Interleukin-2 May Induce Prolonged Remissions in Advanced Acute Myelogenous Leukemia

By Giovanna Meloni, Robin Foa, Marco Vignetti, Anna Guarini, Susanna Fenu, Silvia Tosti, Anna Gilio Tos, and Franco Mandelli

The administration of interleukin-2 (IL-2) may induce complete remissions in acute myelogenous leukemia (AML) patients with a low proportion of residual bone marrow (BM) blasts. To confirm this preliminary observation, we treated 14 AML patients with advanced disease and with a residual BM blastosis that ranged between 7% and 24% with repeated 5-day cycles of high-dose recombinant IL-2 administered by daily continuous intravenous infusion. Patients who responded have been subsequently submitted to a monthly maintenance scheme with subcutaneous IL-2 at lower doses. While using this schedule and closely monitoring clinical and laboratory conditions, side effects were acceptable and no toxic deaths recorded. Eight of the 14 patients treated with high-dose IL-2 obtained a complete remission (CR). Five remain in persistent CR (four in third CR and one in fourth CR) after a median follow-up time of 32 months (14, 30, 32, 33, and 68 months, respectively). In all five patients, the IL-2–induced remission is the longest in the natural history of the disease. These findings show that IL-2 displays an antileukemic effect in AML with limited residual disease, and suggest that IL-2 should be considered a therapeutic option for resistant or relapsed AML patients.

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**PATIENTS AND METHODS**

**Patients**

Fourteen patients have been enrolled in this study. All were affected by primary AML, diagnosed cytologically and confirmed by cytochemistry and immunophenotypic analysis. The inclusion criteria were: (1) relapsed or refractory leukemia with more than 5% and less than 30% BM blasts confirmed by at least three marrow aspirates within 1 month (each marrow preparation was seen by at least three independent observers); (2) ineligibility for further intensive chemotherapy programs; (3) absence of central nervous system (CNS) involvement, major organ failure, or active infectious processes; and (4) written informed consent (obtained from parents in patients less than 18 years old).

The characteristics of the patients are shown in Table 1. Median age was 30 years (range 8 to 55); seven patients were males. According to the French-American-British classification, six patients were M4; five patients, M2; two patients, M1; and one patient, M5. The mean percentage of BM blasts was 13% (range, 7% to 24%). Standard chemotherapy had been stopped at least 4 weeks before IL-2, and steroid treatment at least 2 weeks before starting IL-2. Three patients were refractory to two different induction regimens; 11 patients were in hematologic relapse (1 in first, 6 in second, 3 in third, and 1 in fourth) refractory to chemotherapy; 2 of them had also suffered from a previous CNS relapse.

During the course of their disease, all patients received different chemotherapy regimens including anthracyclines and cytosine-arabinoside (ARA-C) at high or intermediate doses. Four patients also underwent an autologous BM transplantation (BMT) (three in first and one in third CR), and one patient received two autologous BMT (one in first and one in second CR, respectively).
total IL-2 dose, dissolved in a saline solution containing 20% of human albumin to a total volume of 10 mL, was infused using a B. Braun microinjection pump (Melsungenag, Germany) over a period of 24 hours.

Proleukin. Each vial (1 mg), containing $3 \times 10^6$ Cetus U ($18 \times 10^6$ IU), was reconstituted with 1.2 mL of sterile water and the total IL-2 dose, dissolved in a 5% glucose solution to a final volume of 10 mL, was infused with the same modalities described above.

**Treatment Plan**

IL-2 was administered intravenously by 24-hour continuous infusion for 5 days, using a daily dose-escalating protocol. IL-2 infusion was then repeated after a rest of at least 72 hours starting from the maximum tolerated dose, for a total of four cycles. The maximum dose to be reached with Glaxo IL-2 was 900 pg/m²/d, starting from 10,000 pg/m²/d, whereas the highest Proleukin dose was $18 \times 10^6$ IU/m²/d, starting from $8 \times 10^6$ IU/m²/d.

