

# Prospective, Randomized Trial of 5-Fluorouracil, Leucovorin, Doxorubicin, and Cyclophosphamide Chemotherapy in Combination With the Interleukin-3/Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) Fusion Protein (PIXY321) Versus GM-CSF in Patients With Advanced Breast Cancer

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We conducted a prospective randomized trial to evaluate the ability of the interleukin-3/granulocyte-macrophage colony-stimulating factor (GM-CSF) fusion protein, PIXY321, to ameliorate cumulative thrombocytopenia after multiple cycles of 5-fluorouracil, leucovorin, doxorubicin, cyclophosphamide (FLAC) chemotherapy compared with GM-CSF in patients with advanced breast cancer. Fifty-three patients were randomized to receive either PIXY321, 375  $\mu\text{g}/\text{m}^2$  twice a day subcutaneously, or GM-CSF, 250  $\mu\text{g}/\text{m}^2$  daily subcutaneously after FLAC chemotherapy. PIXY321 was less well tolerated than GM-CSF, with more patients developing chills and local skin reactions and more patients stopping PIXY321 due to intolerance. While no difference in the neutrophil nadirs was seen with the two cytokines, the duration of the abso-

lute neutrophil count less than 1,000/ $\mu\text{L}$  for all cycles was significantly longer with PIXY321 than with GM-CSF. Fifty percent of patients treated with multiple cycles of FLAC chemotherapy on both study arms developed dose-limiting thrombocytopenia. No differences in platelet nadirs, duration of thrombocytopenia, or need for platelet transfusions were observed with PIXY321 versus GM-CSF. The average delivered doses of FLAC chemotherapy were somewhat higher in the GM-CSF study arm. PIXY321 was not superior to GM-CSF in ameliorating the cumulative thrombocytopenia observed with multiple cycles of FLAC chemotherapy and was less well tolerated.

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SEVERAL HEMATOPOIETIC growth factors are under clinical development to ameliorate the cumulative thrombocytopenia that is dose-limiting in the treatment of advanced breast cancer with high-dose chemotherapy. We have previously shown that a 20% increase in the dose intensity of multiple cycles of 5-fluorouracil, leucovorin, doxorubicin, cyclophosphamide (FLAC) chemotherapy can be achieved with the addition of granulocyte-macrophage colony-stimulating factor (GM-CSF; *Escherichia coli*-derived molgramostim) compared with treatment with FLAC alone; however, cumulative thrombocytopenia over several cycles of chemotherapy limited further dose intensification.<sup>1</sup>

PIXY321 is a genetically engineered fusion protein combining interleukin-3 (IL-3) and GM-CSF into one molecule.<sup>2</sup> IL-3 is a multilineage hematopoietic growth factor that stimulates the proliferation of early progenitors of myeloid, erythroid, and megakaryocytic lineages, while GM-CSF promotes the proliferation of more mature myeloid progenitors.<sup>3</sup> One study has shown additive effects of combined IL-3 and GM-CSF administration in sublethally irradiated primates,<sup>4</sup> and several studies have demonstrated greater proliferation of myeloid and megakaryocyte progenitors as well as improvement in patients' platelet recovery with sequential IL-3 and GM-CSF administration after high-dose chemotherapy.<sup>5-8</sup> We have recently shown that administration of 9 days of IL-3 followed by GM-CSF was superior to either cytokine alone and to concurrent IL-3 and GM-CSF administration in ameliorating cumulative thrombocytopenia over multiple cycles of FLAC chemotherapy.<sup>8</sup> PIXY321 has been shown to be a potent stimulator of multipotential and lineage-restricted progenitors, including colony-forming units-granulocyte, erythroid, monocyte, megakaryocyte (CFU-GEMM).<sup>9</sup> In addition, PIXY321 has been shown to enhance the rate of both neutrophil and platelet recovery in sublethally irradiated primates.<sup>10</sup>

A phase I/II trial of PIXY321 in sarcoma patients being treated with cyclophosphamide, doxorubicin, dacarbazine (Cy-

ADIC) showed that doses of 500 to 1,000  $\mu\text{g}/\text{m}^2/\text{d}$  were biologically effective in preventing cumulative thrombocytopenia over two cycles of therapy compared with historical controls that had been treated with GM-CSF.<sup>11</sup> The preliminary reports of several other phase I/II studies have also shown reduced durations of thrombocytopenia in patients receiving treatment with ifosfamide, carboplatin, etoposide (ICE) chemotherapy<sup>12,13</sup> and in patients receiving high-dose chemotherapy and autologous stem cell reinfusion.<sup>14</sup> In addition, recent data have suggested that twice daily dosing of PIXY321 may be more effective in ameliorating thrombocytopenia than once-a-day dosing.<sup>15</sup>

To evaluate the ability of PIXY321 to ameliorate thrombocytopenia over multiple cycles of chemotherapy, we conducted a prospective, randomized, open-label phase III trial of PIXY321 versus GM-CSF in patients with advanced breast cancer undergoing treatment with five cycles of FLAC chemotherapy.

