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Peripheral Blood Progenitor Cell Cycle Kinetics Following Priming with pIXY321 in Patients Treated with the "ICE" Regimen

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Purpose: Treatment with hematopoietic growth factors increases the percentage of hematopoietic progenitor cells in cell cycle. Following withdrawal of certain growth factors, preclinical data suggest that there is a transient fall in the percentage of progenitor cells in cycle below the baseline, thus providing a window to administer chemotherapy with reduced risk of myelotoxicity.

Patients and Methods: Patients with histologically confirmed, previously untreated neoplasia, were treated with pIXY321 by subcutaneous injection at a dose of 375 μg/m^2 twice daily (total dose 750 μg/m^2/day) for seven days (days -8 to -2), followed by a two-day rest (days -1 to 0). Patients received ICE (ifosfamide, carboplatin and etoposide) on days 1 to 3. On day 4, pIXY321 was resumed until hematologic recovery. Peripheral blood was collected on days -8, -2, - 1, 1, and cell cycle distribution was determined using flow cytometry.

Results: Twenty patients were treated in this study and received a total of 54 cycles. Partial responses were observed in three of 13 patients with non-small cell lung cancer (23 percent) and two of five patients with small cell lung cancer (40 percent). Six of 15 patients had an increased number of cells in S+G_2/M on day 1 of ICE following seven days of pIXY321 and two days off (days -1 to 0). The average increase was 63 percent (range 6-253). Seven patients had a decreased number of cells in S+G_2/M. The average decrease was 55 percent (range 6.3-78). There were no significant differences among the fifteen patients with regards to the observed toxicity of the chemotherapy.

Discussion: pIXY321 in this schedule did not consistently decrease the percentage of cycling progenitor cells in the peripheral blood. Future studies should define whether other growth factors and/or schedules can synchronize progenitor cell cycling and protect the marrow compartment from cycle specific chemotherapy.

INTRODUCTION

Cancer cell resistance to DNA damaging agents can be overcome by increasing the dose intensity [1]. This observation has provided the rationale for the use of high dose chemotherapy combined with growth factors, with or without autologous blood progenitor cell rescue. Studies to date have shown that colony stimulating factors (CSFs)[e] decrease the duration of myelosuppression and its associated morbidity [2, 3]. CSFs

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[e]*Abbreviations:* CSF, colony stimulating factor; G, granulocyte; GM, granulocyte macrophage; ICE, ifosfamide, carboplatin, and etoposide.

reduce the duration of neutropenia both by increasing the percentage of bone marrow progenitor cells in cell cycle and by reducing the duration of the cell cycle. These growth factors may have other effects on progenitor cell cycle kinetics, which could be exploited to further reduce the degree of myelosuppression. Aglietta et al. noted that following completion of a three-day course of GM (granulocyte-macrophage)-CSF, the percentage of bone marrow CFU-GM in S phase was significantly decreased below baseline within 24-72 hours [4]. In a larger group of patients, Vadhan-Raj, Broxmeyer and colleagues confirmed these findings for GM-CSF and reported similar results with pIXY321, but not with G-CSF [5-7]. These observations suggested that a priming schedule of certain growth factors, coupled with the appropriate rest period before the administration of chemotherapy, might reduce the percentage of marrow progenitor cells in cycle at the time the chemotherapy is given and thus render the bone marrow less susceptible to chemotherapy induced toxicity.

pIXY 321 is a recombinant protein that contains both IL-3 and GM-CSF domains [5, 8-10]. pIXY 321 produced significantly enhanced biological effect *in vitro* on both myelo- and thrombopoiesis, perhaps by multiple cross linking of GM-CSF, IL-3 and dual receptors [11, 12]. In clinical trials, pIXY 321 has been well tolerated with the most common reported side-effect being erythema at the injection site and mild constitutional symptoms. There appears to be a dose response effect at doses less than 1000 $\mu g/m^2$/day [13]. Some studies have shown a biological effect at a dose of 125 $\mu g/m^2$/day, though in others a dose of 500-750 $\mu g/m^2$/day was necessary. The optimal biologic dose appears to be 375 $\mu g/m^2$/day administered twice daily [13].

The combination of the DNA damaging agents ifosfamide, etoposide and a platinum analog ("ICE") has demonstrated significant activity in lung cancer, germ cell tumors, lymphomas and pediatric malignancies [14-20]. The major dose limiting toxicity of this drug combination is myelosuppression, and significant dose escalation is feasible with the use of bone marrow rescue or the addition of cytokines. For example, Tepler and colleagues found that a 25 percent increase in the dose intensity of carboplatin was feasible when G-CSF was incorporated into the ICE regimen [19]. Furthermore, they reported that when IL-3 was given in combination with G-CSF following this chemotherapy regimen, the degree and duration of the myelosuppression was further reduced, suggesting that there was synergy between G- or GM-CSF and IL-3.

