A Pilot Study of High-Dose Interleukin-3 Treatment of Relapsed Follicular Small Cleaved-Cell Lymphoma: Hematologic, Immuneological, and Clinical Results

By Anas Younes, Andreas Sarris, Ugo Consoli, Alma Rodriguez, Peter McLaughlin, Yang Huh, Susan Starr, Fernando Cabanillas, and Michael Andreeff

The growth stimulatory effects of interleukin-3 (IL-3) on normal hematopoietic progenitor cells are well established, and clinical trials using IL-3 after bone marrow transplantation for various malignancies including lymphomas are frequently conducted. Although the IL-3 receptor is expressed on the surfaces of follicular small cleaved-cell lymphoma (FSCCL) cells, the in vivo effects of IL-3 on FSCCL have not been studied previously. Because our preclinical data suggested that IL-3 may have dose-dependent inhibitory effects on FSCCL cells in vitro, we treated eight FSCCL patients with high-dose IL-3 in an outpatient setting. Each patient received 1 mg/m² of IL-3 subcutaneously daily for 14 days followed by 7 days without IL-3. After three courses (9 weeks), the patients were evaluated for clinical responses. One patient had a minor response, and four had no responses. Three patients who had progressive disease before IL-3 treatment continued to have progressive disease. In two patients with bone marrow involvement with lymphoma, IL-3 had no effect on FSCCL cells. One patient with peripheral blood involvement with FSCCL cells that expressed IL-3 receptors had temporary growth arrest of the circulating malignant cells. IL-3 significantly increased the absolute neutrophil count in seven patients (87%) but had little effect on the number of normal circulating B cells. There was an increase in the number of circulating natural killer cells and CD8+ cells in four patients. Treatment was very well tolerated; no life-threatening toxicities were observed. The most common toxicities were injected conjunctivae (100%), fever (100%), fatigue (87%), and skin rash (75%). Most of the side effects subsided with the continued use of IL-3. These preliminary results suggest that high-dose IL-3 does not stimulate the growth of FSCCL cells in vivo and, in some instances, may cause growth inhibition.

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From the Departments of Hematology and Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

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Address reprint requests to Anas Younes, MD, Section of Lymphoma (Box 68), The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

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Table 1. Patient Characteristics, Prior Therapy, and Clinical Responses to IL-3

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Prior Therapy</th>
<th>Disease Status Before IL-3</th>
<th>Sites of Disease</th>
<th>Response to IL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60/M</td>
<td>CHOP/ESHAP/NOPP + VP-16 ESHAP</td>
<td>Stable</td>
<td>Lymph nodes, bone marrow</td>
<td>No change</td>
</tr>
<tr>
<td>2</td>
<td>63/F</td>
<td>CHOP-B ESHAP</td>
<td>Progressive disease</td>
<td>Lymph nodes</td>
<td>Progressive disease</td>
</tr>
<tr>
<td>3</td>
<td>67/M</td>
<td>CHOD/ESHAP/NOPP Interferon Taxol Taxol + cyclosporine MINE FND</td>
<td>Stable</td>
<td>Lymph nodes, bone marrow</td>
<td>No change</td>
</tr>
<tr>
<td>4</td>
<td>55/F</td>
<td>CHOP MINE ESHAP FND</td>
<td>Progressive disease</td>
<td>Lymph nodes</td>
<td>Progressive disease</td>
</tr>
<tr>
<td>5</td>
<td>62/F</td>
<td>CHOP/ESHAP/NOPP 5-FU</td>
<td>Stable</td>
<td>Lymph nodes</td>
<td>No change</td>
</tr>
<tr>
<td>6</td>
<td>51/F</td>
<td>Radiation</td>
<td>Progressive disease</td>
<td>Lymph nodes</td>
<td>No change</td>
</tr>
<tr>
<td>7</td>
<td>53/M</td>
<td>CHOP ProMACE ESHAP Interferon FND</td>
<td>Stable</td>
<td>Lymph nodes</td>
<td>Minor response</td>
</tr>
<tr>
<td>8</td>
<td>72/F</td>
<td>FND Taxol ESHAP</td>
<td>Progressive disease</td>
<td>Lymph nodes, bone marrow, peripheral blood</td>
<td>Progressive disease</td>
</tr>
</tbody>
</table>

