Effects of PIXY321, a Granulocyte-Macrophage Colony-stimulating Factor/Interleukin 3 Fusion Protein, on Human Tumor Colony-forming Units Taken Directly from Patients

Miguel A. Izquierdo, Donna Degen, Eric Raymond, Dania Caron, Veronica Ortiz, Peter Banks, and Daniel D. Von Hoff

Cancer Therapy and Research Center of South Texas, Institute for Drug Development, San Antonio, Texas 78229 [M. A. I., D. D. E. R., V. O., D. D. V. H.]; Department of Pathology, University of Texas Health Science Center, San Antonio, Texas [P. B.], and Immunex Corporation, Seattle, Washington [D. C.]

INTRODUCTION

GM-CSF and IL-3 stimulate the proliferation, differentiation, and have synergistic effects in multipotent and lineage-committed hematopoietic stem cells (1–4). Recently, Curtis et al. (5) created a GM-CSF/IL-3 fusion protein, named PIXY321, by constructing a plasmid in which the coding regions of human GM-CSF and IL-3 cDNAs were connected by a synthetic linker sequence. Although GM-CSF and IL-3 share the same receptor β subunit (6), PIXY321 has shown greater receptor affinity and biological activity than either GM-CSF or IL-3 alone in preclinical models (5, 7–9), and it accelerated hematological recovery after chemotherapy in several clinical trials (10).

ABSTRACT

PIXY321, a granulocyte-macrophage colony-stimulating factor/interleukin 3 (GM-CSF/IL-3) genetically engineered hybrid, has shown greater biological activity in stimulating committed myeloid progenitors than either GM-CSF or IL-3 in vitro, in vivo, and in patients treated with high-dose chemotherapy. However, one concern is that PIXY321 may stimulate the proliferation of malignant cells which have functional GM-CSF or IL-3 receptors. Therefore, using a human tumor cloning assay, we have tested the effects of several concentrations of PIXY321 ranging from 0.1 to 100 ng/ml on tumor cells taken directly from 98 patients with solid tumors and Hodgkin’s or non-Hodgkin’s lymphomas. Of the 34 evaluable specimens, including 15 breast cancers, 5 ovarian cancers, 5 lung cancers, and 9 lymphomas, none showed stimulation of tumor growth. Interestingly, a significant inhibition of the tumor proliferation was seen in one breast cancer and in one large cell immunoblastic non-Hodgkin’s lymphoma after continuous exposure of PIXY321. In conclusion, the use of PIXY321 to reduce myelosuppression after high-dose chemotherapy appears unlikely to result in stimulation of the growth of malignant cells in patients with lymphoma or cancers of the breast, lung, and ovary.

MATERIALS AND METHODS

Drugs. The GM-CSF/IL-3 hybrid PIXY321 was obtained from Immunex Corp. (Seattle, WA). Stock solutions of PIXY 321 (2 μg/ml) were prepared in CMRL 1066 medium (Irvine Scientific, Irvine, CA) enriched with 15% horse serum (Sigma, St. Louis, MO), 2% FCS (JRH Biosciences, Lenexa, KS), and stored at −70°C until further use. On the day of an experiment, stock solution was also diluted in enriched CMRL.

Tumor Sample Processing. After written informed consent was obtained according to institutional guidelines, tumor specimens were collected by sterile standard procedures as part of routine clinical measures. A specific procedure aimed to obtain just material for the human tumor cloning assay was not performed. Solid specimens were immediately placed in McCoy’s 5A medium containing 10% newborn calf serum plus 1% penicillin/streptomycin. Solid specimens were minced and repeatedly passed through a 100 stainless steel mesh (EC Apparatus, St. Petersburg, FL) to obtain a single-cell suspension. Malignant effusions were collected with preservative-free heparin (10 units/ml), centrifuged at 150 x g, washed twice with enriched CMRL, and resuspended in enriched CMRL.

Received 7/20/95; revised 6/12/96; accepted 7/3/96.

1 This work was supported in part by a grant from Immunex Corporation.

2 To whom requests for reprints should be addressed, at Cancer Therapy and Research Center, 8122 Datapoint Drive, Suite 700, San Antonio, TX 78229. Phone: (210) 616-5850; Fax: (210) 692-7502.

