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Clinical effects and pharmacokinetics of the fusion protein PIXY321 in children receiving myelosuppressive chemotherapy

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Abstract A hemopoietin with the ability to accelerate both platelet and granulocyte recovery after intensive chemotherapy would have great clinical utility. The recombinant fusion protein composed of human granulocyte-macrophage colony-stimulating factor and interleukin-3 (PIXY321), showed some promise in early adult trials. However, studies for pediatric patients are limited, and there are no systematic data on the pharmacokinetics of PIXY321 given over prolonged periods at current dosage levels. *Purpose*: To determine the safety, clinical effects and plasma concentrations of increasing doses of PIXY321 in children treated with myelosuppressive chemotherapy. *Methods*: A total of 39 children with relapsed or high-risk solid tumors were enrolled in this phase I/II study. PIXY321 was administered once or twice daily by subcutaneous injection in total doses of 500 to 1000 $\mu g/m^2$ per day for 14 days after each course of chemotherapy with ifosfamide, carboplatin, and etoposide (ICE). Pharmacokinetic studies were performed on day 1 of the first course in 33 patients and repeated on day 14 in 13 patients (once-daily schedule only). *Results*: Although mild local skin reactions and fever were frequent, no dose-limiting toxicity was identified at the maximum dose studied (1000 $\mu g/m^2$ per day). There were no statistically significant differences in chemotherapy-induced hematologic toxicity with increasing doses of PIXY321 or with twice-daily vs once-daily dosing. On day 1, the median PIXY321 clearance was 657 ml/min per m^2 (range 77–1804 ml/min per m^2) and the median half-life was 3.7 h (range 2.1–20.8 h). On day 14, clearance increased in all patients studied (median increase 63%), with a corresponding decrease in the median 12-h concentration (from 1.2 to 0.25 ng/ml). Maximum concentrations were <1 ng/ml in 81% of patients, and only two patients had maximum plasma concentrations equivalent to those required for consistent activity in vitro. *Conclusions*: The recombinant fusion protein PIXY321 proved safe in children treated with myelosuppressive ICE chemotherapy but had no demonstrable clinical benefits. The pharmacokinetic studies suggest that the observed lack of hematologic benefit may be explained by low plasma concentrations resulting from increased clearance with prolonged administration. Moreover, the significant increase in PIXY321 systemic clearance in the absence of increased circulating myeloid cells suggests that the upregulation of either extravascular compartment hematopoietic progenitor cells or nonhematopoietic cells may play an important role in controlling circulating concentrations of this unique cytokine. These findings highlight the importance of a thorough assessment of the systemic disposition of cytokines when determining the dose and schedule necessary to achieve clinical activity in patients.

Key words Pharmacokinetics · Children · Solid tumors · PIXY321

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Introduction

Increased dose intensity shows promise in improving the outcome of therapy for relapsed or high-risk solid tumors of childhood. However, dose escalation is often limited by myelosuppressive toxicity. Although the introduction of hematopoietic growth factors has ameliorated treatment-induced neutropenia, none of the currently approved colony-stimulating factors significantly affects platelet recovery [1, 6, 8, 9, 11, 20]. A hemopoietin with the ability to accelerate both platelet and granulocyte recovery would reduce toxicity, lessen the need for blood product support, and potentially permit increased dose intensity. Early suggestions that PIXY321, a recombinant fusion protein, might accelerate recovery from myelosuppression, particularly cumulative thrombocytopenia [30], have not been confirmed in a randomized comparison with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) in adults [22]. Pediatric experience with PIXY321 is very limited [3, 4].

PIXY321 consists of rhuGM-CSF and recombinant human interleukin-3 (rhuIL-3) coupled by a flexible amino acid linker sequence that allows the binding domains to fold into their native conformation [7]. The recombinant product is produced in yeast (*Saccharomyces cerevisiae*) and has a molecular weight of approximately 35 kDa [30]. GM-CSF produces a rapid increase in white blood cells [9, 13], whereas IL-3 has broader, slower, and more sustained effects on hematopoiesis of several cell lineages – particularly platelets and reticulocytes [10, 11]. Because GM-CSF and IL-3 share a common receptor component [23], PIXY321 was created with the expectation that it would exhibit properties of both cytokines [7, 31]. Studies of these two cytokines given sequentially provide support for the potential additive or synergistic effects of PIXY321 on thrombopoiesis [1, 12, 21].