Based on these data, we designed a study to measure the effects of a priming schedule of pIXY321 on hematopoietic progenitor cell kinetics and assess whether priming reduced the myelotoxicity anticipated with ICE chemotherapy.

PATIENTS AND METHODS

Eligibility

Patients with biopsy-proven, previously untreated solid tumors were eligible to participate in this study. All patients were at or over 18 years of age, were not pregnant, had an ECOG performance status ≤ 2, had adequate bone marrow function (defined as an absolute neutrophil count $\geq 1500/\mu l$ and platelets $\geq 100{,}000/\mu l$), had adequate hepatic and renal function (defined as a total bilirubin ≤ 1.5 mg/dl and a creatine clearance ≥ 60 ml/min) and measurable or evaluable disease. Patients excluded from enrollment included those with a serious intercurrent medical illness, a prior diagnosis of an invasive malignancy (unless potentially curative treatment had been rendered and the disease-free interval exceeded five years), patients with an active infection and patients with an anticipated life expectancy of <2 months. Informed, written consent was obtained according to Federal and Institutional guidelines.

Dosages and drug administration

The pIXY321 was administered at a dose of 375 $\mu g/m^2$ given subcutaneously twice daily. The priming dose was delivered over seven days followed by a two-day rest before the start of the chemotherapy. The pIXY321 was resumed at the same dose 24 hours after the completion of chemotherapy (day 4) and was continued for 10 consecutive days or until the WBC exceeded 15,000/μl and the platelet count exceeded 100,000/μl.

Premedication for the chemotherapy consisted of 20 mg of ondansetron or 0.1 mg/kg of granisetron admixed with 10 mg of dexamethasone. The antiemetics were repeated every eight to 12 hours as needed. Mesna at a dose of 400 mg/m^2 was infused just before and then four, eight and 12 hours after the ifosfamide. The final two doses of mesna were sometimes given orally by diluting 800 mg/m^2/dose of mesna in juice. Ifosfamide at a dose of 2 $gm/m^2/d$, and etoposide at a dose of 75 $mg/m^2/d$, were given intravenously on days 1 to 3 of each chemotherapy cycle. Carboplatin was administered at a dose of 400 mg/m^2 on day 1 only of each cycle.

Chemotherapy was repeated every three weeks provided that there was hematologic recovery (absolute neutrophil count >1500/μl and platelet >100,000/μl), complete resolution of mucositis and diarrhea, no evidence of gross hematuria, and no other serious non-hematologic toxicity.

The doses of all chemotherapy drugs were calculated according to the patient's actual body weight, unless the body weight was more than 30 percent of the ideal weight. In this situation, the ideal weight plus 30 percent was used to calculate doses. Dose reduction of 25 percent was permitted on subsequent chemotherapy cycles for grade 3 non-hematologic toxicities, neutropenic fever with documented infection and a nadir platelet count of <20,000/μl.

pIXY321 was supplied by the Immunex Corp (Seattle, WA) in 1.5 mg vials and was reconstituted in 1 ml of bacteriostatic water. For some patients who experienced injection site reactions, 1 mg of hydrocortisone sodium phosphate was injected into the pIXY321 vial before reconstitution.

On-study examinations and criteria for assessment of response and toxicity

A history including assessment of performance status, physical examination, a complete blood count, electrolytes, BUN, creatinine, creatinine clearance, liver function tests (aspartate aminotransferase, lactate dehydrogenase, total and direct bilirubin, alkaline phosphatase), serum calcium and phosphate, total protein, albumin, prothrombin time, urinalysis, pregnancy test (if indicated), chest radiograph and computed tomography scans (if appropriate for staging the disease) were obtained prior to the start of treatment. A physical examination and toxicity assessment were repeated weekly. Toxicity was graded according to the National Cancer Institute common toxicity criteria. A complete blood count and differential were obtained every two to three days while the patients were receiving the pIXY321. The complete blood count and a chemistry profile was repeated before every treatment cycle. Formal tumor measurements were obtained after every two cycles of chemotherapy. Response duration was measured from the time of first documented response until progression. Survival was measured from the time of entry on study and estimated using the Kaplan-Meier method.