Patients who responded to IL-2 entered an intermission program consisting of monthly 5-day courses of lower doses of IL-2 administered on an outpatient basis; in the first three patients, a 6-hour continuous intravenous infusion route was used, whereas in the later patients, IL-2 was given subcutaneously. Glaxo maintenance doses were $100 \mu g/m²$ on day 1 and $200 \mu g/m²$ for four days after, whereas Proleukin doses were $4 \times 10^6$ IU/m² on day 1 and $8 \times 10^6$ IU/m² on days 2 through 5 (Fig 1). After the first year, maintenance was administered every other month at a lower dose ($4 \times 10^6$ IU/m²/d) and continued in all responding patients until relapse. In patients receiving maintenance IL-2, no other treatment was planned until relapse.

**Supportive Care and Monitoring**

During the induction cycles, all patients were nursed in double rooms with the relatives’ assistance. Patients had a single-lumen central catheter and received prophylactic therapy with acetylsalicylic acid 1 g/d, allopurinol 300 mg/m²/d, and ranitidine intravenously 300 mg/d. Cotrimoxazole was also given at a dose of 480 mg twice daily. Intravenous broad-spectrum antibiotics were used only in the presence of a documented infection. Platelet transfusions were given when the platelet count was lower than $20 \times 10^9/L$, body temperature was greater than 38°C, or bleeding was present. Packed red blood cells (RBCs) were transfused when hemoglobin was lower than 11 g/dL. Standard peripheral blood (PB) studies, including complete counts, hepatic and renal function tests, and electrolyte determinations, were performed daily during the induction treatment; echocardiographic monitoring of cardiac function was performed at least before each induction cycle.

BM evaluations were performed in all patients before starting IL-2 and at the end of each induction cycle. A documented progression of disease was mandatory for the discontinuation of IL-2 treatment. Meperidine for chills, loperamide for diarrhea, antihistaminics for itching, and benzodiazepine for anxiety were used as needed. Initial support of hypotension and oliguria consisted of rapid infusion of colloid solutions; low-dose dopamine (2 to 5 mg/kg/min) was added if volume replacement was not sufficient. Furosemide was given only in the case of persistent oliguria with a documented normal central venous pressure.

Toxicity was defined according to the World Health Organization (WHO) grading system. Doses of IL-2 were not increased when simultaneous grade 2 toxicities were present, whereas IL-2 was stopped in the presence of side effects that were considered either acutely life-threatening or likely to become life-threatening on the basis of the known cumulative effects of additional doses.

During treatment with IL-2 and until patients went off-study either for progression or relapse, no other chemoradiotherapy or biologic therapy was installed.

**Immunologic Activation**

Mononuclear PB and BM cells were obtained after fractionation on a Lymphoprep gradient (Nycomed As, Oslo, Norway). The cells recovered from the interface were washed twice and resuspended in RPMI 1640 (Flow Laboratories, Opera, Italy) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Flow). The expression of Leu-11 (CD16) and the IL-2 receptor a chain (CD25) (Becton Dickinson, Mountain View, CA) was monitored using a FACScan flow cytometer (Becton Dickinson).

LAK cells were generated by incubating mononuclear cells at the concentration of $1.5 \times 10^6$ cells/mL in RPMI 1640 supplemented with 10% heat-inactivated FBS, in the presence of 100 U/mL of recombinant IL-2 (rIL-2) (Glaxo Institute for Molecular Biology) for 7 days at 37°C with 5% CO₂ in humidified air. To assess whether the administration of IL-2 was capable of inducing in vivo the generation of IL-2–producing cells, various dilutions of LAK cells were added to rIL-2–stimulated PBMCs for 24 hours. The frequency of IL-2–producing cells was assessed by measuring the increase in the IL-2 concentration in the supernatants using an IL-2 ELISA. The results indicated that LAK cells significantly increased the IL-2 concentration in the supernatants, suggesting that IL-2–producing cells were induced in vivo by the administration of IL-2.