Hematopoietic growth factors are most appropriately evaluated in patients receiving multiagent chemotherapy that consistently produces profound myelosuppression. One such combination is ifosfamide, carboplatin, and etoposide (ICE), a regimen that is active against a wide variety of solid tumors and produces predictable, severe hematotoxicity in children [17–19]. To investigate the safety, efficacy, and pharmacokinetics of PIXY321 in children, we initiated a dose escalation trial of PIXY321 in pediatric patients receiving ICE chemotherapy.

Patients and methods

Patient eligibility

Between April 1993 and November 1994, 39 children and young adults were entered on a clinical trial of ICE chemotherapy plus escalating doses of PIXY321 for the treatment of refractory solid tumors or tumors for which no standard therapy was available. PIXY321 was supplied by Immunex Corporation (Seattle, Wash.). Eligible patients had an Eastern Cooperative Oncology Group (ECOG) performance status of at least 2, a life expectancy of at least 8 weeks, adequate renal and liver function (serum creatinine < 2.0 mg/dl, bilirubin < 1.5 mg/dl, AST not more than three times normal), absolute neutrophil count (ANC) $\geq 1000/\mu l$, packed red cell volume $> 29\%$, and platelet count $\geq 100\,000/\mu l$. This trial was approved by the St. Jude Children's Research Hospital Institutional Review Board, and written informed consent was obtained from patients, parents, and/or guardians, as appropriate.

Clinical and laboratory studies

Before treatment, all patients underwent physical examination and laboratory testing, including complete blood cell count with differential, reticulocyte count, serum chemistry and coagulation profiles, urinalysis, glomerular filtration rate (GFR) measured by ^{99m}Tc-DTPA serum clearance, and tumor assessment with appropriate diagnostic imaging. During each course of therapy, blood counts were monitored at least three times weekly and serum chemistries were monitored weekly. Throughout the study period, patients were systematically evaluated for evidence of toxicity (rated using the NCI Common Toxicity Criteria), need for blood product support, occurrence of fever and infections, antibiotic usage, hospitalization for febrile neutropenia, and tumor response.

Patients who had an ANC less than $500/\mu l$ and an oral temperature > 38.3 °C at any point, or > 38 °C for longer than 1 h, were hospitalized and treated with broad-spectrum antibiotics. The duration of hospitalization was defined as the interval from admission to the point when the patient had been without fever for more than 48 h and all antimicrobials (except trimethoprim-sulfamethoxazole) were discontinued. Platelet transfusions were given only when the platelet count was $\leq 10\,000/\mu l$, unless there was clinically significant bleeding. Red blood cell transfusions were given as clinically indicated.

Treatment

In our studies of ICE chemotherapy, carboplatin doses are based on an estimate of carboplatin clearance predicted from the measured GFR [25]. Carboplatin is eliminated primarily by glomerular filtration, and systemic exposure reliably correlates with response and toxicity, particularly thrombocytopenia [5, 17]. In this trial, carboplatin was administered intravenously to achieve a targeted systemic exposure, or area under the curve (AUC), of 8 mg/ml × min on day 1, followed by ifosfamide (2 g/m^2 per day) and etoposide (100 mg/m^2 per day) on days 2, 3, and 4. Ifosfamide administration was followed immediately by MESNA uroprotection, repeated at 3 and 6 h postdose. Doses of chemotherapy were not modified for subsequent cycles. If a patients's GFR increased or decreased by more than 15%, that patient was taken off the study.

Subcutaneous administration of PIXY321 began the day after each 4-day course of ICE chemotherapy was completed, and continued daily for 14 days. After the first few doses, PIXY321 was administered by patients or families at home, whenever possible. PIXY321 was discontinued on day 19 regardless of blood counts. Consecutive cohorts of 2 to 10 patients were treated with doses of 500, 750, or 1000 μg/m^2 given once daily, or 375 and 500 μg/m^2 administered twice daily. The twice-daily schedule was included to determine whether more frequent administration was more effective than once-daily. No intrapatient escalation of PIXY321 dosage was allowed; patients received from one to six cycles of chemotherapy and PIXY321.