Abbreviations: CHOP-B, cyclophosphamide, hydroxydoxorubicin, vincristine, prednisone, and bleomycin; ESHAP, etoposide, solumedrol, high-dose ara-c, and platinum; NOPP, novantrone, vincristine, procarbazine, and prednisone; MINE, mesna, ifosphamide, novantrone, and etoposide; 5-FU, 5-fluorouracil; FND, fludarabine, novantrone, and decadron; ProMACE, prednisone, methotrexate, Ara-C, and etoposide.

of blood cultures. After three cycles (9 weeks), all patients were reassessed for clinical responses. Peripheral blood lymphocyte subsets were determined by FACscan (Becton Dickinson, San Jose, CA) with monoclonal antibodies against CD4, CD8, CD16/CD56, and CD19 (Becton Dickinson) according to the manufacturer’s instructions and our clinical laboratory guidelines. The absolute number of lymphocytes expressing each antigen was calculated by multiplying the percentage of cells expressing the antigen by the total number of peripheral blood lymphocytes counted in the same day. IL-3 and IL-2 receptor expression was determined as previously described.

RESULTS

Clinical responses and toxicity. All eight eligible patients were assessable for clinical responses and toxicities. Patient characteristics, prior therapy, and clinical responses to IL-3 therapy are shown in Table 1. One patient (12.5%) had a major response in the retroperitoneal lymph node that lasted for 6 months; four (50%) had no responses, including two patients who had lymphoma with bone marrow involvement; and three (37.5%) had progressive disease. All patients who had progressive disease after IL-3 treatment had had progressive disease at the start of the study (Table 1). Disease progression occurred at the previous sites of disease; no new lesions appeared. One patient (patient 8) had a transient enlargement of an axillary lymph node while receiving IL-3. Fine-needle aspiration of the lymph node revealed heavy infiltration with monocytes, neutrophils, and eosinophils. The treatment was well tolerated, and all eight patients completed the planned three cycles IL-3. During the first cycle, two patients received 10 and 12 days of IL-3 instead of 14 days because of unexplained shortness of breath (patient 6) and bacteremia (patient 2). However, both patients continued the next two cycles as planned. No life-threatening toxicities were observed. Toxicities were more pronounced during the first cycle than the subsequent two cycles (Table 2). Injected conjunctivae and fever were the most common side effects and were observed in all patients. Drug-induced fever was observed in all patients and occurred within 30 to 60 minutes of IL-3 administration. In two patients, it was accompanied by chills; one of these patients also had bacteremia related to the presence of an indwelling central venous catheter. Acetaminophen (650 mg orally 60 minutes before IL-3 and after IL-3 administration) prevented the drug-related fevers in all patients during cycles 2 and 3. Fatigue was reported by seven patients (87%); it also became less prominent during the subsequent cycles. No patient had evidence of capillary leak syndrome or pulmonary rales, although two patients complained of unexplained dyspnea on exertion, which was relieved with a short course of diuretics. Headache was ob-
Table 2. Toxicities

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>8 (100)</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>Injected conjunctivae</td>
<td>8 (100)</td>
<td>7 (87)</td>
<td>7 (87)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>7 (87)</td>
<td>1 (13)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>6 (75)</td>
<td>3 (38)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Myalgia/arthralgia</td>
<td>5 (63)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Headache</td>
<td>4 (50)</td>
<td>0 (0)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>4 (50)†</td>
<td>2 (25)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (25)</td>
<td>2 (25)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>2 (25)</td>
<td>1 (13)</td>
<td>1 (13)</td>
</tr>
</tbody>
</table>

* Fever was prevented by premedication with acetaminophen.
† Nausea and vomiting were observed only during the first 2 days of the cycle.

Fever was observed only in 50% of the patients during the first cycle, and the incidence decreased during subsequent cycles. Macular rash on the trunk and extremities was observed in six (75%) patients during the first cycle. It appeared within 3 days of IL-3 administration and peaked around day 10. It was not associated with urticaria and was not related to the injection sites. The intensity of the rash decreased significantly after day 10. The other two patients did not have discrete rash but did have generalized erythema. A skin biopsy performed on one patient revealed nonspecific dermatitis. Although four patients had nausea or vomiting during the first cycle, this symptom occurred only during the first 2 days and occurred within 2 hours of IL-3 injection.