## PATIENTS AND METHODS

Fifty-three patients with stages II (four or more nodes positive), III, or IV histologically confirmed breast cancer were entered on

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*Submitted July 24, 1995; accepted October 30, 1995.*

*Supported in part by the Susan G. Komen Foundation and by the Immunex Corp.*

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this study. Patients with stage II or III disease had received no prior chemotherapy, and patients with stage IV disease were previously untreated for metastatic disease but could have received doxorubicin up to 360 mg/m<sup>2</sup> as adjuvant therapy. An Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 was required, as well as a leukocyte count greater than 4,000/ $\mu$ L and a platelet count above 100,000/ $\mu$ L. Patients were required to have normal renal, cardiac, and hepatic function, unless there was evidence of tumor involvement in liver in which case, up to four-times normal hepatic transaminases were allowed. All patients gave written informed consent according to National Institutes of Health (NIH) Clinical Center guidelines before study entry.

**Treatment plan and study schema.** FLAC chemotherapy was administered every 21 days for five cycles, as follows: 5-fluorouracil (5-FU; Solopak, Franklin Park, IL), 300 mg/m<sup>2</sup> slow intravenous (IV) push days 1, 2 and 3; calcium leucovorin (supplied by the Cancer Therapy Evaluation Program [CTEP], National Cancer Institute [NCI], Bethesda, MD, 500 mg/m<sup>2</sup> IV over 15 to 30 minutes days 1, 2, and 3, given 1 hour before 5-FU; doxorubicin (Adriamycin; Adria Labs, Columbus, OH), 17 mg/m<sup>2</sup> IV days 1, 2, and 3; and cyclophosphamide (Cytosan, Bristol-Myers-Squibb, Evansville, IL), 500 mg/m<sup>2</sup> IV days 1, 2, and 3. No dose escalations were undertaken over the five cycles of therapy. Dose reductions for FLAC chemotherapy were made for grade 3 or 4 (CTEP Common Toxicity Criteria) gastrointestinal toxicity, resulting in a 25% reduction in the 5-FU dose in subsequent cycles. Grade 4 thrombocytopenia (defined in this study as a platelet count less than 20,000/ $\mu$ L) or grade 4 neutropenia (absolute neutrophil count [ANC] less than 500/ $\mu$ L) of greater than 5 days' duration resulted in a 10% reduction in the dose of both cyclophosphamide and doxorubicin in the subsequent cycle. Platelets were transfused for counts less than 10,000/ $\mu$ L to 20,000/ $\mu$ L, and red blood cells were transfused at the discretion of the treating physicians, generally for a hemoglobin level less than 8 mg/dL. Patients were hospitalized for intravenous antibiotic therapy for an ANC less than 500/ $\mu$ L and either one temperature greater than 38.5°C or three repeated temperatures greater than 38°C.

Patients were randomized to receive either PIXY321 (1,500  $\mu$ g per vial; Immunex; supplied by CTEP, DCT, NCI), 375  $\mu$ g/m<sup>2</sup> twice a day subcutaneously from day 4 until neutrophil and platelet recovery, or sargramostim (500  $\mu$ g per vial, Leukine, yeast-derived GM-CSF, Immunex; supplied by CTEP, DCT, NCI), 250  $\mu$ g/m<sup>2</sup>/d subcutaneously until hematologic recovery. Patients were taught to reconstitute and self-administer the cytokine. PIXY321 was reconstituted with 1 mL of bacteriostatic water for injection at a final concentration of 1,500 mg/mL, and sargramostim was reconstituted with 1 mL of sterile water for injection at a final concentration of 500  $\mu$ g/mL. As the data suggesting superiority of a twice daily divided schedule of PIXY321 were not yet available, the first five patients randomized to receive PIXY321 were treated with 750  $\mu$ g/m<sup>2</sup> once a day. Both cytokines were continued until patients' ANCs were greater than 5,000/ $\mu$ L and platelet counts were greater than 100,000/ $\mu$ L. No dose reductions of PIXY321 or GM-CSF were permitted, and the cytokines were discontinued for dose-limiting toxicity that we defined as grade 2 allergic reactions or any grade 3 or 4 toxicity believed to be cytokine-related (allergic reactions, arthralgias/myalgias, chills, fever, headache, local skin reactions). Complete blood counts (CBC) with white blood cell differentials were scheduled to be obtained on Mondays, Wednesdays, and Fridays for cycles 1 through 5 on all patients.