Pharmacokinetic studies

PIXY321 pharmacokinetic studies were performed after the first dose for 33 patients. These studies were repeated after dose 14 for

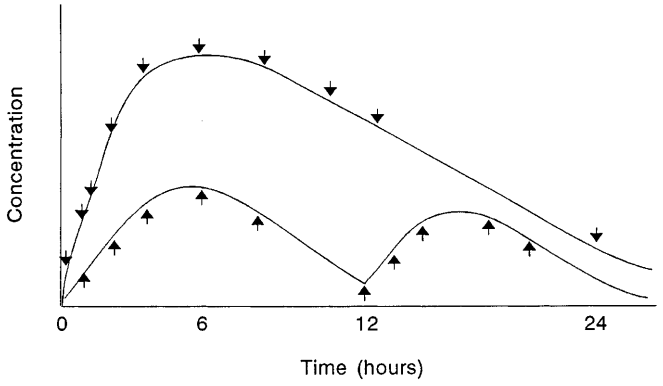

Fig. 1 Predicted concentration vs time curves for patients given once- vs twice-daily PIXY321. *Arrows* indicate when pharmacokinetic samples were drawn. Ten samples were obtained for both the single and twice-daily dose schedules. The sampling schemes for 12- h and 24-h dose schedules were designed to provide comparable pharmacokinetic parameter estimates

patients who received PIXY321 as a single daily injection. On the single daily dose schedule, 3 ml of blood was obtained before the first dose (day 1) and then 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, and 24 h after both the first dose and the last dose (day 14) during cycle 1. Samples for patients on the twice-daily schedule were collected only on day 1, before the morning dose 1, 2, 4, 6, 8, and 12 h postdose, and again 1, 2, 4, and 6 h after the evening dose (Fig. 1). Samples were collected in nonheparinized tubes, centrifuged for 5 min at 3000 *g*, and serum was stored at −20 °C. The PIXY321 ELISAs were performed after patient enrollment was completed. The ELISA had a limit of quantitation of 0.125 ng/ml. Assays were performed by Immunex Corporation (Seattle, Wash.). A one-compartment model with first-order absorption and elimination was fitted to the measured PIXY321 concentrations using maximum likelihood estimation. Each observation was weighted based on an estimate of the variance for the model prediction. The apparent volume of distribution (V), absorption rate constant (k_a), and elimination rate constant (k_e) were estimated, and the apparent clearance (C_L) and elimination half-life ($t_{1/2}$) were calculated. Estimates for V and C_L assumed the fraction absorbed was 1.0. Adapt II software was used for pharmacokinetic modeling (Biomedical Simulation Resource, University of Southern California, Los Angeles). The maximum concentration (C_{max}) was the highest concentration predicted from the pharmacokinetic parameters estimated for each individual patient.

Statistical methods

The outcome measures included ANC nadir, platelet nadir, number of platelet transfusions, number of packed red blood cell (PRBC) transfusions, duration of grade 4 neutropenia, duration of thrombocytopenia (platelet counts $< 50\,000/\mu l$) and severe thrombocytopenia (platelet counts $< 20\,000/\mu l$), and duration of hospitalization for febrile neutropenia during the first course of ICE plus PIXY321. Descriptive statistics were calculated for all outcome measures. The Kruskal-Wallis test was used to assess the correlation between outcome measures and administration schedule [14]. The relationship between variables was assessed by Spearman's correlation with *P*-value testing no correlation. Differences in the pharmacokinetic parameters between dose 1 and dose 14 for patients on the once-daily schedule were analyzed by the Wilcoxon signed rank's test.

Results

Table 1 lists the characteristics and prior treatment of the 39 patients enrolled on this study. The median age of patients at enrollment was 12 years (range 23 months to 23 years). Predominant diagnoses were neuroblastoma, Ewing sarcoma, and brain tumors. Seven patients were treated for newly diagnosed tumors for which no standard therapy was available; the remainder had received extensive prior treatment. A total of 36 patients received at least one dose of PIXY321 and were thus evaluable for toxicity. Response was evaluated in 31 patients who received complete courses of both ICE chemotherapy and PIXY321.