Hematologic effects. One patient had evidence of circulating malignant lymphoma cells at the beginning of the study (patient 8). Her lymphocyte count was 120,000/μL at 40 days before IL-3 therapy began (Fig 1A). The lymphocyte count decreased to 11,000/μL after ESHAP chemotherapy (etoposide, solumedrol, high-dose ara-c, and cis-platinum) but increased to 17,000/μL on the day IL-3 therapy began. After an initial increase in the number of her peripheral blood lymphocytes, which consisted mainly of CD19+ cells, her CD19+ cell count declined and then plateaued, while the number of nonmalignant lymphocytes continued to increase (Fig 1A). This growth arrest lasted for 4 weeks. A review of her peripheral blood smear revealed no evidence of lymphoplasmacytic differentiation. Expression of IL-3 and IL-2 receptors on her circulating malignant B cells was measured by FACscan before the start of IL-3 therapy and after 3 days of IL-3 treatment (Fig 2). Both receptors were expressed before IL-3 administration, and the level of receptor expression increased after 3 days of IL-3 therapy. There was no significant change in the number of normal circulating lymphocytes in the other seven patients.

Seven patients (87%) had significant increases in their peripheral blood absolute neutrophil counts (ANCs), as shown in Figs 1B and 3. The ANC values reached their peaks at the end of the 14-day treatment with IL-3 and declined to the baseline value during the subsequent week. The mean ANC for the seven responding patients increased fivefold from the baseline values. Significant increases in absolute eosinophil counts were observed in five patients (62%); the counts were greater than 5,000/μL in four patients. There was no significant increase in the number of basophils. The effects of IL-3 on the platelet count and hemoglobin levels were variable. Two patients (patients 2 and 6) had significant increases in their platelet count compared with baseline values (increased from 300,000/μL and 250,000/μL to 700,000/μL and 600,000/μL, respectively), and five had decreased platelet counts (Fig 4). In all these patients, the platelet count changes were reversible and returned to the baseline values at the end of IL-3 therapy. Patient 8, who had peripheral blood and bone marrow involvement, continued to have a decreased platelet count because of disease progression. High-dose IL-3 caused a transient decrease in the hemoglobin values by 1 to 3 g/dL in all patients. This decrease also was reversible after discontinuation of IL-3 therapy (Fig 5). None of the patients required platelet or packed red cell transfusion during IL-3 therapy. However, patient 8 was transfused with packed red cells at the end of IL-3 treatment.

Effects on circulating lymphocyte subsets. One patient had circulating lymphoma cells at the time of treatment (Fig 1A). Initially, the number of her CD19+ cells increased and reached its peak by day 14 of the first cycle. Subsequently, the number of CD19+ cells declined and plateaued during the second cycle, and increased and decreased again during...
the third cycle. The number of nonmalignant lymphocytes (which consisted mainly of CD8⁺ cells) continued to increase during all three cycles. In the other seven patients, the absolute number of CD19⁺ cells did not significantly change (Fig 6). The number of natural killer cells, identified by expression of CD56⁺/CD16⁺ and CD3⁺, was moderately and transiently increased in six patients (Fig 6). Similarly, the number of CD8⁺ cells transiently increased in three patients.

Effects on lymphoma cells in the bone marrow. Three patients had bone marrow involvement with lymphoma (Table 1). In two patients (patients 1 and 3), bone marrow aspirates/biopsies and immunophenotypic studies were performed before and after IL-3 therapy. No significant change in the percentage of lymphoma involvement or subsets of normal lymphocytes were observed compared with the baseline values. Patient 8 did not have a bone marrow biopsy at the end of therapy.

**Fig 2.** Expression of IL-2 receptors (IL-2R) and IL-3 receptors (IL-3R) on malignant circulating FSCCL cells before IL-3 therapy (A) and 3 days after the beginning of IL-3 therapy (B).

**Fig 3.** Effects of high-dose IL-3 on peripheral blood (PB) ANCs, lymphocyte counts (Lymph), and eosinophil counts (EOS). Representative data are from patient 2. Shaded areas indicate periods of IL-3 therapy.

