proinflammatory cytokines (which may have had modified toxicity) or proteins involved in positively regulating hematopoiesis, a kinetic analysis (0, 0.5, 1, 2, 4, and 6 hours) of circulating cytokine levels was performed on patients following the subcutaneous injection of PIXY321. None of the patients who received PIXY321 had elevations in the proinflammatory cytokines, interferon gamma, TNF-α, or IL-1α. However, in 5 of 13 evaluable patients (38%) who received high doses (500 μg/m^2 twice a day or 1000 μg/m^2/day) of PIXY321, there was an induction of circulating G-CSF (4–144 pg/mL) and IL-1β (0.2–3.1 pg/mL) over the baseline values of these molecules, which was noted 2–4 hours after subcutaneous PIXY321 injections.

Pixy321 Pharmacokinetics

Samples were obtained on Day 5 of the first course of PIXY321 at baseline, 5 and 30 minutes or 1, 2, 4, 6, 8, and 12 hours after subcutaneous injection. For 3 patients who received 500 μg/m^2/day, the mean area under the curve (AUC) was 9.4 $\pm$ 2.6 ng-hr/mL, C_{max} 1.02 $\pm$ 0.16 ng/mL, and T_{max} 4.5 $\pm$ 3.4 hours. For 7 patients who received 1000 μg/m^2/day, the AUC was 18.6 $\pm$ 5.0 ng-hr/mL, C_{max} 2.53 $\pm$ 0.83 ng/mL, and T_{max} 3.4 $\pm$ 1.9 hours (mean $\pm$ SEM).

DISCUSSION

PIXY321 is a novel fusion protein (GM-CSF/IL-3) that appears to be more potent than either GM-CSF or IL-3 alone in vitro in inducing hematopoietic progenitor colony formation (BFU-E, CFU-GM, and CFU-GEMM).[10] The combination of GM-CSF and IL-3 following radiation-induced bone marrow aplasia in a nonhuman primate demonstrated significant additive effects on hematopoietic recovery compared with either cytokine alone.[15] Sequential IL-3 and GM-CSF following combination chemotherapy with ifosfamide, cisplatin, and etoposide in adults has been demonstrated to improve platelet recovery compared with GM-CSF alone.[16,17] Recently, PIXY321 has also been demonstrated to enhance in vitro human megakaryocytopoiesis significantly, expand peripheral blood progenitor cells ex vivo, and, with the addition of SCF, significantly inhance CFU-GM formation from both cord and adult CD34$^+$ selected stem cells.[18–20]

We have recently demonstrated a significant overall response rate (complete response and partial response) (CR + PR) following ifosfamide, carboplatin, and etoposide in children with recurrent or refractory solid tumors.[1,2] This combination of chemotherapy induced an overall response rate (CR + PR) of 51% in a wide variety of refractory or recurrent pediatric solid tumors.[1] The CR rate in all diagnostic categories was 27%, and the overall response rate (CR + PR) was greater than 60% for patients with Wilms' tumor, Ewing's sarcoma, rhabdomyosarcoma, other soft tissue sarcomas, or lymphomas. Despite increasing the dose of G-CSF (5.0 vs. 10.0 μg/kg/day) following the identical regimen of ICE chemotherapy for a similar group of patients and pretreatment history, we previously demonstrated an 83% incidence of Grade IV neutro-

TABLE 4
Transfusions and Toxicity in Evaluable Patients Receiving ICE and PIXY321 by Dose Level

	All courses dose level[a,b]				Course 1 only dose level[a,b]			
Characteristic	500	750	1000	500 bid	500	750	1000	500 bid
No. evaluable	3	6	9	6	3	6	9	6
Median days requiring red cell transfusions (range)	3 (2–6)	2 (0–4)	1 (0–6)	1 (0–7)	4 (2–5)	1 (0–4)	1 (0–6)	1.5 (0–7)
Median days requiring platelet transfusions (range)	4.5 (1–5)	2 (0–6)	1.5 (0–12)	3 (1–14)	5 (3–5)	2.5 (1–4)	1 (0–12)	3.5 (1–14)
Median days hospitalized for fever or infection (range)	18 (9–28)	7 (0–18)	7.5 (0–28)	4 (0–28)	21 (19–28)	8.5 (0–18)	8 (0–28)	3 (0–28)
Median days of fever and neutropenia (range)	9 (0–25)	3 (0–12)	4 (0–18)	3 (0–16)	15 (13–25)	5 (2–12)	5 (0–18)	2.5 (0–16)

ICE: ifosfamide, carboplatin, and etoposide therapy; bid: twice a day.
[a] Doses are expressed in $\mu g/m^2$/day.
[b] Figures in parentheses represent the range.