**Statistical analysis.** All patients entered on study were assessable for toxicity. Only cycles in which patients received all four chemotherapy agents were included in the analyses of hematologic toxicity. If a patient was taken off of the hematopoietic growth factor due to toxicity, the subsequent cycles of FLAC chemotherapy without the cytokine were also included in the analysis of hemato-

logic toxicity that was an intention-to-treat analysis. Differences in the incidence of nonhematologic toxicities for PIXY321 versus GM-CSF were assessed using an exact version of the Cochran-Armitage test.

For analysis of platelet and neutrophil counts, any cycle that had less than three observations within the 2-week period centered around the nadir was judged to have too little data to be reliable and was omitted from all analyses of the nadirs and the durations of cytopenia. Only in the analysis of the percentage of cycles with recovery of counts by day 22, any cycle that had no observations after day 13 or that had no recovery of counts on or before day 17 and no data after day 17 was omitted for the same reason.

The day on which a patient's count crossed a threshold (eg, ANC less than 500/ $\mu$ L or 1,000/ $\mu$ L or platelet count less than 50,000/ $\mu$ L) was estimated by linear interpolation between the logarithms of the consecutive counts above and below the threshold. Fractional days arising in the calculation of durations were rounded to the nearest full day. When a platelet transfusion produced a transient increase above a threshold, the increased was ignored, but if the increase above the threshold was sustained for the rest of the cycle, then that increase was interpreted as the end of the duration of thrombocytopenia. The distributions of durations are well represented by their means and standard errors, but the distributions of nadirs are highly skewed, and therefore, medians are reported in all calculations of nadirs. For the comparisons between the two arms of the study, the mean duration or the median nadir for each patient was calculated over the assessable cycles among the first five cycles, or among cycles 3 through 5 as indicated, and the distributions of these statistics were tested using the Wilcoxon rank sum test.<sup>16</sup> In cases where some durations were right-censored, the estimate of the mean duration was based on the Kaplan-Meier estimate of the duration distribution. The *P* values reported for individual tests have not been corrected for the large number of tests performed. Because of the correlations between the four outcomes tested, we have estimated that *P* values less than .025 represent significant results.

The recovery of counts by day 22 is correlated across cycles for each patient; that is, recovery is more likely in one cycle if it occurred in an earlier cycle. Nonetheless, the overall proportions of patients with recovery by day 22 are essentially equal in cycles 3, 4, and 5. Therefore, the cycles with recovery were modeled as correlated binary observations with constant correlation between consecutive cycles, maximum likelihood estimation was used to estimate the marginal probability and correlation parameters, and comparisons between study arms were made using the likelihood ratio test. The average delivered dose intensities for the individual drugs were expressed in mg/m<sup>2</sup>/wk, and 21 days were added to the durations of therapy to account for the interval after administration of cycle 5 of FLAC.<sup>17</sup> The potential contribution of leucovorin was not included in the dose intensity analyses, because there was no modification of this drug dose. Objective antitumor responses in stage IV patients with bidimensionally measurable disease (excluding patients with bone-only disease) were determined according to standard criteria.<sup>18</sup>

## RESULTS

**Patient characteristics.** Fifty-three patients were randomized to receive five cycles of FLAC chemotherapy with either PIXY321 (26 patients) or GM-CSF (27 patients). The patient characteristics are listed in Table 1. The patients were well balanced on the two arms of the study for disease stage, number of patients who had received prior adjuvant chemotherapy, or radiation therapy. More patients in the PIXY321 arm had two or more sites of metastatic disease: 8 of 26 versus 3 of 27 patients for PIXY321 and GM-CSF, respec-

**Table 1. Patient Characteristics**

	PIXY321	GM-CSF
No. of patients	26	27
Stage II	8	6
Stage III	3	5
Stage IV	15	16
Median age (yr)	45	46
Stage IV patients		
Prior adjuvant chemotherapy	8	10
Prior radiotherapy	7	6
Bone disease	8	5
No. of disease sites		
0	8	7
1	10	17
2	5	2
≥3	3	1

tively. Eight patients who received PIXY321 had bony metastatic breast cancer versus five with GM-CSF.

In the detailed analyses of hematologic toxicity, 26 of the 27 GM-CSF patients were assessable for cycle 1 of FLAC (one patient died during cycle 1 of sepsis), 26 in cycle 2, 25 in cycle 3, 23 in cycle 4, and 22 in cycle 5. For the 26 patients randomized to PIXY321, 24 were assessable for hematologic toxicity for cycle 1 (one patient received only two doses of PIXY321 because she developed symptoms of cardiac ischemia, and a second patient developed clinically apparent brain metastases during cycle 1 and was taken off PIXY321), 24 for cycle 2, 20 for cycle 3, 20 for cycle 4, and 18 for cycle 5. For patients randomized to PIXY321, one patient developed progressive disease during cycle 1, one patient was removed from study due to noncompliance, one patient refused further therapy after cycle 2 due to poor tolerance of chemotherapy, and the other five patients were taken off PIXY321 due to grade 3 or 4 toxicity during cycle 1 or 2 (one allergic reaction, one renal failure, one prolonged pancytopenia, one cardiac ischemia, one fevers with grade 3 arthralgias). On the GM-CSF arm, one patient died of sepsis in cycle 1, two patients developed progressive disease after cycles 2 and 3, respectively, one patient was removed from study because of a significant drop in her cardiac ejection fraction, and one patient went on to receive high-dose chemotherapy with autologous stem cell rescue after four cycles of FLAC plus GM-CSF.