3 The abbreviations used are: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-3, interleukin 3; CMRL, Connaught Medical Research Laboratories.

Downloaded from clincancerres.aacrjournals.org on October 15, 2017. © 1996 American Association for Cancer Research.
concentrations of PIXY321 were adjusted to 0.1 ng/ml in CMRL Medium, as previously described (33). Enriched plates (Corning, Corning, NY) were incubated in a mixture of 0.3% agar in broth (Difco), and 100 μg/ml asparagine (Life Technologies, Inc.), a mixture of McCoy’s 5A medium as described above, 0.6% soy cations. Base layers contained 0.5% agar (Difco, Detroit, MI) in Hamburger and Salmon (32) with several modifications. The cloning assay was performed using the two-layer soft agar system determined the final concentration of plated cells.

Positive control increased the reproducibility and validity of the agents. The use of the ≥30% colony formation criterion in the cloning assay to predict clinical response to single anticancer agents were adequate in a prospective study showing the usefulness of the cloning assay. Thus, ≥20 colonies per control plate were adequate in a prospective study showing the usefulness of the cloning assay to predict clinical response to single anticancer agents. The use of the ≥30% colony formation criterion in the positive control increased the reproducibility and validity of the cloning assay in a large methodological study evaluating its applicability to large-scale anticancer drug screening. For the lymphoma specimens, a more conservative criterion was used based on the reduced growth capacity of this tumor type in the cloning assay system (35). Lymphoma specimens were considered evaluable if more than five clusters per plate with more than eight cells per cluster were obtained.

Statistical Analyses. Inhibition of colony formation was defined as a reduction in growth of more than 50%. No effect corresponded to 50–150% growth of the control, and stimulation was noted when >150% of the control without PIXY321 exceeded. Data were expressed as means ± SD of triplicate determinations. Percentage of survival was calculated by expressing the mean number of tumor colony-forming units from cytokine-treated cells divided by the mean number of tumor colony-forming units from untreated controls × 100.

RESULTS

Tumors from 98 patients were tested for the effects of PIXY321 in a human tumor cloning assay (Table 1). Sixty-two specimens derived from epithelial solid tumors and 36 from lymphomas. The histological classification of the lymphomas is shown in Table 2. Overall, 34 specimens were considered evaluable, including the 15 breast cancers, 5 ovarian cancers, 5 lung cancers, 8 non-Hodgkin’s lymphomas, and 1 Hodgkin’s lymphoma. No

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Specimens evaluable/tested (%)</th>
<th>No effect (%)a,b</th>
<th>Growth inhibition (%)a</th>
<th>Growth stimulationb,c,d,e,f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>15/36 (42)</td>
<td>14 (93)</td>
<td>1 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>9/36 (25)</td>
<td>8 (89)</td>
<td>1 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>5/18 (28)</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovary</td>
<td>5/8 (63)</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34/98 (35)</td>
<td>32 (94)</td>
<td>2 (6)</td>
<td>0</td>
</tr>
</tbody>
</table>

a Percentage of evaluable tumors.

b At any concentration of PIXY321.

c >44%, 52%, 44%, and 32% of the control.

d >87%, 39%, 27%, and 21% of the control.

e >150% of the control.

f Growth reduced to <50% of the control without PIXY321.

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. of specimens tested</th>
<th>No. of specimens evaluable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma*</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Low grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small lymphocytic</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Follicular small cleaved cell</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Follicular mixed</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse small cleaved cell</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse mixed</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>High grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large cell immunoblastic</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Lymphoblastic</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Small noncleaved cell</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* According to the Working Formulation classification.
patient received growth factors (granulocyte-CSF, GM-CSF, IL-3, or PIXY321) within 6 months prior to the tumor biopsy.