TABLE 5
Peripheral Blood Progenitors following PIXY321 (1000 $\mu g/m^2$/day) after ICE Chemotherapy in Children with Refractory or Recurrent Solid Tumors[a]

	Colony forming units/10^4 MNC mean ± SEM	% Increase from baseline	*P* value (baseline vs. post tx)
CFU-GM	50 ± 17	2500	< 0.01
BFU-E	49.8 ± 19.1	5000	< 0.01
CFU-GEMM	30.0 ± 30	3000	< 0.01

ICE: ifosfamide, carboplatin, and etoposide therapy; CFU-GM: colony forming unit–granulocyte - macrophage; BFU-E: burst forming unit–erythroid; CFU-GEMM: colony forming unit–granulocyte, erythroid, monocyte, megakaryocyte; MNC: mononuclear cell; SEM: standard error of the mean; tx: toxicity; WBC: white blood cell count.
[a] Course 1 when WBC reached 1000/mm^3.

TABLE 6
Peripheral Blood Receptor Expression following PIXY321 (1000 $\mu g/m^2$/day) after ICE in Children with Refractory or Recurrent Solid Tumors[a]

Receptor	% Increase from baseline mean ± SEM	*P* value (baseline vs. post tx)
CD34+	389.7 ± 143.0	< 0.01
IL-3	320.0 ± 91.1	< 0.01
IL-6	525.4 ± 153.0	< 0.01
GM-CSF	184.9 ± 70.6	< 0.01
c-*kit*	728.0 ± 710.0	< 0.05

ICE: ifosfamide, carboplatin, and etoposide therapy; SEM: standard error of the mean; tx: toxicity; IL: interleukin; GM-CSF: granulocyte-macrophage–colony stimulating factor.
[a] Course 1 when WBC reached 1,000/mm^3.

penia and an 80% incidence of Grade IV thombocytopenia (CCG-0894).[1] Most importantly, 25% of patients treated on CCG-0894 during Course 1 required at least 35 days to recover their platelet count of ≥100,000/mm^3.[1,2] To identify new hematopoietic growth factors that might enhance platelet recovery following dose-intensive chemotherapy (ICE), we sought to determine the toxicity and possible hematologic efficacy of PIXY321 in a similar group of children with recurrent or refractory tumors. The principal goal was to determine the MTD on clinical criteria. Analysis of progenitor cell assays was conducted to explore the correlation between dose and response. A study to ascertain the dose-response correlation would have required a prohibitive number of patients.

PIXY321 was well tolerated in this pediatric Phase I trial. After 9 patients were evaluated at 1000 $\mu g/m^2$/day, an additional 6 patients were studied at 500 $\mu g/m^2$ twice a day, based on reports suggesting that twice-daily dosing of PIXY321 may be more effective in ameliorating thrombocytopenia than daily dosing.[21,22] The twice-daily dosing of 500 $\mu g/m^2$ dose compared with 1000 $\mu g/m^2$/day did not seem to offer any advantage relative to hematologic recovery or reduced toxicity. The small number of patients enrolled at the two dose levels precluded detection of even moderate differences in time to platelet recovery. There was only one dose-limiting toxicity (chills) in the current study (750 $\mu g/m^2$/day), but the MTD was not reached in this trial. The most common toxicities associated with PIXY321 identified in this pediatric Phase I trial included chills, bone pain, and local reactions. Local reactions usually included redness and itching of mild-to-moderate severity. Comparatively, adults who received similar doses of PIXY321 had a higher incidence of other constitutional symptoms, including headache, malaise, and fatigue.[13] However,

headache, malaise, and fatigue were rarely identified in this pediatric trial.