**Fig 4.** Effects of high-dose IL-3 on platelet counts. Representative data are from patient 1. Shaded areas indicate periods of IL-3 therapy.

**Fig 5.** Effects of high dose IL-3 on hemoglobin levels. Representative data are from patient 2. Shaded areas indicate periods of IL-3 therapy.
that IL-3 caused FSCCL cells to transiently differentiate to Epstein-Barr virus-transformed human B cell lines in vivo. More recently, Kramer et al.\textsuperscript{11} reported transgenic mice expressing antisense IL-3 RNA developed IL-3 were reported only recently. Von Hoff et al.\textsuperscript{4} demonstrated that high-dose IL-3 as the primary treatment for patients with progressive disease before IL-3 therapy developed stable disease in response to the last chemotherapy regimen administered. After three courses of high-dose IL-3, three patients with progressive disease at the beginning of the study continued to have progressive disease, and three patients continued to have refractory stable disease. One patient who had progressive disease before IL-3 therapy developed stable disease, and one patient (patient 7) who had no response before IL-3 therapy had a minor response to it. Although these findings do not support the hypothesis that IL-3 can inhibit the growth of FSCCL in vivo, they refute the notion that IL-3 stimulates the growth of FSCCL. However, the case of patient 8, who had circulating FSCCL, is noteworthy. After an initial increase in the number of circulating malignant cells, their numbers declined and then plateaued for almost 4 weeks. During this transient growth arrest, the number of benign lymphocytes continued to increase, as did the number of neutrophils, in response to IL-3. A peripheral blood smear did not show any evidence of morphologic differentiation to plasmalympocytic type, as reported previously by Kramer et al.\textsuperscript{11} Therefore, this growth arrest may have been due to a direct cytostatic effect of IL-3 on the malignant cells through its IL-3 receptor. IL-3 receptor expression appeared to be increased 3 days after the beginning of IL-3 therapy, but no follow-up receptor studies were performed. In addition, IL-2 receptor expression appears to have increased after 3 days of IL-3 therapy. While prolonged treatment with IL-3 may have caused downregulation of IL-3 receptor expression and, therefore, abolished cytostatic effects of IL-3 on the malignant cells, this is unlikely, because IL-3 continued to increase the number of neutrophils and nonmalignant lymphocytes. Although the effect of IL-3 on neutrophil count was significant and reproducible in each cycle in seven (87%) patients, the effects of high-dose IL-3 on platelets counts appeared to be modest and were observed in only two patients (25%). The remaining patients had either no change in platelet counts or a trend to decreased platelet counts. A similar trend in decreased hemoglobin levels was observed. Although early reports suggested that IL-3 may have a clinical use as a growth factor for inducing thrombocytosis\textsuperscript{19} and erythropoiesis,\textsuperscript{5,6} the effects of IL-3 may be paradoxical when given at high doses, perhaps because of stimulation of inhibitory secondary cytokines or direct cytostatic effects. It is not clear if similar paradoxical effects on lymphoma cells can be obtained with different doses of IL-3.

In this report, IL-3 caused a modest increase in the number of natural killer cells and CD8\textsuperscript{+} cells in four patients. This increase was probably mediated through secondary cytokines such as IL-2, as CD19-negative mononuclear cells do not express IL-3 receptors (A.Y. and U.C., unpublished observation, April 1995).

Finally, the toxicity profile we observed was somewhat similar to those reported earlier by others.\textsuperscript{20,23} However, the skin rash was not previously reported. It was not associated with urticaria or elevated blood basophil levels and was more severe during the first course. A skin biopsy did not reveal the etiology of the rash. Also, chills were not as common in this study as in trials in which IL-3 was administered intravenously.

Our preliminary data suggest that IL-3 does not stimulate the growth of FSCCL in vivo and may cause transient growth inhibition of the malignant cells. The use of IL-3 after bone marrow transplantation for FSCCL appears to be safe and, in special circumstances, could be advantageous.

REFERENCES


A Younes, A Sarris, U Consoli, A Rodriguez, P McLaughlin, Y Huh, S Starry, F Cabanillas and M Andreeff