Analysis of hematopoietic progenitor cell kinetics

On days -8 and -2 (the start and completion of the priming schedule of pIXY321) and day 1 of chemotherapy during first and second cycles, 15 ml of blood was obtained in anticoagulated tubes. Blood samples from patients treated at the Cancer Institute of New

Jersey and at affiliated hospitals were shipped by overnight courier mail. Mononuclear cells were isolated from the blood samples by centrifugation through a ficoll-Hypaque density gradient. The mononuclear cells were washed, treated with RNase A, incubated with a fluorocein-conjugated anti-CD34 monoclonal antibody and then stained with propidium iodide. The samples were analyzed in a flow cytometer, and the cell cycle distribution in the CD34 cell population was determined.

RESULTS

Twenty patients were entered into the study and received a total of 54 cycles (Table 1). Four patients experienced allergic reactions from pIXY321 and were removed from the study prior to the administration of chemotherapy. An additional two patients withdrew following the first cycle of chemotherapy due to unacceptable side effects (1) or personal reasons (1). These two patients were considered evaluable for both toxicity and response.

Response and survival

Among the 11 patients with non-small cell lung cancer, one patient had a partial response, which lasted six months. Six patients had stable disease lasting five to seven months. Among the four evaluable patients with small cell lung cancer, there were two partial responses lasting five months each. One patient with poorly-differentiated carcinoma had stable disease lasting 11 months. The median survival for the 20 patients was 15 months. Seventy-one percent of the patients were alive at one year. No difference in survival was noted between responders and non-responders.

Hematologic toxicity

Neutropenia was the most prominent toxicity. Severe (grade 3-4) neutropenia was noted during the first cycle in 42 percent of the patients, and four patients required hospitalization for neutropenic fever. Myelosuppression during subsequent treatment cycles was common. Dose modifications were required in 30 percent of the patients by cycle 3, 63 percent of the patients during cycle 4 and in all three of the patients who received five or

Table 1. Patient characteristics.

Patients	20
Sex	
Female	11
Male	9
Age, years	
Median	51.5
Range	33-68
ECOG performance status	
0	13
1	6
2	1
Tumor histology/stage	
Non-small-cell lung cancer	
Stage III	2
Stage IV	11
Small-cell lung cancer	
Extensive disease	4
Local disease	1
PDC (Unknown primary site)	2

Table 2. Hematologic toxicities.

Cy	No. of patients treated	Patients dose modified no. (percent)	Patients transfused with platelets no. (percent)	Patients transfused with red blood cells no. (percent)
1	16	0 (0)	2 (13)	0 (0)
2	15	0 (0)	2 (13)	1 (7)
3	10	3 (30)	5 (50)	4 (40)
4	8	5 (63)	0 (0)	2 (25)
5	3	3 (100)	1 (33)	1 (33)
6	2	2 (100)	0 (0)	0 (0)

more cycles, indicating that the myelosuppression was cumulative. Treatment was discontinued in one patient due to prolonged neutropenia following the fourth cycle of therapy. Thrombocytopenia occurred frequently, and platelet transfusions were required in 19 percent of the treatment cycles. Severe anemia occurred in 7 percent of the patients during cycle 2, and 30 percent of the patients in cycle 3, and red blood cell transfusions were frequently required (Table 2).

Non-hematologic toxicities

The most common non-hematological toxicities were mild fatigue and weight loss, which were observed in 11 of 19 patients. Two patients developed mental status changes during chemotherapy, which were attributed to the ifosfamide, and the patients had chemotherapy discontinued after three cycles and two cycles, respectively. Gastrointestinal side effects were uncommon, and consisted of mild stomatitis (1) and diarrhea (2).

Local erythema and edema at the site of the pIXY injection was seen in seven patients. Four of these patients experienced grade 2 erythema/edema at the site of injection, and an additional patient experienced grade 2 nausea/vomiting after the pIXY321 was administered. In two patients, hydrocortisone was mixed with the pIXY321 before injection, and this reduced the severity of the local irritation. In an additional four patients, however, allergic reactions were severe and precluded further participation in the study. Three of these patients developed symptoms following the first or second injection of pIXY321 consisting of sneezing, tongue swelling, difficulty swallowing, facial flushing, drooling, chest pressure and dyspnea. The fourth patient experienced respiratory distress and flushing following the first cycle of therapy.

Three thrombotic events occurred in patients in this study. One patient experienced a myocardial infarction and cardiac arrest after the second cycle of chemotherapy. This patient had no prior anginal syndrome and had a hemoglobin of 8.9 g/dl at the time of the infarct. Two patients had documented deep venous thromboses. All three of these patients had carcinoma of the lung and may have had activation of coagulation pathways prior to the initiation of therapy.

