

Postremission Therapy with Low-dose Interleukin 2 with or without Intermediate Pulse Dose Interleukin 2 Therapy Is Well Tolerated in Elderly Patients with Acute Myeloid Leukemia: Cancer and Leukemia Group B Study 9420¹

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ABSTRACT

Purpose: The purpose of the study is to investigate the tolerability of interleukin 2 (IL-2) after intensive chemotherapy in elderly acute myeloid leukemia (AML) patients in first complete remission (CR).

Experimental Design: AML patients ≥ 60 years in CR after induction and consolidation chemotherapy on Cancer and Leukemia Group B study 9420 were eligible if they had neutrophils $\geq 1 \times 10^9$ /liters and platelets $\geq 75 \times 10^9$ /liters. Patients received low-dose IL-2 (1×10^6 IU/m²/day s.c. for 90 days) or low-dose IL-2 with intermediate pulse doses ($6\text{--}12 \times 10^6$ IU/m²/day s.c. for 3 days) every 14 days (maximum five pulses). In a subset of patients, we investigated the expression of NKG2D ligands by leukemic cells because they are likely important mediators of natural killer cytotoxicity.

Results: Of 35 CR patients receiving IL-2, 34 were evaluable for toxicity. Median age was 67 (range, 60–76) years. Thirteen of 16 patients receiving low-dose IL-2 com-

pleted the planned therapy, and 11 of 18 who also received intermediate pulse dose IL-2 therapy completed all five pulses. The spectrum of toxicity in both groups was similar, with predominantly grade 1–2 fatigue, fever, injection site reactions, nausea, anemia, and thrombocytopenia. Grade 3–4 hematological and nonhematological toxicity were more frequent in patients also receiving intermediate pulse dose IL-2 therapy. Grade 3–4 fatigue and hematological toxicity, although uncommon, were the major causes for discontinuing or attenuating therapy. In 8 cases, mRNA for one or more NKG2D ligands was detected in leukemic cells obtained at diagnosis before treatment.

Conclusions: Low-dose IL-2, with or without intermediate pulse dose therapy, given immediately after chemotherapy in first CR to elderly AML patients is well tolerated. Expression of NKG2D ligands by leukemic cells was detected in the majority of cases tested and should be assessed for correlation with response to IL-2 in future studies.

INTRODUCTION

Approximately 25% of patients with *de novo* AML³ are cured after intensive chemotherapy. The majority of these patients, however, are young. Multicenter trials that included patients above and below the age of 60 years have consistently indicated that older age is associated with a poorer response to initial therapy, as well as shorter DFS and OS (1–4). In part, the inferior outcome in older patients reflects a different biology of the disease in this population. Compared with younger patients, a greater proportion of AML cases in patients ≥ 60 years is associated with poor prognostic features, including cytogenetic findings such as $-5/\text{del}(5q)$, $-7/\text{del}(7q)$ or complex karyotypes, as well as high expression of the multidrug resistance glycoprotein MDR1 (5–7). However, poor tolerance to intensive treatment by older patients is also an important factor. Although intensive induction chemotherapy is frequently well tolerated (1, 2), postremission therapy may be less well tolerated. Only 29% of patients > 60 years of age could tolerate repetitive cycles of high-dose cytarabine, compared with 62% of younger patients in a recent CALGB trial investigating high-dose cytarabine postremission therapy (1). The biology of the disease and the

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³ The abbreviations used are: AML, acute myeloid leukemia; DFS, disease-free survival; OS, overall survival; CR, complete remission; CALGB, Cancer and Leukemia Group B; NK, natural killer; IL-2, interleukin 2; ULBP, FAB, French-American-British; UL-16 binding protein; PB, peripheral blood; PBMC, peripheral blood mononuclear cells BM, bone marrow; RL4, RAE-1-like transcript 4; RT-PCR, reverse transcriptase-polymerase chain reaction.

limited tolerance to intensive postremission therapy in older AML patients indicate the need for alternative, well-tolerated, noncross-resistant therapies in the postremission period to prevent relapse.