Tolerability of PIXY321

In general, PIXY321 was well tolerated by the 36 evaluable patients. Three of the 39 patients enrolled did not receive PIXY321 because of parental refusal to administer this agent twice daily, a severe allergic reaction to etoposide, and the development of hemorrhagic cystitis, respectively. The most frequent side effects were

Table 1 Characteristics of 39 patients enrolled on the ICE plus PIXY321 trial

Characteristic	Number of patients	Percent
Diagnosis		
Neuroblastoma	10	26
Ewing sarcoma	7	18
Brain tumor	5	12.6
Germ cell tumor	3	8
Peripheral neuroepithelioma	2	5.2
Wilms' tumor	2	5.2
Desmoplastic small round cell tumor	1	2.5
Clear cell sarcoma	1	2.5
Chondrosarcoma	1	2.5
Hodgkin's disease	1	2.5
Osteosarcoma	1	2.5
Retinoblastoma	1	2.5
Rhabdomyosarcoma	1	2.5
Triton tumor	1	2.5
Neuroendocrine carcinoma	1	2.5
Peripheral nerve sheath tumor	1	2.5
Age (years)		
0–2	1	2.5
2–10	17	44
10–23	21	53.5
Sex		
Male	29	74
Female	10	26
Performance status (ECOG)		
0	28	72
1	8	21
2	3	8
Prior treatment		
Radiation	25	64
Chemotherapy	30	77
Number of drugs 0–8, median 5		
Autologous bone marrow transplant	11	28
No prior therapy	7	18

Table 2 Side effects in 36 children who received 91 evaluable courses of PIXY321. Data are presented as the number of episodes of toxicity/number of evaluable courses; toxicities were less than grade 3 unless otherwise indicated

Side effect	Dose ($\mu g/m^2$/day) and schedule[a]				
	500 once daily ($n = 3$)	750 once daily ($n = 6$)	1000 once daily ($n = 8$)	375 twice daily ($n = 9$)	500 bid twice daily ($n = 10$)
Fever	5/9	7/9	10/24	5/22	12/27
Local reaction	1/9	6/9	13/24	6/22	13/27
Generalized rash	0	1/9	1/24[a]	0	0
Chills	3/9	1/9	1/24	1/24	0
Fatigue/malaise	3/9	3/9	0	1/24	6/27
Anorexia	0	1/9	1/24	0	3/27
Headache	0	1/9	1/24	1/24	0
Chest pain	0	0	0	1/24[b]	1/27[c]
Generalized aching	1/9	0	1/24	1/24	1/27
Nausea	1/9	0	0	1/24	0
Vomiting	0	1/9	0	0	1/27
Leg cramps/pain	0	1/9	4/24	0	3/27
Flushing	0	0	1/24	0	0
Pruritus	0	0	1/24	0	0
Tachycardia	0	0	1/24[a]	0	0
Capillary leak	1/9[d]	0	0	0	0

[a] Grade 3 generalized rash with grade 2 tachycardia after first dose of PIXY321; patient taken off study
[b] Cardiac and GI evaluation revealed esophagitis as cause of pain (unrelated to PIXY321)
[c] Chest pain resolved upon stopping PIXY321; cardiac evaluation was unremarkable
[d] Postmortem examination revealed bowel necrosis unrelated to PIXY321 as reason for "capillary leak"

fever and local erythema. Side effects associated with each dose level are presented in Table 2. Of the 36 evaluable patients, 26 had fever (grade 2 or less in all cases), which peaked 2–4 h after the injection in most cases. Local skin reactions (22 patients) were characterized by mild erythema, induration, and warmth. Three children developed a generalized rash. In one of these, the rash developed after the first dose (1000 $\mu g/m^2$ once daily) and was associated with significant pruritus, hives, and tachycardia, requiring discontinuation of PIXY321. Most dermatologic reactions peaked within 12 h of injection, resolved over the next day or two, and were not bothersome. Seven patients had mild chills, usually coincident with a rise in temperature. None of these toxicities was dose-related or dose-limiting.