**Table 1. Patient Characteristics**

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<th>UPN</th>
<th>Sex/Age</th>
<th>FAB</th>
<th>Status at IL-2</th>
<th>Previous Longer CR (mos)</th>
<th>BM Blasts (%)</th>
<th>Induction Cycles</th>
<th>ASD Ratio</th>
<th>Response</th>
<th>Maintenance Cycles</th>
<th>Outcome (mos)</th>
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<td>4th CR</td>
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<td>CCR (88+)</td>
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<td>M4E</td>
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<td>3rd CR</td>
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<td>51</td>
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<td>9</td>
<td>CCR (14+)</td>
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</table>

Abbreviations: ASD, administered/scheduled dose; CCR, continuous CR.

* First CR obtained after two different induction schedules.
of LAK cells, the lytic activity against Raji cells was also tested on PB and marrow lymphocytes before and after treatment in the absence of any further in vitro preincubation with IL-2. The natural killer (NK) activity was measured using the classic \(^{51}Cr\) release assay against the K562 cell line as previously described in detail. The results reported always referred to a final effector:target ratio of 100:1.

**Evaluation of Response**

Response to the IL-2 induction treatment was evaluated at the end of the last cycle. A CR was defined as a cellular marrow with less than 5% blasts, a normalization of PB counts and a disappearance of any clinical features related to the leukemia that persisted more than 1 month.

BM aspirates have also been performed monthly before each maintenance cycle to confirm the persistence of response. A patient was defined in relapse when a BM blastosis greater than 25% was observed in two consecutive BM aspirates performed after a 1-week interval.

**RESULTS**

**Toxicity**

The usual IL-2–related side effects (ie, fever, nausea and/or vomiting, and hemodynamic and metabolic toxicities) were recorded in all patients. Figure 2 shows WHO side effects greater than grade 2 that occurred at least once during induction treatment. The administration of IL-2 was adjusted according to toxicity. The median percentage of IL-2–planned dose administered is shown in Table 1 (administered/scheduled dose [asD] ratio). In 7 of 14 patients, the infusion of IL-2 had to be stopped because of oliguria (five cases, nos. 1, 3, 6, 13, and 14), hypotension and tachycardia (one case, no. 8), or increase in creatinine (one case, no. 10). Six patients developed sepsis (four by *Staphylococcus aureus*, one by *Staphylococcus epidermidis*, and one by *Streptococcus viridans*); one of them (no. 5) also developed a sepsis by *Candida*. In all these patients, IL-2 treatment was withheld at the moment of microbiologic documentation and intravenous antibiotic therapy (plus antifungine in one case, no. 5) was administered. Upon resolution, IL-2 was resumed in patients who had not completed the planned treatment. Nine patients developed severe thrombocytopenia that reversed within 5 to 7 days from the end of IL-2 treatment in all but three patients who developed a progression of disease. Grade 2 and 3 anemia occurred in nine patients, who received packed RBC transfusion. Nine patients developed severe hypotension (grade 3 WHO in seven cases, grade 4 in two) that required pressors and was resolved in all cases. Five patients had a cutaneous rash with a clinical picture similar to a graft-versus-host reaction. Despite these side effects, the lytic activity against Raji cells was also tested on PB and marrow lymphocytes before and after treatment in the absence of any further in vitro preincubation with IL-2. The natural killer (NK) activity was measured using the classic \(^{51}Cr\) release assay against the K562 cell line as previously described in detail. The results reported always referred to a final effector:target ratio of 100:1.

**Fig 1.** rIL-2 schedules used in refractory/relapsed AML patients with limited disease.
INTERLEUKIN-2 IN ADVANCED AML

effects, no IL-2-related deaths occurred during the induction period. During maintenance therapy, only mild fever (WHO grade 2), fatigue, and occasional nausea were observed.

Hematologic Modifications

During the administration of IL-2, the proportion of lymphocytes always decreased, whereas a rebound absolute lymphocytosis was observed in all cases 24 to 48 hours after stopping therapy. The mean lymphocyte count before IL-2 was $1.06 \times 10^9/L$ (range, 0.2 to 1.8) and the peak after the last induction cycle was $6.44 \times 10^9/L$ (range, 2.2 to 9.1). The subsequent IL-2 course was often started when a high lymphocyte count was still present, and lymphopenia occurred within 48 to 72 hours from the start of IL-2. The neutrophil and, mainly, eosinophil counts increased during the cycles, whereas platelets decreased slowly during the entire induction therapy and reached the minimum at the end of the last cycle in all but one patient.