Several studies indicate that NK cells can mediate an antileukemic effect (8, 9). Lysis of leukemic cells (and other targets) by NK cells is regulated by a balance of activating and inhibitory surface receptors that interact with specific MHC class I and class I-like molecules expressed on target cells (10–12). Under physiological conditions, recognition of ‘self’-MHC class I molecules on target cells by inhibitory receptors on NK cells of the killer cell immunoglobulin receptor and CD94/NKG2 families generates signals that lead to termination of NK cell activation. Autologous tumor cells missing MHC class I molecules or expressing altered self-MHC class I molecules trigger activation of NK cells and lysis. Recently, it was demonstrated that triggering signals mediated by the activating receptor NKG2D can override inhibitory signals generated by the interaction of killer cell immunoglobulin receptor with MHC class I molecules (13, 14). Expression of NKG2D ligands, including MHC class I chain-related molecules MICA and MICB, and molecules of the ULBP family by autologous tumor cells that also express unaltered MHC class I molecules may therefore be relevant for NK cell killing. To date, the expression of NKG2D ligands on fresh leukemic blasts has not been reported.

IL-2 can augment the cytotoxic effect of NK cells (15). IL-2-activated NK cells can lyse fresh leukemic blasts that are generally resistant to killing by unstimulated NK cells (16, 17). Furthermore, IL-2 alone or in combination with *ex vivo*-generated IL-2-activated NK cells has been effective in treating leukemia in animals (17–19) and has induced clinical remissions in AML patients with overt relapse (20–22). The dose and schedule of IL-2 in these studies have been highly variable, and in the majority of cases, high doses of IL-2 ($3\text{--}18 \times 10^6$ IU/m²/d) were used, usually with significant toxicity. Caligiuri *et al.* (23–25) demonstrated that low-dose infusion of IL-2 results in the *in vivo* selective expansion of a normally small subset of NK cells (CD56^{bright} CD16^{dim}) expressing the high-affinity IL-2 receptor. The expanded NK cell population, however, demonstrated cytotoxic activity against NK-resistant cells only on incubation in higher concentrations of IL-2, which saturate the intermediate-affinity receptors. On the basis of the hypothesis that NK cell expansion results from engagement of high-affinity IL-2 receptors with low-dose IL-2 and that cytotoxic activity requires the activation of intermediate-affinity receptors only achieved with intermittent pulse doses with higher concentrations of IL-2, we designed a regimen of extended low-dose IL-2 with interval intermediate-dose IL-2 pulsing (26). This regimen was well tolerated when used in patients with a variety of solid tumors (26). In the latter study, however, patients were relatively young and IL-2 therapy did not follow intensive cytoreductive antileukemic chemotherapy.

The primary aim of this study was to investigate the tolerability of low-dose IL-2, with or without intermediate pulse doses, as postremission therapy in AML patients ≥ 60 years after intensive remission induction and consolidation chemotherapy. As a secondary aim, in a subset of patients where leukemic cells from diagnosis were available, we also examined

for the expression MICA and MICB and the ULBPs by leukemic cells. Expression of these ligands by primary leukemic cells has not been previously reported but is likely to be an important mediator of NK cell killing of autologous target cells.

PATIENTS AND METHODS

Patient Eligibility. All patients were required to be treated on the AML therapy protocol CALGB 9420 and to have no morphological evidence of leukemia after completion of induction and consolidation therapy. Eligibility criteria for CALGB 9420 included age ≥ 60 years, biopsy proven AML with FAB M0-M2 or M4-M7 defined by standard criteria (27), no prior treatment for AML, no antecedent hematological disorder or history of prior chemotherapy exposure, and adequate renal and hepatic function. Patients with acute promyelocytic leukemia (AML-M3) were excluded and treated on other protocols. The protocol was reviewed and approved by local institutional review boards, and written consent for both AML chemotherapy and IL-2 therapy phases of the study was obtained from all patients.