Progenitor cell cycle kinetics

In five patients, there were insufficient numbers of progenitor cells detected in the peripheral blood to determine cell cycle distribution at baseline. In the fifteen patients in whom flow cytometric data are available, the average percentage of peripheral cells in $S+G_2/M$ was 9.6 percent. As shown in Figure 1, six patients had an increased percentage of cells in $S+G_2/M$ on day 1 of ICE following seven days of pIXY321 and two days off (days -8 to 1). The average increase was 63 percent (range 6-253 percent). Seven patients

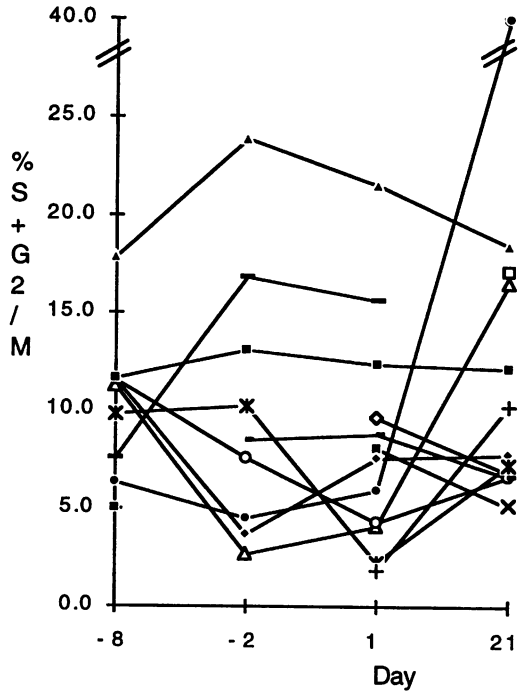

Figure 1. Cell cycle kinetics in peripheral CD34+ progenitor cells. Peripheral blood was sampled and cell cycle distribution analyzed during priming with pIXY321 as described in the Methods. Each line represents data from an individual patient.

Table 3. Bone marrow progenitor cell kinetics following growth factor priming.

Priming schedule[a] (CSF/days on/days off)	Assay method[b]	Pts (no.)	Percent progenitors in S phase: Baseline	End Prime	2-4d off	Ref.
GM-CSF/3d/1-4 d	[^{3}H]Tdr Suicide	7	19	32	13[d]	[4]
GM-CSF/14d/1-4d	[^{3}H]Tdr Suicide	14	18	55	8	[7]
GM-CSF/10d/1-7d	BUDR LI	5	4.7	8.2	7.4	[32]
GM-CSF/7d/1-4d	BUDR LI	9	12.8	20.2	5.2	[25]
G-CSF/7d/1-4d	BUDR LI	9	4.9	19.6	20	[25]
G-CSF/8d/2-4d	[^{3}H]Tdr Suicide	6	27	51	43	[6]
pIXY321/14d/2d	[^{3}H]Tdr Suicide	15	36	62	15[d]	[5]
pIXY321/7d/2d	PI stain CD34+ PB	17	10.4	9.4	8.1	[e]

CSF = colony stimulating factor; PI = propidium iodide; PB = peripheral blood; BUDR LI = bromodeoxyuridine labeling index.

[a]Days on = no. of days priming growth factor given; days off = no. of days before start of chemo.

[b]Marrow was obtained at baseline, at the end of priming schedule, and then two to four days after the last dose of growth factor

[c]DNA synthesis was determined by a [^{3}H]Tdr suicide assay in CFU-GM or by *in vivo* bromodeoxyuridine labeling index.

[d]$p < .05$ compared with data from baseline.

[e]This study.

had a decreased percentage of cells in S+G_2/M. The average decrease was 55 percent (range 6.3-78 percent). Nine patients also had flow cytometric data available on day -2 (at the end of priming). In five of these patients, the percentage of peripheral cells in S+G_2/M was decreased on day 1 of ICE compared to day -2. The mean decrease was 21 percent, and the median was 6.1 percent. In the four patients in whom there was an increase in the percentage of peripheral cells in S+G_2/M on day 1 of ICE, the average increase was 47 percent, with the median being 52 percent. No correlation between number or percentage of progenitor cells in S phase on day 1, and subsequent myelosuppression or transfusion requirement was identified.