**Nonhematologic toxicity.** The nonhematologic toxicities associated with FLAC chemotherapy and PIXY321 versus GM-CSF are listed in Table 2. With regard to toxicities that were believed to be cytokine-related, one patient receiving GM-CSF developed transient grade 3 fatigue that did not require stopping the cytokine, and six patients receiving PIXY321 developed dose-limiting toxicities (two patients with grade 3 local skin reactions [erythema, induration, warmth, and pruritus], one patient with a grade 2 allergic reaction, one with grade 3 possible cardiac ischemia, one with grade 3 renal failure [occurring in a dehydrated patient with a history of hypertension, and therefore, renal failure was only possibly related to PIXY321], and one with grade 3 arthralgias). The incidence of grade 2 and 3 local skin

reactions was greater with PIXY321 ( $n = 21$ ) than with GM-CSF ( $n = 6$ ;  $P < .001$ ), and grade 1 chills also developed more commonly in patients who received PIXY321. Overall, six patients on the PIXY321 arm stopped the cytokine due to toxicity compared with none on the GM-CSF arm. No significant differences in the nonhematologic toxicities that were likely related to administration of high-dose chemotherapy were noted on the two arms of the study (Table 2). One patient who was treated with five cycles of FLAC plus PIXY321 developed a secondary acute myelogenous leukemia (French-American-British [FAB] subtype M5) 9 months after completing therapy with cytogenetics consistent with an abnormality of chromosome 11q23.

**Hematologic toxicity.** Of the patients treated with GM-CSF or PIXY321, 96% developed grade 4 neutropenia (ANC less than 500/ $\mu$ L) during the five cycles of FLAC chemotherapy. Of patients treated with GM-CSF, 48% (13 of 27) developed grade 4 thrombocytopenia (platelet count less than 20,000/ $\mu$ L), and 26% (7 of 27) developed grade 3 thrombocytopenia (platelet count less than 50,000/ $\mu$ L). Of patients treated with PIXY321, 50% (13 of 26) developed grade 4 thrombocytopenia, and 31% (8 of 26), grade 3 thrombocytopenia at some time during the five cycles of FLAC chemotherapy.

The hematologic toxicities observed over five cycles of FLAC chemotherapy for patients treated with PIXY321 versus GM-CSF are listed in Table 3. There was no significant difference in the median neutrophil nadirs in patients treated with PIXY321 or GM-CSF over five cycles of FLAC. Although no difference in the mean duration of grade 4 neutropenia was observed for PIXY321 versus GM-CSF, the duration of neutropenia less than 1,000/ $\mu$ L was significantly longer with PIXY321 than with GM-CSF: 8.3 versus 7.0 days, respectively ( $P = .015$ ).

As shown in Table 3, there was no difference in platelet nadirs or duration of platelet nadirs less than 20,000/ $\mu$ L or

**Table 2. Number of Patients With Nonhematologic Toxicities as a Result of FLAC Plus PIXY321 Versus GM-CSF**

Toxicity	PIXY321 (N = 26)				GM-CSF (N = 27)			
	Gr 1	Gr 2	Gr 3	Gr 4	Gr 1	Gr 2	Gr 3	Gr 4
Allergic reactions		1*				2		
Arthralgia/myalgia	7	8	1*		4	8		
Chills	8	1			2	1		
Fatigue	4	18			3	20	1	
Fever	3	18	3		5	13	2	
Headaches	4	2			3	2		
Local skin reaction	5	19	2*		15	6		
Thrombosis		2				1		
Cardiac ischemia			1*					
Renal insufficiency		1	1*					
Diarrhea	2	9	4*		5	8	3*	
Mucositis	2	10	6*		10	10	5*	
Nausea	4	13	6		5	14	5	1
Vomiting	5	13	3	2	5	11	2	
Dysuria/hematuria	3	2			1	1		

Abbreviation: Gr, grade.

\* Dose-limiting toxicity.

50,000/ $\mu\text{L}$  over five cycles of FLAC chemotherapy between patients treated with PIXY321 and those treated with GM-CSF. Also, there was no difference observed in the median hemoglobin nadirs over five cycles of FLAC with PIXY321 versus GM-CSF.