Induction and Consolidation Therapy. Protocol CALGB 9420 was a Phase I study undertaken to define the appropriate doses of daunorubicin to be given together with cytarabine, etoposide, and the multidrug resistance glycoprotein 1 modulator PSC-833 (28). Induction and consolidation consisted of cytarabine- and anthracycline-based chemotherapy with or without PSC-833. The details of induction and consolidation therapy, together with the results and toxicity of this portion of the protocol, have been reported previously (28).

IL-2 Therapy. Patients were eligible for treatment with recombinant human IL-2 (Proleukin; Chiron/Cetus Corporation, Emeryville, CA) if they were in continuing CR as documented by the presence of $< 5\%$ blasts in the BM on an aspirate and biopsy performed after hematological recovery from postremission chemotherapy. In addition, patients were required to have recovered from all nonhematological toxicity, a neutrophil count of $\geq 1.0 \times 10^9$ /liters and a platelet count of $\geq 75 \times 10^9$ /liters without growth factor or platelet transfusion support, respectively, and a satisfactory hepatic and renal function. Initially, patients received IL-2 at a dose of 1×10^6 IU/m²/day by s.c. injection for a total of 90 days. After the first 16 patients were treated, the low-dose IL-2 therapy was judged to be tolerable. The protocol was then modified to incorporate intermittent 3-day intermediate-dose pulse IL-2 in the dose schedule shown in Fig. 1. IL-2 was initiated at 1×10^6 IU/m²/day by s.c. injection and continued on days 1–28, 33–42, 47–56, 61–70, and 75–84 of therapy. Beginning on day 29, 3-day sequences of s.c., escalating intermediate-dose pulse IL-2 were administered on days 29–31 (6×10^6 IU/m²/day), days 43–45 (9×10^6 IU/m²/day), and days 57–59, 71–73, and 85–87 (12×10^6 IU/m²/day). Patients did not receive IL-2 on days 32, 46, 60, and 74. Low-dose IL-2 was interrupted for 2 days if any grade 3–4 toxicity developed and then recommenced at 0.8×10^6 IU/m²/day. If grade 3–4 toxicity recurred, low-dose IL-2 was stopped again for 2 days and then recommenced at 0.6×10^6 IU/m²/day. Continued grade 3–4 toxicity resulted in discontinuation of IL-2. Similarly, intermediate-dose IL-2 therapy was interrupted for 2 days if grade 3–4 toxicity developed and was then recom-

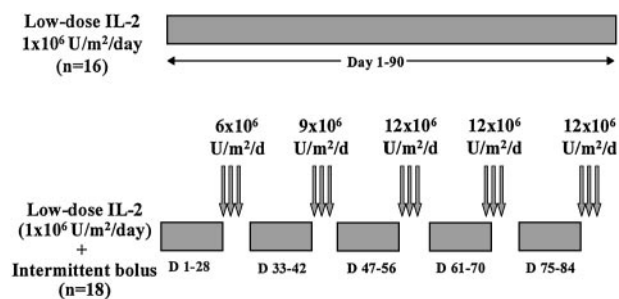


Fig. 1 Schema of IL-2 postremission therapy phase of CALGB 9420. IL-2 was given once/day by s.c. injection.

menced with a dose reduction of 1.5×10^6 IU/m²/day. Intermediate-dose IL-2 therapy was again interrupted and recommenced with an additional 1.5×10^6 IU/m²/day dose reduction for recurrent toxicity. Intermediate-dose pulse IL-2 was discontinued if grade 3–4 toxicity recurred, although low-dose IL-2 therapy was continued. During therapy, patients received supportive care with acetaminophen and nonsteroidal anti-inflammatory drugs, and RBC and platelet transfusions as required.

Assessment of *in Vivo* Lymphokine-activated Killing.

The function of the *in vivo* IL-2-expanded NK cells was tested. Patient PB was collected both before and 24 h after receiving an intermediate-dose IL-2 bolus (usually after the second 3-day sequence). PBMCs were isolated using Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation. RBCs were lysed, after which PBMCs were washed in RPMI 1640 supplemented with 10% human AB serum (C-six Diagnostics, Mequon, WI) and counted with a hemocytometer. PBMCs were immediately used without addition of any cytokines in a standard 4-h *in vitro* ⁵¹Cr release assay with the NK-resistant COLO 205 cells as targets at an E:T ratio of 25:1 (29). Results are expressed as percentage of specific lysis calculated by the following formula: [(experimental release – spontaneous release)/(maximal release – spontaneous release)] × 100. Aliquots of each PBMC sample were also stained with CD56-PE and CD3-FITC monoclonal antibodies and analyzed by flow cytometry for the percentage of CD56+CD3⁻ NK cells using standard techniques (30).