DISCUSSION

Although most clinical trials have reported that pIXY321 is well tolerated, the frequency of certain non-hematologic toxicities in this study was notable. Four patients (20 percent) had allergic reactions severe enough to preclude continued treatment on the study. This is higher than the incidence of severe allergic reactions reported in other studies of pIXY321 and much higher than the incidence observed with either GM-CSF or G-CSF. Three of the 20 patients experienced thrombotic events. Proposed mechanisms by which these cytokines may contribute to hypercoagulability include induction of adhesion molecule expression on neutrophils and enhancement of platelet aggregation [21-23]. In a recent meta-analysis, the incidence of thrombotic events among patients receiving GM-CSF was 6.6 percent compared with 3.6 percent in controls, with an odds ratio of 1.67 ($p > .05$) [24]. An unexpectedly high incidence of thrombotic events has not been previously reported with pIXY321 and may reflect selection in this small patient cohort. If pIXY321 is further developed, however, additional investigation into its thrombogenic potential should be performed.

Some previous studies have suggested that certain cytokines given prior to the administration of chemotherapy decrease the percentage of progenitor cells in cycle, thus making the bone marrow protentially more resistant to the myelosuppressive effects of chemotherapy (Table 3). For example, an Italian group found differences in the kinetic effects induced by GM- and G-CSF [25]. They reported that the bromodeoxyuridine (BUDR) labeling index in CD34+ marrow cells fell significantly below baseline two days after stopping GM-CSF but was still elevated two to four days after stopping G-CSF. Differences in these observed kinetic effects may be related to the population of cells expanded by a particular growth factor. Expansion of more primitive cell compartments might be necessary to affect feedback loops regulating hematopoiesis [26] and may contribute to the variable results summarized in Table 4. In addition to the growth factor and the schedule, the results observed in Table 4 may also be related to biologic differences between CD34+ progenitor cells obtained in the peripheral blood and CD34+ progenitor cells residing in the marrow [27]. Although Paccagnella et al. reported that priming produced comparable results on the kinetics of these two cell populations [28], in a limited number of samples using different assay techniques we found poor agreement between the priming effects on marrow and peripheral blood progenitor cells. Future studies should analyze marrow samples until effective priming schedules are defined and subsequently correlated with kinetics in peripheral progenitor cells.

Evaluation of a priming schedule of growth factor administration as a means of reducing myelotoxicity has been clinically tested in a limited number of patients. Aglietta et al. demonstrated reduced myelotoxicity in a small group of patients randomized to treatment with a priming schedule of GM-CSF [29]. In this study, none of the patients received growth factor support following chemotherapy. Janick et al. treated 24 evaluable patients

Table 4. Impact of growth factor priming on subsequent myelosuppresion.

Priming schedule CSF/days on/days off	Chemo regimen	Patients (no.)[b]	Results	Ref.
GM-CSF/10d/7d	P/E/E	5/42	Compared to patients treated with only post-chemo GM-CSF, no further reduction in myelotoxicity noted in patients receiving priming.	[28]
GM-CSF/11d/3d	C/E/Ctx	18	Compared to patients treated only with GM-CSF, less myelotoxicity if PBSC infused.	[33]
+/- GM-CSF/3d/4d (Randomized)	CEF	6/12	No post-chemo GM-CSF given. Significantly higher ANC and less treatment delays in primed group.	[29]
+/- GM-CSF/5d/2d (Randomized)	VP-16, p.o. 21d schedule	9/27	Priming increased myelosuppresion (not significant) c/w no GM-CSF support.	[34]
+/- GM-CSF/5d/3d (Randomized, all patients post-chemo G-CSF)	HDCEP	36/72	Priming reduced PBSC yield without reducing myelotoxicity	[31]
+/- GM-CSF/5d/1d (Randomized)	Topotecan	14/27	Priming reduced gr 4 neutropenia in cycles 1 and 2.	[30]
pIXY321/14d/7d	CyADIC	23	At low doses of pIXY321 (25-250 $\mu g/m^2/d$), no difference between prime only (cycle 1) vs. post-chemo pIXY (cycle 2). At doses of 500-1000 $\mu g/m^2/d$, reduced myelotoxicity in cycle 2.	[13]
G-CSF/8d/2d	Melphalan	4/16	No difference in myelosuppression c/w patients treated with prime.	[35]
G-CSF/5d/2d	Topotecan/ CDDP	5/35	No difference in myelosuppression c/w 6 patients treated at same chemo doses without prime.	[36]

CEF/CMF = cyclophosphamide, epirubicin, 5-fluorouacil alternating with cyclophosphamide, methotrexate, 5-fluorouacil; C/E/Ctx = carboplatin, etoposide, cyclophosphamide; P/E/E = cisplatin, epirubicin, etoposide; CyADIC = cyclophosphamide, doxorubicin, dacarbazine; HDCE = high-dose cyclophosphamide, etoposide, cisplatin; PBSC = peripheral blood stem cells; CDD = cisplatin.