Of the 36 evaluable patients, 5 (including the one described above) received an incomplete first course of PIXY321. The four other patients had their first course interrupted for a variety of reasons. One patient developed significant substernal chest pain after a single dose of PIXY321 (375 $\mu g/m^2$ twice daily cohort), and no further drug was given. A cardiac evaluation was negative and esophagitis, unrelated to PIXY321, was diagnosed. A second patient, at the same dose, had PIXY321 discontinued by his local physician after 4 days and refused further follow-up. A third patient developed ascites and pitting edema with unstable blood pressure, prompting discontinuation of PIXY321 after 9 doses at 500 $\mu g/m^2$ once daily. Postmortem examination revealed bowel necrosis from other causes. The fourth patient developed mild chills (grade 2), and his parents removed him from the study. PIXY321 was discontinued in an additional patient during the second course of therapy because of chest pain, after 11 doses at 500 $\mu g/m^2$ twice daily. Cardiac evaluation was unremarkable.

Hematologic effects of PIXY321

Granulocyte response

A total of 84 complete courses of ICE chemotherapy followed by 14 days of PIXY321 (range 1–6 courses, median, 2) were administered to 31 patients who were evaluable for hematologic response. Because hematologic effects in sequential cycles of chemotherapy cannot be considered independent, response was evaluated only for each patient's first cycle. Data are presented for the 31 evaluable patients. Data were also analyzed separately for the subset of 21 previously treated patients (excluding the 7 patients with newly diagnosed tumors and 3 patients who had received ICE chemotherapy for relapsed disease immediately prior to enrollment). All 21 previously treated patients had received multiagent chemotherapy (median, five agents) and all but five had received radiation therapy; six patients had undergone autologous bone marrow transplantation.

As shown in Table 3, the duration of grade 4 neutropenia did not differ significantly in relation to the PIXY321 dose level. When the 21 previously treated patients were analyzed separately there also was no noticeable difference ($P = 0.94$; data not shown). The duration of hospitalization for febrile neutropenia was shortest at a dose of 500 $\mu g/m^2$ twice daily for all 31 evaluable patients and for the 21 previously treated patients (median, 5 days for both groups), although

Table 3 The effect of dose and schedule of PIXY321 on neutropenia, hospitalization for febrile neutropenia, and thrombocytopenia (all patients, $n = 31$)

Group	Once daily ($\mu g/m^2$/day)			Twice daily ($\mu g/m^2$/dose)		*P*-value
	500	750	1000	375	500	
Grade 4 neutropenia						
Number treated	2	6	6	7	10	
Days (median)	15	23	12.5	8	14.5	0.3
Days (range)	11–19	7–42	8–18	7–15	4–33	
Hospitalization for febrile neutropenia						
Number treated	2	6	6	7	10	
Days (median)	9.5	7.5	8	9	5	0.26
Days (range)	5–14	0–17	0–36	0–31	0–14	
Grade 3 thrombocytopenia						
Number treated	2	6	6	7	10	
Days (median)	11.5	20.5	9.5	8	15	0.72
Days (range)	5–18	12–39	4–16	0–18	8–43	
Severe thrombocytopenia (< 20 000 platelets/μl)						
Number treated	2	6	6	7	10	
Days (median)	7	13.5	3.5	2	6.5	0.37
Days (range)	4–10	4–28	2–9	0–11	0–22	

differences were not significant. Given the absence of a detectable dose-response effect, we combined cohorts to examine the effects of schedule in the previously treated patients. The median duration of grade 4 neutropenia was 14 days (range 4–33 days) with the twice-daily schedule and 12.5 days (range 7–28 days) with once-daily dosing ($P = 0.94$). In addition we compared the 21 previously treated patients with a group of 14 historical controls who had received ICE chemotherapy after similar prior treatment and found no significant difference in the duration of grade 4 neutropenia ($P = 0.98$, data not shown).

Platelet response

The dose of PIXY321 had no statistically significant effect on the duration of thrombocytopenia (platelets < 50 000/μl and < 20 000/μl; Table 3). When dose levels were combined, there was no significant difference between once- and twice-daily administration in the duration of severe thrombocytopenia ($P = 0.36$). There was no statistical evidence of a dose effect on numbers of platelet ($P = 0.4$) or PRBC transfusions ($P = 0.9$). Comparison of the 21 previously treated patients with the historical controls suggested that use of PIXY321 resulted in an increase in duration of grade 3 thrombocytopenia (median 16 days vs 10 days for historical controls, $P = 0.05$). There was no difference between the two groups in duration of severe thrombocytopenia (< 20 000/μl; $P = 0.12$, data not shown).