Analysis of MICA, MICB, and ULBP Expression by Leukemic Blasts.

BM or PB samples were obtained from patients at diagnosis and from consenting healthy adult donors and cryopreserved in liquid nitrogen or used directly. Thawed samples were analyzed for mRNA expression of the NKG2D ligands MICA and MICB, the ULBP-1, ULBP-2, and ULBP-3, and RAE-1-like transcript 4 (RL4), a putative NKG2D ligand and fourth member of the ULBP family (GenBank accession no. AY054974). BM and PBMC were isolated by density centrifugation over a Ficoll-Hypaque (Sigma) gradient at $400 \times g$ for 30 min. Two million mononuclear cells from PB and BM were each harvested for RNA at this point. In the case of cells obtained from healthy donors, the remaining mononuclear cells were enriched for CD34⁺ cells by magnetic separation (Milyeni Biotech), according to the manufacturer's protocol. The purity of this population ($\geq 95\%$) was demonstrated by staining with anti-CD34-PE and visualization on a FACStar Plus cell sorter

(Becton Dickinson). MCF7 and LNCaP cell lines were obtained from American Type Culture Collection (Manassas, VA) as positive controls. The HTB-148 cell line was generously provided by Dr. Stephan Tanner.

For reverse transcriptase-PCR, 1–2 million cells were harvested for RNA using RNeasy Mini Kit (Qiagen), according to the manufacturer's protocol. Two μ g of RNA were reverse transcribed to cDNA using 300 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) with 20 pmol each of random hexamer and oligodeoxythymidylic acid primers. Approximately 200 ng of cDNA were used as template in PCR using 1.25 units of AmpliTaq DNA polymerase (Perkin-Elmer Applied Biosystems) on a GeneAmp PCR System 9700 instrument (Perkin-Elmer Applied Biosystems). All primer oligonucleotides were purchased from Sigma-Genosys. Primer sequences and cycling parameters for MICA and MICB were as published (31). Primers for ULBP1, ULBP2, ULBP3, and RL4 were designed using PrimerSelect 4.0 (DNASTAR): ULBP1 (522-bp product) sense, 5'CCGCCAGCCCCGCTTCTCT3' and antisense, 5'CATCCCTGTTCTTCTCCACTTCT3'; ULBP2 (478-bp product) sense, 5'GTCACAACGGCCTGGAAAGCACAA3' and antisense, 5'TGAAGCAGGGGAGGATGATGAGGA3'; ULBP3 (454-bp product) sense, 5'CGGGCCGACGCTCACTCT3' and antisense, 5'GTCCGC-TATCCTTCTCCACTTCT3'; and RL4 (465-bp product) sense, 5'GGAGAAGTGGGGCGAGACCT3' and antisense 5'TTGCCACCAGACACAGATGAGAA3'. Amplification was performed for 35 cycles (30 s at 95°C, 30 s at 60°C, and 45 s at 72°C), followed by 7-min extension step at 72°C. PCR products were separated on 0.8% agarose gels and visualized with ethidium bromide.

Statistical Considerations.

Descriptive statistics were used to describe the baseline characteristics of patients enrolled into the IL-2 therapy component of the study. Toxicity was measured according to the CALGB-expanded common toxicity criteria. OS and DFS were assessed from the start of IL-2 therapy, and the survival distributions were estimated using standard statistical methodology (32).

RESULTS

Patient Population.