[a]Days on = no. of days priming growth factor given; days off = no. of days before start of chemo.

[b]Patients receiving priming schedule/total patients treated in study.

with topotecan followed by seven days of GM-CSF [30]. Half of the patients were randomized to also receive a five-day priming course of GM-CSF, which was completed 24 hours before the start of chemotherapy. A significant decrease in the number of patients experiencing grade 4 neutropenia (27 percent vs. 77 percent, p = .02) was noted in the patients treated with the priming schedule. In contrast, Schwartzberg et al. found no advantage with a five-day GM-CSF priming schedule when used in combination with a dose-intensive regimen containing cyclophosphamide, cisplatin, and etoposide [31].

The clinical efficacy observed by Janik et al. may be in part related to the use of a cycle-specific chemotherapy regimen. In a murine model, de Haan et al. recently demonstrated that the combination of stem cell factor and IL-11 given for seven days followed by a one day rest before cycle-specific chemotherapy (5-fluorouracil) reduced the percentage of progenitor cells in cycle at the time of chemotherapy, and protected the animals from treatment-related toxicity [26]. In contrast to alkylating drugs, the cytotoxicity of the camptothecins is highly dependent on cells transversing the cell cycle. Since the dose limiting toxicity of this class of agents is myelosuppression, they are uniquely suited for evaluation of priming schedules designed to reduce the percentage of marrow progenitor cells in cycle. Therefore, future studies that test priming schedules of cytokines such as GM-CSF should be designed utilizing cycle-specific agents such as the camptothecins.

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REFERENCES

1. Skipper, H. Data and analysis having to do with the influence of dose intensity and duration of treatments single drugs and combinations) on lethal toxicity and on the therapeutic response of experimental neoplasms. Southern Research Institute, 1987.
2. Laporte, J.P., Fouillard, L., Douay, L., Eugene-Jolchine, I., Isnard, F., Stachowiak, J., Najman, A., and Gorin, N.C. GM-CSF instead of autologous bone-marrow transplantation after BEAM regimen. Lancet 338:601-602, 1991.
3. Crawford, J., Ozer, H., Stoller, R., Johnson, D., Lyman, G., Tabbara, I., Kris, M., Grous, J., Picozzi, V., Rausch, G., Smith, R., Gradishar, W., Yahanda, A., Vincent, M., Stewart, M., and Glaspy, J. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small cell lung cancer. N. Engl. J. Med. 325:164-70, 1991.
4. Aglietta, M., Piacibello, W., Sanavio, F., Stacchini, A., Apra, F., Schena, M., Mossetti, C., Carnino, F., Caligaris-Cappio, F., and Gavosto, F. Kinetics of human hematopoietic cells after *in vivo* administration of granulocyte-macrophage colony-stimulating factor. J. Clin. Invest. 83: 551-557, 1989.
5. Broxmeyer, H., Benninger, L., Cooper, S., Hague, N., Benjamin, R., and 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. 23: 335-340, 1995.
6. Broxmeyer, H., Benninger, L., Patel, S., Benjamin, R.S., and Vadhan-Raj, S. Kinetic response of human marrow myeloid progenitor cells to *in-vivo* treatment of patients with granulocyte colony-stimulating factor is different from the response to treatment with granulocyte macrophage colony-stimulating factor. Exp. Hematol. 22:100-102, 1994.
7. Vadhan-Raj, S., Broxmeyer, H.E., Hittleman, W.N., Papadopoulos, N.E., Chawla, S.P., Fenoglio, C., Cooper, S., Buescher, E.S., Frenck, R.W.J., Holian, A., Perkins, R.C., Scheule, R.K., Gutterman, J.U., Salem, P., and Benjamin, R.S. Abrogating chemotherapy induced myelosuppression by recombinant granulocyte-macrophage, colony-stimulating factor in patients with sarcoma: protection at the progenitor cell level. J. Clin. Oncol. 10:1266-1277, 1992.
8. Lapidot, T., Pflumio, F., Doedemo, M., Murdoch, B., Williams, D.E., and Dick, J.E. Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. Science 255:1137-1141, 1992.
9. Runowicz, C.D., Mandeli, J., Speyer, J., Wadler, S., Hochster, H., Garrison, L., and Holland, J.F. Phase I/II study of PIXY321 in combination with cyclophosphamide (CTX) and carboplatin in the treatment of ovarian cancer, Am. J. Obstetric. Gynecol. 174:1151-1159, 1996.