Pharmacokinetics of PIXY321

Pharmacokinetic studies were completed for 33 patients on day 1 of PIXY321 therapy. Data were available for the first treatment day for all 17 patients on the once-daily schedule (500 $\mu g/m^2$, $n = 3$; 750 $\mu g/m^2$, $n = 6$; 1000 $\mu g/m^2$, $n = 8$) and 16 of 19 patients treated twice daily (7 at 375 $\mu g/m^2$ per dose and 9 at 500 $\mu g/m^2$ per dose). Repeat studies were completed after dose 14 in 13 patients who received PIXY321 once daily. Pharmacokinetic parameters determined on day 1 did not differ significantly for single vs twice-daily dosing or between dose levels (Table 4). Concentrations rose in a generally proportional fashion with increasing doses but were highly variable (Table 5).

Univariate analysis identified weak but significant correlations between PIXY321 clearance and body surface area (BSA; $\rho = 0.49$; $P = 0.003$) and patient age ($\rho = 0.43$; $P = 0.01$). There was no discernible relationship between PIXY321 clearance normalized to BSA and white blood cell count ($P = 0.18$), platelet count ($P = 0.22$), or hemoglobin level ($P = 0.07$). Pronounced variability between patients was seen for systemic clearance (coefficient of variation 57%) and volume of distribution (coefficient of variation 68%).

Table 4 Summary of PIXY321 pharmacokinetic parameters after dose 1 ($n = 33$)

Pharmacokinetic parameter	Median value	Range
Clearance ($ml/min/m^2$)	657	78–1805
Volume (l/m^2)	226	31–874
k_a (h^{-1}, $n = 32$)	0.24	0.01–1.32
$t_{1/2}$ (h)	3.67	2.11–20.81
k_e (h^{-1})	0.19	0.03–0.33

Table 5 PIXY321 concentrations (ng/ml) day 1. Values are median (range)

	Dose ($\mu g/m^2$)			
	375 ($n = 7$)	500 ($n = 12$)	750 ($n = 6$)	1000 ($n = 8$)
C_{max}	0.8 (0.4–1.1)	0.95 (0.4–2.3)	1.1 (0.6–2.1)	1.55 (0.9–16.6)
$C_{12\ h}$	0.2 (< 0.1–1.1)	0.6 (0.1–1.1)	0.75 (0.5–1.3)	0.8 (0.3–12.3)