The median time to recovery of an ANC of $\geq$1000/mm^3 after Course 1 following PIXY321 (1000 μg/m^2/day) was 22 days (range, 5–33 days). For children with similar tumors and pretreatment history who received the identical doses of ICE chemotherapy followed by either 5.0 or 10.0 μg/kg/day of G-CSF administered in a similar fashion, the median days to ANC recovery $\geq$1000/mm^3 were 21 days and 20 days, respectively.[1,2] The median days to recovery of a platelet count of $\geq$100,000/mm^3 after Course 1 following PIXY321 (1000 μg/m^2/day) in the current trial was 20 days (range, 5–31 days). The median time to platelet recovery $\geq$100,000/mm^3 for children with similar tumors and pretreatment history who received identical ICE chemotherapy in a similar time frame following G-CSF (5.0 vs. 10.0 μg/kg/day) was 27 days and 27 days, respectively.[1,2] Most importantly, the median time to restart chemotherapy, i.e., ANC $\geq$1000/mm^3 and platelet count $\geq$100,000/mm^3 during Course 1 following PIXY321 (1000 μg/m^2/day) in the current trial was 23 days (range, 5–33 days). The Phase I adult trial of PIXY321 ($\geq$750 μg/m^2/day) following cyclophosphamide, doxorubicin, and dacarbazine therapy suggested that PIXY321 significantly reduced the degree and duration of neutropenia and increased the mean nadir platelet count relative to GM-CSF alone when compared with historical controls.[13] Both the pediatric and adult Phase I PIXY321 trials, therefore, suggest that higher doses of PIXY321 (750 or 1000 μg/m^2/day) may enhance hematologic recovery, especially platelet recovery, following myelosuppressive combination chemotherapy compared with G-CSF or GM-CSF.

The median number of platelet transfusions in the current study following PIXY321 (1000 μg/m^2/day) during Course 1 was 1 (range, 0–12). Children with similar solid tumors and pretreatment history who received identical doses of ICE chemotherapy and 10 μg/kg/day of G-CSF had a median number of 12 days during which platelet transfusions were required.[1,2] It is noteworthy that, despite repeated courses of ICE chemotherapy in this trial, the median number of platelet transfusions following PIXY321 (1000 μg/m^2/day) was 1.5 (range, 0–12), suggesting a continued effect of PIXY321 despite repeated courses of myelosuppressive chemotherapy.

We assessed the number of peripheral blood progenitor cells following PIXY321 (1000 μg/m^2/day) after ICE chemotherapy during Course 1 when the WBC returned to $\geq$1000/mm^3. Our studies demonstrated that PIXY321 (1000/μg/m^2/day) significantly increased circulating hematopoietic progenitors compared with baseline values. No dose response to PIXY321 was identified. In the Phase I PIXY321 trial following the administration of cyclophosphamide, doxorubicin, and dacarbazine to adults, peripheral blood progenitor cells were measured on Day 14 of treatment (regardless of WBC) compared with our trial, in which peripheral blood progenitor cell colony formation was measured only when the WBC was $>$1000/mm^3.[23] The Phase I study of adults did not demonstrate any consistent changes among BFU-E, CFU-GM, CFU-G, CFU-M, or CFU-GEMM following PIXY321.[23] This difference in results may have been secondary to the obtainment of progenitor samples at different times following hematologic recovery. However, in the adult trial, PIXY321 was demonstrated to increase significantly the cycling rate (the percentage of progenitors in S-phase) of all bone marrow progenitors.[23]

During the first course, we measured peripheral blood progenitor receptor expression following PIXY321 (1000 μg/m^2/day) in children who received ICE chemotherapy. The expression of CD34$^+$, IL-3, IL-6, GM-CSF, and c-*kit* all significantly increased compared with baseline values. The increase in circulating committed progenitor cells and the increase in receptor expression of CD34$^+$ and other cytokine receptors suggest that PIXY321 (1000 μg/m^2/day) may, in conjunction with chemotherapy, be useful as a mobilization strategy for obtaining peripheral blood stem cells for future myelosuppressive therapy and autologous stem cell transplantation.

With respect to the quantitation of circulating cytokine levels following PIXY321 injection, the absence of induction of proinflammatory cytokines is consistent with the clinical tolerability of PIXY321. In addition, the very modest induction of G-CSF cannot account for the hematopoietic effects observed in these patients. The fact that there was a modest induction of circulating G-CSF and IL-1β levels following subcutaneous PIXY321 injections in some patients suggests that the fusion protein may be able to bind to GM-CSF receptors on endothelial cells, which are a source of these two growth factors. The absence of IFN-γ and TNF-α suggest that macrophages were not stimulated by PIXY321 via GM-CSF receptor binding.

We also studied PIXY321 pharmacokinetics following subcutaneous administration. At 1000 μg/m^2/day, the AUC was 18.6 $\pm$ 5.0 ng-hr/mL, C_{max} was 2.53 $\pm$ 0.83 ng/mL, and T_{max} was 3.4 $\pm$ 1.9 hours (mean $\pm$ SEM). In adults, the T_{max} following subcutaneous administration of PIXY321 at 1000 μg/m^2/day also appears to be approximately 4 hours.[24] Although only 6 patients were entered at 500 μg/m^2 twice a day in our pediatric Phase I trial, we were unable to demonstrate any difference compared

with 1000 $\mu g/m^2$/day as a single dose with respect to hematologic recovery, transfusion requirements, or toxicity. However, reliable statistical comparisons cannot be made because of the small number of patients evaluated.