10. Taylor, C., Modiano, M., Garrison, L., List, A., Alberts, D., Hersh, E., and Taetle, R. PIXY plus carboplatin (C) and adriamycin (A) in patients with advanced gastrointestinal (G) malignancy. Proc. Amer. Soc. Clin. Oncol. 12:195, 1993. Abstract 569.
11. Williams, D. and Park, L. Hematopoietic effects of a granulocyte-macrophage colony stimulating factor/interleukin-3 fusion protein. Cancer 67 (suppl 10): 2705-2707, 1991.
12. Curtis, B., Williams, D., Broxmeyer, H., Dunn, J., Farrah, I., Jeffrey, E., Clevenger, W., de Roos, L., Martin, U., Friend, D., Craig, V., Gayle, R., Price, V., Cosman, D., March, C., and Park, L. Enhanced hematopoietic activity of a human granulocyte/macrophage colony-stimulating factor-interleukin 3 fusion protein. Proc. Natl. Acad. Sci. USA. 88:5809-5813, 1991.
13. Vadhan-Raj, S., Papadopoulis, N.E., Burgess, M.A., Linke, K.A., Patel, S.R., Hays, C., Arcenas, A., Plager, C., Kudelka, A.P., Hittelman, W.N., Broxmeyer, H.E., Williams, D.E., Garrison, L., and Benjamin, R.S. 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. 12:715-724, 1994.
14. Marina, N.M., Rodman, J., Shema, S.J., Bowman, L.C., Douglass, E., Furman, W., Santana, V. M., Hudson, M., Wilimas, J., and Meyer, W. Phase I study of escalating targeted doses of carboplatin combined with ifosfamide and etoposide in children with relapsed solid tumors. J. Clin. Oncol. 11:554-560, 1993.
15. Ettinger, D. Overview of ifosfamide in small cell lung cancer. Semin. Oncol. 19(suppl 1):59-67, 1992.
16. Wilson, W., Jain, V., Bryant, G., Cowan, K.H., Carter, C., Cottler-Fox, M., Goldspiel, B., Steinberg, S.M., Longo, D.L., and Wittes, R.E. Phase I and II study of high-dose ifosfamide, carboplatin, and etoposide with autologous bone marrow rescue in lymphoid and solid tumors, J. Clin. Oncol. 10:1712-1722, 1992.
17. Loehrer, P., Einhorn, L., and Williams, S. VP-16 plus ifosfamide plus cisplatin as salvage therapy in refractory germ cell cancer. J. Clin. Oncol. 4:528-536, 1986.
18. Le Cesne, A., Antoine, E., Spielmann, M., Le Chevalier, T., Brain, E., Toussaint, C., Janin, N., Kayitalire, L., Fontaine, F., and Genin, J. High dose ifosfamide: circumvention of resistance to standard-dose ifosfamide in advanced soft tissue sarcomas. J. Clin. Oncol. 13:1600-1608, 1995.
19. Tepler, I., Hamm, J.T., Shulman, L., Ritch, P., Nemunaitis, J., Fitch, T., Young, D.C., Felser, J., Resta, D., Gaynes, L., Millenson, M., Strauss, G., Lynch, T., Skarin, A., Schnipper, L.E., and Elias, A.D. Combination cytokine therapy with recombinant human interleukin-3 (IL-3) and granulocyte colony stimulating factor (G-CSF) after "ICE" chemotherapy for lung cancer: sequential and simultaneous schedules. Proc. Amer. Soc. Clin. Oncol. 12:333, 1993. Abstract 1110.
20. Krigel, R., Palackdharry, C., Padavick, K., Haas, N., Kilpatrick, D., Langer, C., and Comis, R. Ifosfamide, carboplatin, and etoposide plus granulocyte-macrophage colony-stimulating factor: a phase I study with apparent activity in non-small cell lung cancer. J. Clin. Oncol. 12:1251-1258, 1994.
21. Gasson, J., Weisbart, R., Kaufman, S., Clark, S., and Golde, D. Purified human granulocyte-macrophage colony-stimulating factor and platelet aggregation. Science 226:1339-1342, 1984.
22. Shimoda, K., Okamura, S., Inaba, S., Okamura, T., Ohga, S., Ueda, K., and Niho, Y. Granulocyte colony-stimulating factor and platelet aggregation. Lancet 371:633, 1993.
23. Arnaout, M., Wang, E., Clark, S., and Sieff, C. Human recombinant granulocyte macrophage colony-stimulating factor increases cell-to-cell adhesion and surface expression of adhesion-promoting surface glycoproteins as mature granulocyte. J. Clin. Invest. 78:597-601, 1986.
24. Barbui, T., Finazzi, G., Grassi, A., and Marchioli, R. Thrombosis in cancer patients treated with hematopoietic growth factors: a meta-analysis. Thromb. Haemost. 75:368-371, 1996.
25. Danova, M., DeRenzis, R., Rosti, V., Mazzini, G., Cazzola, M., and Riccardi, A. Cell kinetics of hematopoietic progenitor cells following chemotherapy (CT) plus colony-stimulating factors (CSF's) in advanced breast cancer. Proc. Amer. Assoc. Cancer Res. 36:221, 1995. Abstract 1317.
26. de Haan, G., Dontje, B., Engel, C., Loeffler, M., and Nijhof, W. Prophylactic pretreatment of mice with hematopoietic growth factors induces expansion of primitive cell compartments and results in protection against 5-fluorouracil-induced toxicity. Blood 87:4581-4588, 1996.
27. Roberts, A. and Metcalf, D. Noncycling state of peripheral blood progenitor cells mobilized by granulocyte colony-stimulating factor and other cytokines. Blood 86:1600-1605, 1995.
28. Paccagnella, A., Favaretto, A., Riccardi, A., Danova, M., Ghiotto, C., Giordano, M., Pappagallo, G., Comis, S., Panozzo, M., and Chieco-Bianchi, L. Granulocyte-macrophage colony-stimulating factor increases dose intensity of chemotherapy in small cell lung cancer. Cancer 72:697-706, 1993.