Figure 1A and B depicts the patterns of the median neutrophil nadirs and the mean number of days with the ANC less than 500/ $\mu\text{L}$  for FLAC cycles 1 through 5 for PIXY321 versus GM-CSF. No significant differences were noted in the patterns of the neutrophil nadirs across cycles. There was a trend towards a longer duration of grade 4 neutropenia during cycles 3 through 5 for PIXY321 compared with GM-CSF; however, these comparisons were not statistically significant. A similar pattern was seen for the duration of ANC less than 1,000/ $\mu\text{L}$  and less than 1,500/ $\mu\text{L}$  over cycles 1 through 5, with PIXY321 being associated with a longer duration of neutropenia over cycles 3 through 5 compared with GM-CSF (data not shown).

Figure 2A and B shows the patterns of the median platelet nadirs and the mean number of days with the platelet count less than 50,000/ $\mu\text{L}$  (grade 3) for cycles 1 through 5 for PIXY321 versus GM-CSF. No difference in the patterns of cumulative thrombocytopenia was seen comparing the two cytokines. A longer duration of grade 3 thrombocytopenia was observed during cycles 2 and 3 with PIXY321 compared with GM-CSF; however, these differences were not statistically significant. No significant difference in the duration of the platelet counts less than 20,000/ $\mu\text{L}$  was seen for PIXY321 versus GM-CSF over the five cycles of FLAC chemotherapy (data not shown). Figure 3A and B shows the patterns of the platelet nadirs and recoveries for patients treated with GM-CSF or PIXY321 during cycle 3 of FLAC chemotherapy. It is interesting to note that the platelet counts on day 1 of cycle 3 were somewhat higher in patients who received GM-CSF than in patients treated with PIXY321,

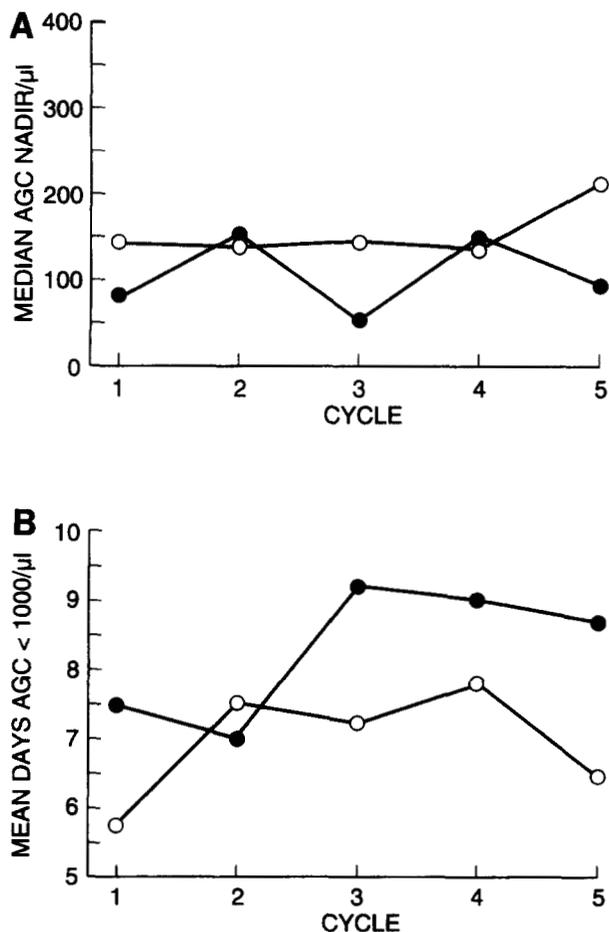


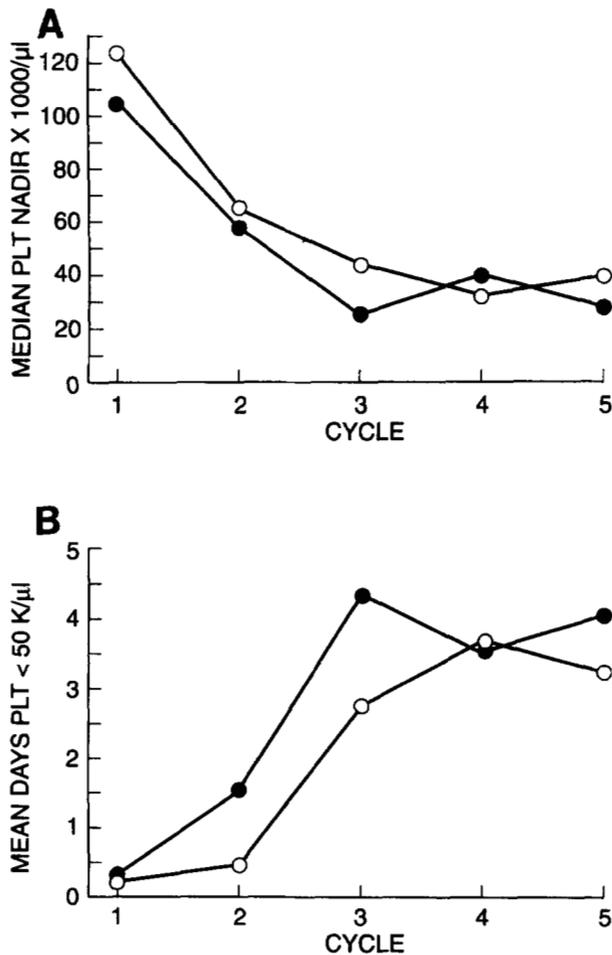
Fig 1. Median neutrophil nadirs (A) and mean duration of the ANC less than 500/ $\mu\text{L}$  (B) over FLAC cycles 1 to 5 for PIXY321 (●) versus GM-CSF (○).