Recent Phase II/III randomized studies of adults that compared PIXY321 with GM-CSF following either myeloablative therapy and autologous bone marrow transplantation or myelosuppressive chemotherapy (with 5-flourouracil, leucovorin, doxorubicin, and cyclophosphamide) failed to demonstrate any superiority of PIXY321 with respect to hematologic reconstitution.[25,26] Specifically, Vose et al.[25] reported that PIXY321 did not improve time to platelet independence compared with GM-CSF following autologous bone marrow transplantation in adults with non-Hodgkin's lymphoma. Furthermore, O'Shaughnessy et al.[26] also could not demonstrate that PIXY321 could ameliorate cumulative thrombocytopenia or the need for platelet transfusions compared with GM-CSF in adults with breast carcinoma following FLAC chemotherapy. These adult Phase II/III trials have strongly suggested that PIXY321 does not have any advantage over GM-CSF with respect to hematologic reconstitution following myelosuppressive or myeloablative chemotherapy.

In summary, this pediatric Phase I trial involving children with refractory or recurrent ST demonstrated that PIXY321 was well tolerated and that the MTD was not reached. Hematologic recovery following PIXY321 (1000 $\mu g/m^2$/day) and ICE chemotherapy suggests that the median days to platelet recovery (100,000/mm^3) was 20 days and the median number of days requiring platelet transfusions was 1.5 days. In addition, we have demonstrated that PIXY321 induces a significant increase in the number of peripheral blood progenitor cells and induces an increase in a subset of cells in the circulating peripheral blood that express specific cytokine receptors. Future double-blind, randomized prospective Phase II/III trials would be required to determine whether PIXY321 provides earlier hematologic recovery, reduced transfusion requirements, and/or dose intensification of chemotherapy. However, based on the recent negative results with PIXY321 in randomized Phase II/III trials involving adults, PIXY321 is currently not available for future randomized trials involving children.

REFERENCES

1. Cairo MS, Shen W-P, Miser J, Krailo M, Blazar B, Reaman G. A randomized trial of two doses of G-CSF (5.0 vs. 10.0 $\mu g/kg/d$) following ifosfamide, carboplatin, and etoposide (ICE) chemotherapy in children with recurrent solid tumors (RST): significant clinical activity but no improvement in hematopoietic recovery (HR) with increased dose of G-CSF [abstract]. *Proc Am Soc Clin Oncol* 1995;14:255.
2. Cairo MS, Krailo M, Miser J, Shen V, Bergeron S, Liu W, et al. Excellent results of the use of ifosfamide, carboplatin, etoposide (I.C.E.) in children with recurrent or refractory solid tumors [abstract]. *Proc Am Soc Clin Oncol* 1996;15: 468.
3. Miser JS, Kinsella TJ, Triche TJ, Tsokos M, Jarosinski P, Forquer R, et al. Ifosfamide with mesna uroprotection and etoposide: an effective regimen in the treatment of recurrent sarcomas and other tumors of children and young adults. *J Clin Oncol* 1987;5:1191–8.
4. Chard RL, Krivit W, Bleyer WA, Hammond D. Phase II study of VP-16–213 in childhood malignant disease: a Children's Cancer Study Group report. *Cancer Treat Rep* 1979;63:1755–9.
5. Gaynon PS, Ettinger LJ, Baum ES, Siegel SE, Krailo MD, Hammond GD. Carboplatin in childhood brain tumors: a Children's Cancer Study Group phase II trial. *Cancer* 1990; 66:2465–9.
6. Frappaz D, Michon J, Hartmann O, Bouffet E, Lejars O, Rubie H, et al. Etoposide and carboplatin in neuroblastoma: a French Society of Pediatric Oncology phase II study. *J Clin Oncol* 1992;10:1592–1601.
7. Pratt C, Douglass E, Etcubanas E, Goren M, Green A, Hayes F, et al. Ifosfamide in pediatric malignant solid tumors. *Cancer Chemother Pharmacol* 1989;24:24–7.
8. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. *Blood* 1991;78:2791–2808.
9. Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood* 1991;77:1131–45.
10. Curtis B, Williams D, Broxmeyer H, Dunn J, Farrah T, Jeffery E, et al. Enhanced hematopoietic activity of a human granulocyte-macrophage colony-stimulating factor/interleukin 3 fusion protein. *Proc Natl Acad Sci U S A* 1991;88:5809–13.
11. Williams DE, Park LS. Hematopoietic effects of a granulocyte-macrophage colony-stimulating factor/interleukin-3 fusion protein. *Cancer* 1991;67:2705–7.
12. Williams DE, Dunn JT, Park LS, Frieden EA, Seiler FR, Farese AM, et al. A GM-CSF/IL-3 fusion protein promotes neutrophil and platelet recovery in sublethally irradiated rhesus monkeys. *Cancer* 1993;4:17–29.
13. Vadhan-Raj S, Papadopoulos NE, Burgess MA, Linke KA, Patel SR, Hays C, et al. Effects of PIXY321, a granulocyte-macrophage colony-stimulating factor/interleukin-3 fusion protein, on chemotherapy-induced multilineage myelosuppression in patients with sarcoma. *J Clin Oncol* 1994;12:715–24.
14. Kalbfleisch J, Prentice R. The statistical analysis of failure time data. New York: John Wiley & Sons, Inc., 1980.
15. Farese AM, Williams DE, Seiler FR, MacVittie TJ. Combination protocols of cytokine therapy with interleukin-3 and granulocyte-macrophage colony-stimulating factor in a primate model of radiation-induced marrow aplasia. *Blood* 1993;82:3012–8.
16. Brugger W, Frisch J, Schulz G, Pressler K, Mertelsmann R, Kanz L. Sequential administration of interleukin-3 and granulocyte-macrophage colony-stimulating factor following standard-dose combination chemotherapy with etoposide, ifosfamide, and cisplatin. *J Clin Oncol* 1992;10: 1452–9.