29. Aglietta, M., Monzeglo, C., Pasquino, P., Carnino, F., Stern, A.C., and Gavosto, F. Short-term administration of granulocyte-macrophage colony stimulating factor decreases hematopoietic toxicity of cytostatic drugs. Cancer 72:2970-2973, 1993.
30. Janik, J., Miller, L., Smith, J., Kopp, W., Alvord, G., Gause, B., Curti, B., Urba, W.J., and Longo, D.L. Prechemotherapy granulocyte-macrophage colony-stimulating factor (GM-CSF) prevents topotecan-induced neutropenia. Proc. Amer. Soc. Clin. Oncol. 12:437, 1993. Abstract 1507.
31. Schwartzberg, L., West, W., Birch, R., Heffernan, M., Tauer, K., Kalman, L., Middleman, E., Pendergrass, K., and Leff, R. Randomized prospective trial +/- pretreatment with GM-CSF prior to high-dose cyclophosphamide, etoposide, and cisplatin +G-CSF. Proc. Amer. Soc. Clin. Oncol. 12:452, 1993. Abstract 1568.
32. Riccardi, A., Danova, M., Paccagnela, A., Giordano, M., Favaretto, A., Panozzo, M., Ghiotto, C., Comis, S., Fiorentino, M., and Chieco-Bianchi, L. Bone marrow myeloid cell kinetics during treatment of small cell carcinoma of the lung with chemotherapy not associated and associated with granulocyte-macrophage colony-stimulating factor. Ann. Hematol., 1993.
33. Kritz, A., Crown, J., Motzer, R., Reich, L.M., Heller, G., Moore, M.P., Hamilton, N., Yao, T.J., Heelan, R.T., and Schneider, J.G. Beneficial impact of peripheral blood progenitor cells in patients with metastatic breast cancer treated with high-dose chemotherapy plus granulocyte-macrophage colony-stimulating factor. Cancer 71:2515-2521, 1993.
34. Schaffer, D., Smith, L., Burris, H., Clark, G., Eckhardt, J., Fields, S., Weiss, G., Rinaldi, D., Bowen, K., Kuhn, J., and Von Hoff, D. A randomized phase I trial of chronic oral etoposide with or without granulocyte-macrophage colony-stimulating factor in patients with advanced malignancies. Cancer Res. 53:5929-5933, 1993.
35. Morstyn, G., Campbell, L., Lieschke, G., Layton, J.E., Maher, D., O'Connor, M., Green, M., Sheridan, W., Vincent, M., and Alton, K. Treatment of chemotherapy-induced neutropenia by subcutaneously administered granulocyte colony-stimulating factor with optimization of dose and duration of therapy. J Clin Oncol. 7:1554-1562, 1989.
36. Miller, A., Hargis, J., Lilenbaum, R., Fields, S., Rosner, G., and Schilsky, R. Phase I study of topotecan and cisplatin in patients with advanced solid tumors: a Cancer and Leukemia Group B study. J. Clin. Oncol. 12: 2743-2750, 1994.