and that the slopes of the platelet recovery curves appear somewhat steeper in many patients who received GM-CSF compared with those treated with PIXY321. Similar patterns of platelet recovery were also seen during cycle 4 of FLAC with PIXY321 versus GM-CSF (data not shown).

Because the cumulative hematologic toxicity observed with high-dose FLAC chemotherapy is most significant in cycles 3, 4, and 5 (see Figs 1 and 2), we analyzed the percentage of cycles in which adequate ANC recovery (ANC  $\geq 1,500/\mu\text{L}$ ) and adequate platelet count recovery (platelet count  $\geq 100,000/\mu\text{L}$ ) occurred by cycle day 22 of cycles 3 through 5, thereby allowing administration of the next cycle of FLAC chemotherapy on time. As seen in Table 3, 92% versus 86% of cycles 3 through 5 had adequate ANC recovery by cycle day 22 for GM-CSF versus PIXY321, respectively. For platelets, 91% versus 82% of cycles 3 through 5 had adequate recovery by cycle day 22 for GM-CSF versus PIXY321, respectively. For GM-CSF and PIXY321, respectively, 90% and 79% of cycles were associated with both adequate neutrophil and platelet recovery by cycle day 22 ( $P = .14$ ). Figure 4 shows the percentage of cycles with adequate recovery of both neutrophils and platelets for cycles

Table 3. Hematologic Toxicity (Cycles 1 to 5)

	PIXY321 (N = 24)	GM-CSF (N = 26)	P
Median ANC nadir ( $\mu\text{L}$ )	102	148	.089
Mean no. of days ANC < 500/ $\mu\text{L}$ ( $\pm$ SEM)	5.6 (0.39)	4.5 (0.41)	.13
Mean no. of days ANC < 1,000/ $\mu\text{L}$ ( $\pm$ SEM)	8.3 (0.4)	7.0 (0.36)	.015
Median platelet nadir ( $\times 1,000/\mu\text{L}$ )	56	48	.98
Mean no. of days platelet count < 20,000/ $\mu\text{L}$ ( $\pm$ SEM)	0.37 (0.13)	0.31 (0.13)	.7
Mean no. of days platelet count < 50,000/ $\mu\text{L}$ ( $\pm$ SEM)	2.5 (0.46)	2.0 (0.33)	.6
% Cycles 3-5 where ANC $\geq 1,500/\mu\text{L}$ by day 22	86% (42/52)	92% (57/63)	.68
% Cycles 3-5 where platelet count $\geq 100,000/\mu\text{L}$ by day 22	82% (46/56)	91% (61/69)	.4
Median hemoglobin nadir (mg/dL)	8.1	8.3	.71

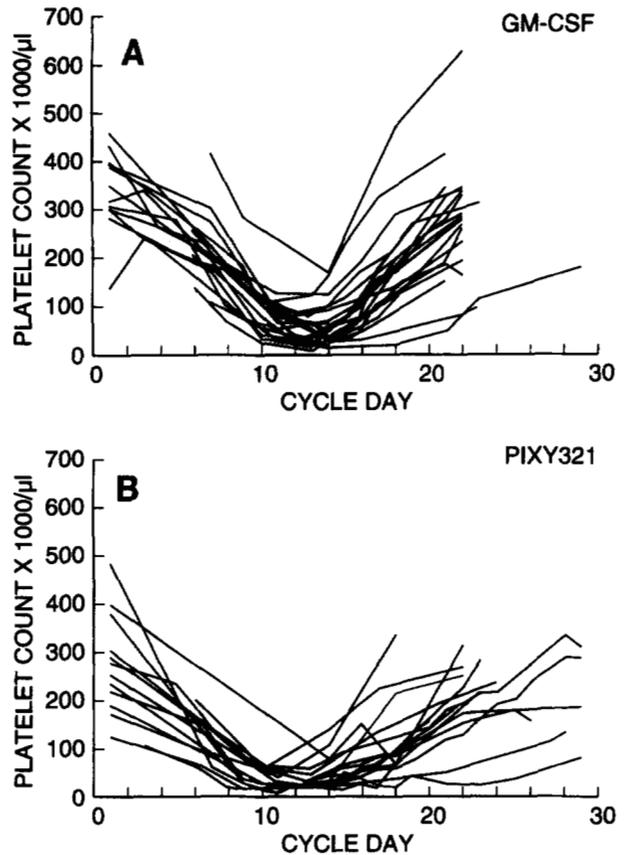


**Fig 2. Median platelet nadirs (A) and mean duration of platelet counts less than 50,000/μL (B) over FLAC cycles 1 to 5 for PIXY321 (●) versus GM-CSF (○).**