17. Ganser A, Lindemann A, Ottmann OG, Seipelt G, Hess U, Geissler G, et al. Sequential in vivo treatment with two recombinant human hematopoietic growth factors (interleukin-3 and granulocyte-macrophage colony-stimulating factor) as a new therapeutic modality to stimulate hematopoiesis: results of a Phase I study. *Blood* 1992;79:2583–91.
18. Bruno E, Briddell RA, Cooper RJ, Brandt JE, Hoffman R. Recombinant GM-CSF/IL-3 fusion protein: its effect on *in vitro* human megakaryocytopoiesis. *Exp Hematol* 1992;20:494–9.
19. McAlister IB, Teepe M, Gillis S, Williams DE. Ex vivo expansion of peripheral blood progenitor cells with recombinant cytokines. *Exp Hematol* 1992;20:626–8.
20. Cairo M, Law P, van de Ven C, Plunkett J, Williams D, Ishizawa L, et al. The in vitro effects of stem cell factor and PIXY321 on myeloid progenitor formation (CFU-GM) from immunomagnetic separated $CD34^+$ cord blood. *Pediatr Res* 1992;32:277–81.
21. Vadhan-Raj S, Broxmeyer HE, Burgess M, Papadoupoulos N, Linke K, Patel S, et al. Schedule-dependent reduction in CyADIC chemotherapy (CT)-induced multilineage hematopoietic toxicity by PIXY321 in patients with sarcoma [abstract]. *Proc Am Soc Clin Oncol* 1994;13:475.
22. Raptis G, Gilewski T, Caron D, Jakubowski A. PIXY321 abrogates cumulative myelosuppression: once (QD) vs. twice (BID) daily dosing [abstract]. *Proc Am Soc Clin Oncol* 1995; 14:260.
23. Broxmeyer HE, Benninger L, Cooper S, Hague N, Benjamin RS, Vadhan-Raj S. Effects of in vivo treatment with PIXY321 (GM-CSF/IL-3 fusion protein) on proliferation kinetics of bone marrow and blood myeloid progenitor cells in patients with sarcoma. *Exp Hematol* 1995;23:335.
24. PIXY321 Investigator Drug Brochure, 1994.
25. Vose JM, Pandite AN, Beveridge RA, Geller RB, Schuster MW, Anderson JE, et al. Granulocyte-macrophage colony-stimulating factor/interleukin-3 fusion protein versus granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for non-Hodgkin's lymphoma: results of a randomized double-blind trial. *J Clin Oncol* 1997;15:1617–23.
26. O'Shaughnessy JA, Tolcher A, Riseberg D, Venzon D, Zujewski J, Noone M, et al. Prospective, randomized trial of 5-fluorouracil, leucovorin, doxorubicin, and cyclophosphamide chemotherapy in combination with interleukin-3/granulocyte-macrophage colony-stimulating factor (GM-CSF) fusion protein (PIXY321) versus GM-CSF in patients with advanced breast cancer. *Blood* 1996;87: 2205–11.