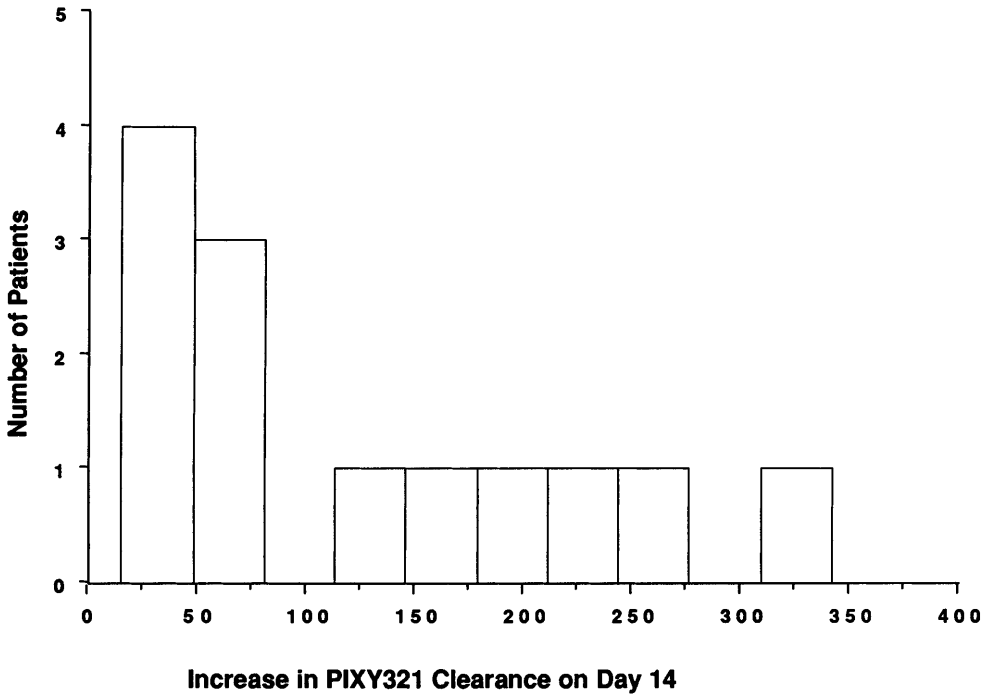

Fig. 2 Percentage increase in clearance (ml/min per m^2) of PIXY321 from day 1 to day 14 for 13 patients

Of the patients on the once-daily schedule, 13 had pharmacokinetic studies performed on both day 1 and day 14. Systemic clearance on day 1 for these 13 patients did not differ from the values for all 33 patients (Table 4). Clearance increased ($P < 0.0002$) over the course of PIXY321 therapy in all 13 patients (Fig. 2), and the increase was greater than 50% in 9. The median clearance increased from 559 at day 1 to 1270 ml/min per m^2 at day 14. There was no significant change in apparent volume of distribution between day 1 and day 14 (medians 215 l/m^2, range 31–806 l/m^2], and median 286 l/m^2, range 57–1540 l/m^2, respectively). Although median C_{max} on day 1 (1.70 ng/ml) and day 14 (1.6 ng/ml) did not differ significantly ($P = 0.29$), 12-h postdose concentrations decreased significantly from day 1 to day 14 (0.8 ng/ml vs 0.3 ng/ml, $P < 0.0004$). Neither predose blood counts nor PIXY321 dose showed any relationship with the observed increase in clearance normalized to BSA, or to the decrease in PIXY321 concentrations.

Discussion

The recombinant fusion protein PIXY321 was well tolerated by children with refractory or high-risk solid tumors who received intensive chemotherapy with ICE. Low-grade fever and local erythema were the most frequent side effects. No dose-limiting toxicities were identified at the highest dose tested (1000 μg/m^2). Unfortunately, no significantly beneficial clinical effects were identified at any dose level. Additionally, the use of a twice-daily schedule had no discernible effect on activity. Systematic pharmacokinetic studies provide important clues to the absence of clinical utility in these pediatric patients.

An early study by Vadhan-Raj et al. [30] confirmed the expectation that PIXY321 would have effects on multiple hematopoietic cell lineages, particularly platelets. These authors reported attenuation of cumulative thrombocytopenia in adults receiving multiple cycles of cyclophosphamide, doxorubicin, and DTIC. In addition, a preliminary study on 25 children with a variety of solid tumors who received PIXY321 after ICE chemotherapy showed more rapid recovery from neutropenia (defined as an absolute neutrophil count $< 1000/\mu l$) and thrombocytopenia (platelets $< 100\,000/\mu l$), compared with historical controls who received identical chemotherapy followed by G-CSF [3, 4]. However, a prospective randomized comparison of PIXY321 (375 μg/m^2 twice daily) vs GM-CSF (250 μg/m^2 once daily) in 53 adults treated with 5-fluorouracil, leucovorin, doxorubicin, and cyclophosphamide (FLAC) identified no differences between these treatment groups in the duration of thrombocytopenia or the need for platelet transfusions [22]. In contrast to the study by Vadhan-Raj et al. [30], in which patients were chemotherapy naive, a significant proportion of our children as well as those in the adult trial of FLAC with PIXY321 [22] were heavily pretreated. In patients without extensive prior therapy, PIXY321 may still have significant beneficial effects on the amelioration of thrombocytopenia.