1 through 5 for PIXY321 versus GM-CSF. A greater proportion of cycles was associated with adequate hematologic recovery with GM-CSF than with PIXY321, allowing on-time FLAC administration.

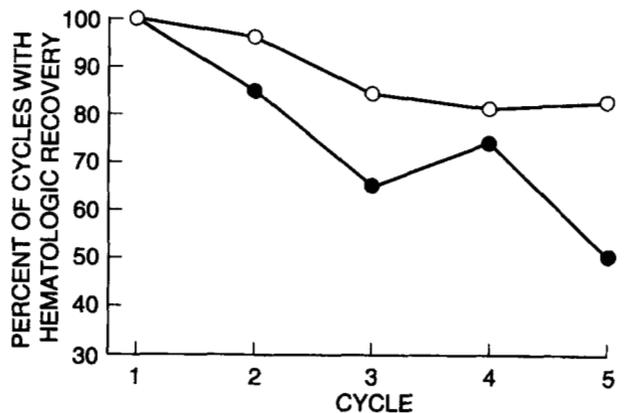
There were no significant differences in the requirements for platelet or red blood cell transfusions for patients treated with PIXY321 versus GM-CSF. In 29% and 28% of all cycles, respectively, PIXY321- versus GM-CSF-treated patients required red blood cell transfusions, and in 13% and 7% of cycles, respectively, PIXY321- versus GM-CSF-treated patients required platelet transfusions. Nine patients who received PIXY321 required an average of 3.2 platelet transfusions versus six patients treated with GM-CSF who required an average of two platelet transfusions over the course of FLAC chemotherapy. In 36% and 20% of cycles, patients treated with PIXY321 versus GM-CSF, respectively, developed a fever greater than 38.5°C associated with an ANC less than 500/μL requiring intravenous antibiotic administration.

The average delivered dose intensities for cyclophosphamide, doxorubicin, and 5-FU over all five cycles of therapy



**Fig 3. Patterns of the platelet nadirs and recoveries for patients treated with GM-CSF (A) or PIXY321 (B) during cycle 3 of FLAC chemotherapy.**

were 435 mg/m<sup>2</sup>/wk (88%), 16 mg/m<sup>2</sup>/wk (94%), and 274 mg/m<sup>2</sup>/wk (91%), respectively for GM-CSF-treated patients compared with 429 mg/m<sup>2</sup>/wk (86%), 15 mg/m<sup>2</sup>/wk (88%), and 253 mg/m<sup>2</sup>/wk (84%), respectively, for PIXY321. Fifteen patients who were treated with GM-CSF and 16 patients



**Fig 4. Percentage of FLAC cycles 1 to 5 with adequate recovery of both neutrophils (ANC ≥ 1,500/μL) and platelets (platelet count ≥ 100,000/μL) for PIXY321 (●) versus GM-CSF (○).**

who received PIXY321 required a dose reduction at some point in their therapy. Of the 15 patients with stage IV disease on both study arms who had bidimensionally measurable disease, 12 patients achieved an objective response. No difference in antitumor activity was seen with FLAC plus PIXY321 versus GM-CSF.

#### DISCUSSION

In this study of dose-intensive FLAC chemotherapy, no significant difference in the incidence of cumulative hematologic toxicity was observed in patients who were randomized to receive PIXY321 versus GM-CSF. Early phase I studies have suggested that PIXY321 could ameliorate thrombocytopenia associated with multiple cycles of chemotherapy. In contrast, no beneficial effects of PIXY321 on platelet toxicity were observed in this randomized study of moderately myelosuppressive FLAC chemotherapy. Although there were more patients randomized to receive PIXY321 who had two or more sites of metastatic disease and who had bony metastatic disease compared with GM-CSF, a somewhat greater number of patients randomized to GM-CSF had received prior adjuvant chemotherapy. It is unlikely that these small degrees of imbalance in factors that can influence bone marrow integrity can explain the overall lack of platelet stimulatory effects of PIXY321 observed in this study. The actual delivered dose intensity of cyclophosphamide, doxorubicin, and 5-FU over five cycles of therapy was somewhat higher in patients treated with GM-CSF, and therefore, the lack of platelet stimulatory effects observed with PIXY321 cannot be explained by an imbalance in the actual doses of drug delivered. The failure to demonstrate an anticipated difference can sometimes be attributed to inadequate numbers of patients in a study. However, this does not appear to be an important consideration in this study, because, if anything, there was a suggestion of greater cumulative neutrophil and platelet toxicity on the PIXY321 study arm.