During maintenance treatment, a progressive increase in eosinophils was present during the course of treatment, and a lymphocytosis has been often observed after 24 to 48 hours from the end of IL-2. These changes were less pronounced compared with those observed during the induction cycles.

Clinical Results

By the end of the induction cycles, a CR was achieved in 8 of the 14 patients treated. One (no. 14), received IL-2 in first hematologic relapse that occurred after a first CR obtained with a second line treatment and lasted 4 months. This patient was treated with 9% BM blasts and received three induction cycles because of toxicity (fever, hypercreatininemia). For this reason, maintenance was performed with a very low dose ($4 \times 10^6/IU/d$ total dose). Fourteen months after starting IL-2, the patient is in persistent CR.

Five patients received IL-2 in second relapse. All had been treated with the current chemotherapy protocol to obtain the first CR (anthracyclines plus ARA-C in induction and consolidation), and one of them also received an autograft; the median first CR duration was 11 months (range, 5 to 16 months). After achieving their third CR with IL-2, they were all started on the monthly IL-2 maintenance protocol. Two of the five patients are still in CR and on maintenance after 30 and 32 months, whereas three relapsed after 5, 5, and 9 months, respectively; this last patient (no. 2) was then induced in fourth CR with low-dose ARA-C and retreated with low-dose maintenance IL-2. Patient no. 1 received IL-2 in third relapse after a short third CR duration (3 months). This patient also underwent two autologous BMT, one in first and one in second CR. Starting from the third year, IL-2 was reduced to $8 \times 10^6$ IU/d total dose every 3 months. Sixty-eight months after starting IL-2, the patient remains in persistent fourth CR.

The last patient (no. 5) received IL-2 in fourth relapse; he had undergone an autologous BMT in third CR. The fifth CR obtained with IL-2 is still lasting, under IL-2 maintenance, 33 months later.

Six patients did not respond to IL-2 and went off-study (three were primary refractory to more than two induction regimens, one was in second and two were in third relapse).

Immunologic Studies

Reevaluation of the immune status of the patients at the end of the induction cycles of IL-2 showed a marked phenotypic and functional activation, both on circulating and on BM lymphocytes. The results obtained are shown in Fig 3, A and B. Lymphocyte activation was documented by the increase in the expression of the $\alpha$ chain of the IL-2 receptor. The expansion of the cytotoxic compartment was shown phenotypically on the basis of CD16 expression. Functionally, the administration of IL-2 induced a notable enhancement of NK and LAK activity, as well as the generation of endogenous LAK effectors.

DISCUSSION

The results of the present study confirm on a larger series of patients with an adequate follow-up the preliminary evidence of an antileukemic efficacy of IL-2 in AML patients with a relatively small proportion of residual marrow blasts. Using IL-2 alone, we could successfully induce a CR in 57% of the treated patients (8/14). All had advanced disease, were heavily pretreated, had a proportion of residual marrow blasts that ranged between 7% and 24%, and had a low
probability of obtaining a new and durable response with further conventional therapeutic approaches. So far, in four of the eight patients, the IL-2–induced CR has lasted over 2.5 years. One such patient persists in fourth CR more than 5.5 years after starting IL-2. A fifth patient remains in persistent remission 14 months after starting IL-2. For all five cases, the IL-2–induced CR is the longest in the clinical history of each patient. Because, at the time of starting IL-2, these five patients were in refractory second or third relapse, their prognosis with conventional treatment was extremely poor. Thus, it appears highly likely that the persistent CR can be ascribed to the administration of IL-2. Though the possibility that IL-2 may trigger a proliferative signal on myeloid blasts may be of concern,20 it is worth noting that IL-2 did not induce progression of disease in any of the 14 patients treated. These findings are in agreement with earlier preclinical data10 and with the recent evidence that human myeloid and lymphoid acute leukemia cells transduced with the IL-2 gene do not modify their proliferative and growth features.21