Between January 1995 and July 1997, 111 patients were registered on CALGB 9420. One patient was never treated. Of the remaining 110 patients, 50 (45%) achieved CR. Of the complete responders, 35 patients started the IL-2 phase of the protocol. Reasons for not proceeding on to IL-2 therapy included relapse before treatment ($n = 5$), withdrawal or refusal ($n = 3$), persistent toxicity from preceding chemotherapy ($n = 2$), initiation of other therapy ($n = 2$), other intercurrent disease ($n = 1$), death ($n = 1$), and physician's decision to remove patient from protocol ($n = 1$). Of the 35 patients who started IL-2, 1 patient received only 1 day of the drug and was taken off study because of relapse and is not considered evaluable for assessing toxicity for this phase of the study. Table 1 shows the baseline characteristics of the 34 patients who are the subject of this report. There were 17 males and 17 females. The median age was 67 years (range, 60–76 years). The majority presented with FAB AML-M1, M2, and

Table 1 Patients characteristics

	No. of patients
No. of patients treated with IL-2	34
Gender	
Male	17
Female	17
Median age (range), years 67 (60–76)	
FAB morphology	
M0	1
M1	10
M2	8
M4	8
M5a	4
M5b	3
Cytogenetics	
46, XY	12
t(8;21)(q22;q22)	1
t(9;11)(p22;q23)	1
inv(3)(q21–q26)	1
Sole +8	2
t(2;11)(p23–q23)	1
Complex abnormalities	2

M4 subtypes. Twenty of these patients had evaluable cytogenetics; 8 patients (40%) had a karyotypic abnormality.

IL-2 Therapy. The median time from CR to the start of IL-2 therapy was 56 days (range, 6–164 days). Of the 34 evaluable patients, 16 received only low-dose IL-2 for 90 days, and after modification of the protocol, 18 patients received low-dose IL-2 with intermediate pulse dose therapy. Thirteen of 16 patients assigned to low-dose IL-2 completed all 90 days of planned therapy. Treatment was discontinued in 2 patients after 21 and 73 days because of excessive fatigue and in a third for intercurrent lumbar disk surgery. For those completing the planned low-dose IL-2 treatment, dose reduction by 25% was required in 3 patients and by 50% in 1 patient because of grade 3 or 4 thrombocytopenia.

Of those receiving low-dose IL-2 in combination with intermediate pulse dose therapy, 11 of the 18 eligible patients received all five of the planned intermediate pulse dose treatments. Four patients did not complete treatment, one because of leukemia relapse before the first pulse, two after the second pulse dose, and one after the third pulse dose. One patient received only four intermediate pulse dose IL-2 treatments in error. Pulse dose therapy was discontinued in two patients after the second and third pulses, respectively, because of grade 3 hematological toxicity (neutropenia and thrombocytopenia), although low-dose IL-2 was continued to completion. In the 11 patients who completed all five IL-2 pulses, 3 patients required 20–40% dose reduction of intermediate-dose IL-2.

Toxicity. Side effects that occurred at least once in patients receiving low-dose IL-2 therapy and low-dose IL-2 with intermediate pulse dose therapy are depicted in Table 2. In patients receiving low-dose IL-2, most toxicity was grade 1 or 2, consisting predominantly of fatigue, fever, injection site reactions with local pain, erythema or induration, nausea or vomiting, anemia, and thrombocytopenia. All were easily managed with simple supportive measures. Grade 3 or 4 toxicities were uncommon and were mostly hematological, with lymphopenia, neutropenia, anemia, and thrombocytopenia. Although 4 pa-

tients required dose reductions for grade 3 or 4 toxicities, only 2 patients discontinued therapy because of nonhematological toxicity (grade ≥ 3 fatigue in both instances). All grade 3 and 4 toxicity was rapidly reversible on discontinuation of IL-2.