We have previously shown that treatment of advanced breast cancer with concurrent IL-3 and GM-CSF with FLAC chemotherapy was associated with longer durations of grade 3 thrombocytopenia and delayed platelet recovery by cycle day 22 than treatment with sequential IL-3 and GM-CSF or with GM-CSF alone.<sup>8</sup> The results of the current study with PIXY321 appear to support the idea that concurrent administration of IL-3 and GM-CSF is not effective in ameliorating FLAC-induced myelosuppression.

The results of our study are somewhat surprising when contrasted with other reports of PIXY321 administered together with combination chemotherapy. Vadhan-Raj et al,<sup>11</sup> in the first human trial of PIXY321, showed that 750  $\mu\text{g}/\text{m}^2$  of PIXY321 given in divided doses twice daily was the optimal dose in ameliorating cumulative thrombocytopenia after CyADIC chemotherapy. In this study, the mean platelet nadir after cycle 2 of CyADIC was significantly higher in patients treated with PIXY321 compared with the platelet nadirs in cycle 1 of CyADIC without PIXY321 and with historical control patients who had been treated with GM-CSF. In the present study, no amelioration of platelet toxicity from PIXY321 was observed over five cycles of FLAC chemotherapy. It would be interesting to evaluate the effects of

PIXY321 over multiple cycles of CyADIC chemotherapy to determine whether any beneficial effects of PIXY321 on platelets may be related to the specific chemotherapy regimen being administered. It is possible, for example, that the addition of 5-FU and leucovorin to cyclophosphamide and doxorubicin, drugs common to both regimens, resulted in greater platelet toxicity that could not be overcome by PIXY321.

Two recent preliminary studies suggest that administration of PIXY321 to patients in whom a prolonged period of thrombocytopenia is expected can significantly reduce the duration of severe thrombocytopenia. In pediatric patients with cancer who were treated with high-dose ICE chemotherapy and PIXY321, the duration of the platelet counts less than 20,000/ $\mu\text{L}$  was 4 days compared to 13.5 days for historical controls treated with ICE alone, and 11 days for historical controls treated with GM-CSF.<sup>12</sup> In the second phase I study, administration of  $\geq 750 \mu\text{g}/\text{m}^2$  of PIXY321 after ICE chemotherapy to children with recurrent solid tumors substantially reduced the duration of the platelet counts less than 100,000/ $\mu\text{L}$ .<sup>13</sup> In addition, a study of PIXY321 administration after high-dose chemotherapy and autologous bone marrow transplant has shown a decrease in the time to platelet independence compared with GM-CSF-treated historical controls (17 v 26 days, respectively).<sup>14</sup> These studies suggest that PIXY321 may be effective in ameliorating thrombocytopenia in patients treated with chemotherapy regimens that induce very prolonged periods of severe thrombocytopenia. In contrast, the duration of severe thrombocytopenia observed with administration of FLAC chemotherapy was generally 1 to 2 days in the 50% of patients who developed grade 4 thrombocytopenia.

Treatment with PIXY321 was less well tolerated in patients receiving FLAC chemotherapy than was GM-CSF. Systemic toxicity with chills as well as significant local skin reactions were more common with PIXY321 than with GM-CSF. In addition, other clinically significant toxicities were seen with PIXY321, including one case of reversible acute renal failure in a patient with long-standing hypertension who became dehydrated, one case of possible cardiac ischemia in a patient with substernal chest pain who did not have coronary artery disease found at catheterization, and one allergic reaction with chest tightness and shortness of breath. One patient developed a presumed secondary acute myelogenous leukemia after treatment with FLAC chemotherapy and PIXY321. Secondary leukemias after treatment with high-dose cyclophosphamide and doxorubicin with granulocyte colony-stimulating factor have been previously reported.<sup>19</sup> At present, it is unclear whether the addition of hematopoietic growth factors and/or the higher doses of chemotherapy are contributing to the incidence of secondary leukemias observed in breast cancer patients treated with high-dose chemotherapy.

In conclusion, the addition of PIXY321 to dose-intensive FLAC chemotherapy in the treatment of patients with advanced breast cancer was not superior in ameliorating platelet toxicity compared with GM-CSF. Dose-limiting thrombocytopenia occurred in one half of the patients treated with high-dose FLAC chemotherapy with PIXY321 or GM-CSF.

In addition, PIXY321 was less well-tolerated than GM-CSF. Efforts to develop other thrombopoietic agents to ameliorate the thrombocytopenia associated with multiple cycles of moderately intensive chemotherapy are warranted.

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