None of the evaluable specimens exhibited tumor stimulation by PIXY321. The addition of PIXY321 had no significant effect on in vitro tumor growth in a large majority of specimens, since a total of 32 tumors were in the no-effect category (94%). Inhibition of tumor growth to more than 50% of the control was observed in two specimens (6%), including one breast carcinoma and one non-Hodgkin’s lymphoma (Table 2). In the breast carcinoma, growth inhibition was concentration dependent, with growth reduced to 87%, 39%, 27%, and 21% of the control at 0.1, 1, 10, and 100 ng/ml of PIXY321, respectively. The lymphoma was histologically classified as a high-grade diffuse large cell immunoblastic, B-cell type and showed a relatively flat dose-response curve to the addition of PIXY321 (44%, 52%, 44%, and 32% at 0.1, 1, 10, and 100 ng/ml of PIXY321, respectively; Table 3). Furthermore, none of the specimens with inadequate growth in the control plates demonstrated recruitment of colony formation in the presence of PIXY321, which might indicate stimulation of proliferation.

**DISCUSSION**

PIXY321 showed potent activity in stimulating myeloid stem cells (5, 8, 9), and the concern has been that PIXY321 may direct part of its activity to stimulate tumor growth. This concern is supported by some reports indicating stimulation of lymphoma cells by IL-3 (21, 25) and by one report showing synergistic effects between GM-CSF and IL-3 on the proliferation of pancreas and gastric cancer cell lines (12). However, cell lines are long-term selected tumor cells and usually differ from primary tumors in the ability to take advantage of several growth factors to proliferate (33). Therefore, we decided to address the effect of PIXY321 on tumors taken directly from patients using the human tumor cloning assay. This method has been extensively used to determine the effects of chemotherapy and growth factors on human tumors (35). Our data show that no proliferation of human tumor colony-forming units was observed among 34 tumors taken directly from patients after exposure to several concentrations of PIXY321. Because of a lack of cell numbers from these tumors taken directly from patients, we did not have enough cells to perform the Scatchard binding analysis for detection of high-affinity receptors. Therefore, whether our results reflect a lack of receptor expression by tumor cells rather than a lack of growth stimulation remains to be determined.

Previous studies showed that tumor cell growth could be achieved with high concentrations of GM-CSF. The concentrations of PIXY321 chosen in this experiment (0.1–100 ng/ml) closely parallel the sustained levels seen with optimal dosing after administration of intensive chemotherapy. Pharmacokinetic data collected from a pediatric setting revealed that 1000 μg/m²/day PIXY321 is well tolerated, effective, and allows the level to reach a peak plasma concentration of 2.5 ± 0.8 ng/ml and an area under the curve of 18.5 ± 5.0 ng/ml/h (30). This confirms that the range of concentrations of PIXY321 used in our study (from 0.1 to 100 ng/ml) includes clinically relevant concentrations. Our data support the conclusion that stimulation is absent at pharmacologically achievable levels of PIXY321 in human patients. Moreover, our results are in agreement with our previous studies (18, 19) and with another report (17) showing that either IL-3 or GM-CSF alone did not significantly affect the growth of human tumors taken directly from patients. Therefore, the expected benefit of PIXY321 in reducing the chemotherapy-associated myelosuppression in patients with breast, lung, and ovarian cancers or lymphomas seems to outweigh the risk of inducing tumor growth in patients.

Interestingly, we observed a significant growth inhibition in 1 breast carcinoma among 25 solid tumor specimens and in 1 non-Hodgkin’s lymphomas. Inhibition of tumor growth has been reported in a preclinical study in cells expressing GM-CSF receptors on their surface. However, tumor growth inhibition with growth factors appears to be marginal in several preclinical studies, and our study supports the notion that PIXY321, like either IL-3 or GM-CSF alone, has a limited inhibitory capacity against tumors growing in vitro (17, 19).

Our results indicate that growth stimulation by PIXY321 of malignant cells derived from patients with solid tumors, in particular breast cancer and lymphoma, is unlikely. However, since some published results have shown that stimulation of tumor cell growth in cell lines is possible, the long-term effects of PIXY321 on the stimulation of tumor growth will only be answered by a careful clinical evaluation of tumor response in patients.

**ACKNOWLEDGMENTS**

The excellent secretarial assistance of Peggy Durack is gratefully acknowledged.

**REFERENCES**


Effects of PIXY321, a granulocyte-macrophage colony-stimulating factor/interleukin 3 fusion protein, on human tumor colony-forming units taken directly from patients.

M A Izquierdo, D Degen, E Raymond, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/2/10/1713

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.