In patients receiving low-dose IL-2 with intermediate pulse dose therapy, the spectrum of reported toxicity was similar to that seen in the low-dose IL-2-treated patients. There was mostly grade 1–2 fever and chills, nausea and vomiting, injection-site skin reactions, anemia, thrombocytopenia, neutropenia, and lymphopenia. Mild elevation in the blood urea nitrogen was also seen, but no cases of frank acute renal failure were seen. Grade 3 and 4 nonhematological toxicity occurred more frequently than in patients who received low-dose IL-2 only (five *versus* two episodes). These included fatigue, fever/chills, infection, hypotension, and elevation of liver enzymes. All were reversible and easily managed. Grade 3 and 4 hematological toxicity also occurred more frequently than in patients who received low-dose IL-2 only therapy. In particular, grade 3 and 4 anemia, neutropenia, and thrombocytopenia were more common in patients who also received intermittent pulse IL-2. The latter toxicity, however, was easily managed. Only 2 patients received less than the planned number of IL-2 pulses because of grade ≥ 3 neutropenia and thrombocytopenia, which occurred during preceding low-dose IL-2 treatment. Therefore, although overall toxicity was at least twice as common in the intermediate-dose pulse IL-2 therapy cohort, it was easily managed with simple supportive therapy. Importantly, no deaths were attributable to either low-dose or intermediate pulse dose therapy.

Functional Activity of IL-2-expanded NK Cells. A subset of patients was examined for the ability of the intermediate-dose bolus IL-2 to activate the expanded NK cells *in vivo*. Patient PBMCs were collected both immediately before and 24 h after receiving their intermediate dose IL-2 bolus and tested for their ability to lyse NK-resistant COLO 205 target cells. All 4 patients tested demonstrated similar significant ($P < 0.05$) *in vivo* increases in cytotoxicity against NK-resistant tumor targets, whereas having similar NK cell percentages both before and 24 h after the IL-2 bolus (Fig. 2). As the percentage of NK cells present at both time points was very similar, these data provide direct evidence in support of *in vivo* lymphokine-activated killing.

Expression of MICA, MICB, and ULBP Transcripts by Leukemic Cells. The expression of transcripts of the known activating ligands for the NKG2D receptor was examined by reverse transcriptase-PCR in BM leukemic cells obtained at the time of diagnosis from 8 patients, where this was available. Fig. 3 shows that MICA transcripts were detected in only 2 cases, whereas MICB transcripts were detected in 5 cases. However, MICB was also detectable in normal PB, BM, and highly enriched CD34⁺ hematopoietic progenitor cells. Only 1 case (case 4) coexpressed MICA and MICB. Expression of ULBPs was more frequent. ULBP-1 was found in 4 cases examined, whereas expression of ULBP-2 was found in 5 cases. Expression of ULBP-3 was expressed in all cases of AML, but like MICB, the ligand was expressed on normal leukocytes, BM, and highly enriched CD34⁺ cells. RL4 was expressed in 2 AML cases only. The absence of MICA, ULBP-1, ULBP-2, and RL4 transcripts in normal blood cells suggests that expression of these ligands may be specific to leukemic cells.

Table 2 Toxicity during low-dose IL-2 therapy (n = 16) and low-dose IL-2 with intermediate-dose pulse therapy (n = 18)

	Number (%) of patients within grade of toxicity							
	Grade 1		Grade 2		Grade 3		Grade 4	
	LD ^a	LD + IP	LD	LD + IP	LD	LD + IP	LD	LD + IP
Fatigue	2 (13)	5 (28)	1 (6)	4 (22)	0	1 (6)	0	0
Injection site reactions	6 (38)	3 (17)	7 (44)	5 (28)	1 (6)	0	0	0
Fever/chills	2 (13)	3 (17)	13 (81)	5 (28)	0	1 (6)	1 (6)	0
Nausea/vomiting	5 (31)	2 (11)	3 (19)	4 (22)	0	0	0	0
Myalgia/arthralgia	3 (19)	3 (17)	2 (13)	0	0	0	0	0
Alopecia	1 (6)	3 (17)	0	4 (22)	0	0	0	0
Neurological	2 (13)	3 (17)	1 (6)	2 (11)	0	0	0	0
Infection	2 (13)	0	0	3 (17)	0	1 (6)	0	0
Edema	3 (19)	1 (6)	1 (6)	0	0	1 (6)	0	0
Hypotension	1 (6)	1 (6)	1 (6)	0	0	0	0	0
Anemia	3 (19)	4 (22)	10 (63)	6 (33)	0	3 (17)	0	0
Thrombocytopenia	9 (56)	7 (39)	3 (19)	3 (17)	2 (13)	3 (17)	0	1 (6)
Neutropenia	2 (13)	4 (22)	3 (19)	0	1 (6)	2 (11)	1 (6)	2 (11)
Lymphopenia	0	2 (11)	2 (13)	2 (11)	6 (38)	2 (11)	4 (25)	3 (17)
Eosinophilia	0	0	1 (6)	0	0	2 (11)	0	0
Elevated BUN	8 (50)	5 (28)	0	0	0	0	0	0
Liver enzyme abnormalities	2 (13)	1 (6)	1 (6)	1 (6)	0	1 (6)	0	0