With regard to feasibility and toxicity, the continuous intravenous daily dose-escalating protocol allows the doses of IL-2 to be modulated on the basis of the individual tolerance of each patient, enabling each patient to receive the highest tolerated dose without the risk of overtreatment. This appears to be a relevant issue, both in terms of individual compliance and because compelling evidence of a dose-dependent activity of IL-2 is still lacking. In addition to the well-known side effects commonly observed in IL-2–treated individuals, six leukemic patients (43%) showed a documented infection, with multiple-positive blood cultures, mainly caused by Staphylococci. These occurred only during the high-dose IL-2 administration. This relatively high incidence of gram-positive septicemia, which is in agreement with previous reports,22 prompted to implement for the subsequent patients treated with IL-2 a prophylactic protocol for gram-positive organisms (teicoplanine) as first antibiotic approach.23 Unlike the high-dose induction cycles, the maintenance schedule has been feasible on an outpatient basis, with doses of IL-2 that can be considered as intermediate (up to 8 × 10^9 IU/m^2/d); side effects have been moderate and controllable, and in no case was hospitalization required.

The clinico-hematologic changes included neutrophilia with eosinophilia during the infusion of IL-2, whereas 24 to 48 hours after the end of each induction cycle, an increase in the white blood cell count with an absolute lymphocytosis is observed. The morphologic changes occur also in the marrow. These are likely to be contributed by the different cytokines that are known to be released in vivo in patients with solid tumors or hematologic malignancies treated with IL-224–25 (and personal data). During maintenance, limited but detectable modifications of the PB picture—with an increase in the lymphocyte count, granular lymphocytes, and eosinophils—could be documented at the end of the administration, suggesting that IL-2, even at lower doses, may still exert a certain degree of activity.

The immunologic studies extended previous observations26–27 and showed that the high doses of IL-2 administered during the induction cycles produced a marked activation of the immune system of the host both in PB and BM lymphocytes. This was documented by the expression of the IL-2 receptor and by a twofold to 10-fold amplification of the NK and LAK compartments, which included the generation of endogenous LAK effectors. It is worth noting that these phenotypic and functional changes are much more evident compared with those observed in acute leukemia patients with a larger marrow blastosis.14 However, despite the marked immunologic modifications, no clearcut correlation between clinical response, outcome, and in vitro data could be established.

Taken together, the results of this study indicate that (1) high doses of IL-2 can be safely administered to AML patients in advanced phase of their disease if clinical and laboratory conditions are closely monitored; (2) complete and prolonged remissions may be obtained in patients with limited disease; (3) IL-2 induces a marked circulating and marrow immune activation in this category of patients; and (4) subcutaneous maintenance treatment with IL-2 can also be safely performed on an outpatient basis for prolonged periods (up to over 5 years) without sequela.

One relevant but as of yet unclarified issue is the heterogeneous clinical responses observed in patients with a similar clinico-hematologic picture. It is tempting to hypothesize the presence of specific antitumor cytotoxic T lymphocytes (CTLs) in some cases that may be amplified by the administration of IL-2. In experimental tumors,28–30 and more recently in human melanoma,31 it has been shown that the IL-2 released constitutively by IL-2–gene-transduced tumor cells is capable of generating CTLs directed specifically against the autologous tumor. Thus, it is conceivable that this may have occurred in a proportion of acute leukemia patients treated with exogenous IL-2.

In conclusion, these data have documented the antileukemic effect of IL-2 alone in AML and suggest that IL-2 should be considered a therapeutic option for AML patients with a proportion of residual chemoresistant blasts.

The possibility of a major efficacy of rIL-2 in patients treated with less advanced disease before the development of chemotherapy resistance should be also considered. AML patients in CR, both after ABMT and after chemotherapy, who are undergoing randomized trials, will probably contribute to a better understanding of this important issue.

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INTERLEUKIN-2 IN ADVANCED AML


Interleukin-2 may induce prolonged remissions in advanced acute myelogenous leukemia

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