Our pharmacokinetic studies of PIXY321 provide a plausible rationale for inconsistent hematologic effects. Variability in both absorption and systemic clearance contributed to variability in plasma concentrations. Coefficients of variation were 57% for clearance and

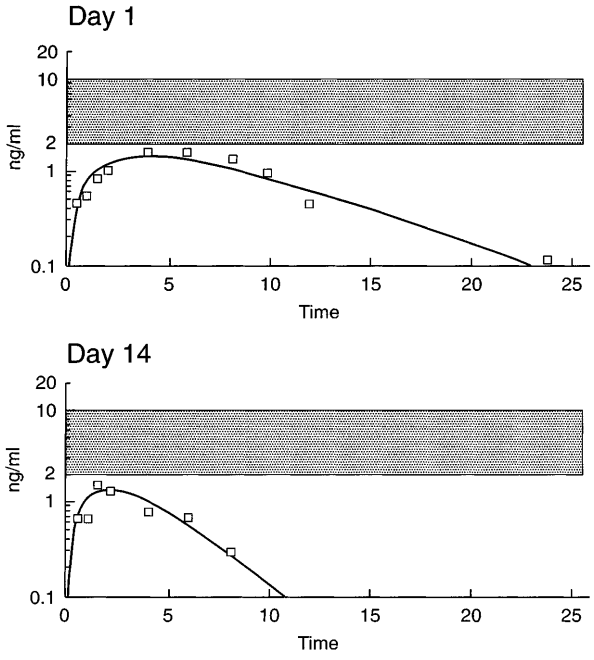

Fig. 3 Concentration vs time (hours) curve for PIXY321 for a representative patient on day 1 (*top panel*) compared with day 14 (*bottom panel*). *Shaded areas* represent concentrations of PIXY321 (2–10 ng/ml) that have been associated with maximal hematologic responses in vitro [2, 26]

181% for 12-h postdose concentrations. The apparent clearances for PIXY321 are considerably higher than values previously published for rhuIL-3 [15] or rhuGM-CSF [28]. The physicochemical characteristics of the larger PIXY321 fusion protein may differ from rhuGM-CSF or rhuIL-3 resulting in incomplete absorption and lower plasma concentrations.

In vitro studies [2, 26] suggest that PIXY321 concentrations above 2 ng/ml may be necessary for an effect on human hematopoietic progenitor cells, and concentrations of 5 to 10 ng/ml are necessary for maximum activity. In our study, even at doses of 500 $\mu g/m^2$ twice daily, plasma concentrations may not have been sufficient to produce a consistent measurable effect on platelets or granulocytes. Of further interest is the consistent and substantial increase in PIXY321 systemic clearance over the course of therapy. As shown in Fig. 3, concentrations of PIXY321 were consistently below the values associated with activity in vitro, and increased clearance substantially reduced systemic exposure over the course of therapy. This pattern has been previously reported for both G-CSF [27] and GM-CSF [24], but in those studies was associated with substantial increases in target cells (e.g. granulocytes). The hypothesis has been put forward that increased systemic clearance of colony-stimulating factors is associated with an upregulation of cellular compartments with the capacity to remove the cytokine from the circulating blood volume [16, 29]. In the present study, increased clearance occurred in the absence of an elevated concentration of circulating target cells. Neither granulocytes nor platelets were increased and there was no discernible relationship between hematologic responses and the increased systemic clearance of PIXY321. It is possible that target cells outside the plasma compartment were upregulated, or that the increased clearance is mediated by other organs of clearance such as the liver. An alternative explanation for these results is that neutralizing antibodies were produced in these patients over the course of treatment. Although antibodies for PIXY321 were not assessed in these children, this explanation is unlikely. Of 142 adults studied for PIXY321 antibody development after multiple courses of PIXY321 in the cancer chemotherapy setting, only 5 developed neutralizing antibody (PIXY321 Investigator Brochure; 1 December 1994).

Numerous in vitro experiments suggest that the most effective stimulation of multilineage hematopoiesis is achieved with a combination of cytokines. It was hoped that the fusion of GM-CSF and IL-3 would result in a single agent capable of accelerating both myeloid and megakaryocyte recovery after chemotherapy [32]. The disappointing clinical results may, in part, be explained by failure to achieve substantial systemic PIXY321 concentrations over sufficient time periods to achieve the activity predicted from in vitro studies [2, 26]. Further clinical trials with PIXY321 at higher doses might yield more consistent effects on multiple cell lineages, including platelets, but dosage adjustments would be required to adjust for changes in clearance with continued dosing. The pharmacokinetic findings from this study highlight the importance of a thorough assessment of the systemic disposition of cytokines when determining the dose and schedule necessary to achieve clinical activity in patients.

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