^a LD, low-dose IL-2; LD + IP, low-dose IL-2 with intermediate-dose pulse therapy.

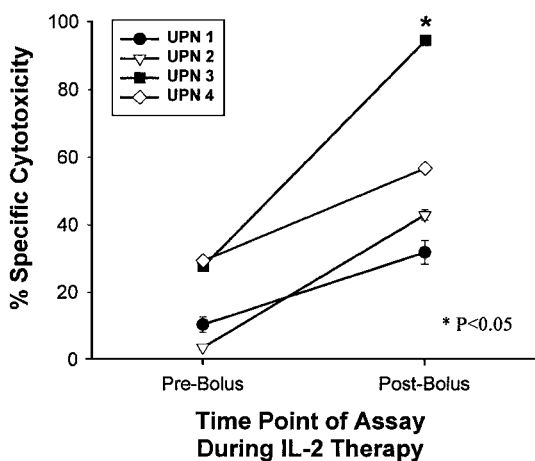


Fig. 2 Intermediate pulse dose IL-2 activates NK cells *in vivo* in patients with AML to kill NK-resistant tumor targets *in vitro*. Four patients receiving low-dose IL-2 therapy with intermediate pulse dose were assayed before and 24 h after bolus 4–6. Fresh blood mononuclear cells were used as effector cells in a ⁵¹Cr release assay against the NK-resistant COLO 205 cell line. There was a significant increase in the specific lysis mediated by PBMC after *in vivo* IL-2 pulse, compared with prepulse ($P < 0.05$). At these time points, similar percentages of CD56⁺ NK cells were present, suggesting that lymphokine-activated killer cells were generated *in vivo* after pulse dose IL-2.

Clinical Outcome. The estimated DFS and OS distributions measured from the start of IL-2 therapy for the 34 evaluable patients are shown in Fig. 4. The median DFS is 0.6 years, with 7 patients remaining in continuing CR for 1.5–4.8 years. The median OS for the group is 1.1 years. Seven patients (21%) are alive and free of disease at last follow-up between 2.2 and 4.9 years from the start of IL-2 therapy.

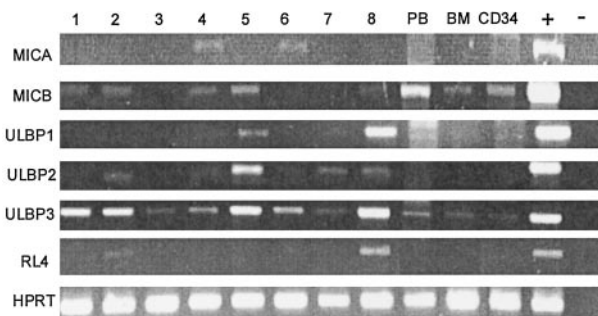


Fig. 3 Reverse transcriptase-PCR analysis of known and putative NKG2D ligands in AML blast cells from CALGB 9420 patients at diagnosis (patients 1–8) and PB, BM, and purified CD34⁺ cells from normal donors. Positive (+) control cDNA was from MCF7 (MICA, MICB), LNCaP (ULBP1, ULBP2, ULBP3), and HTB148 (RL4, HPRT) cell lines. No template was added to the negative (–) control reactions.

DISCUSSION

Studies have demonstrated that IL-2 can be effective in patients with overtly relapsed AML, with clinical reduction in leukemic burden and, in some instances, induction of CRs (20–22). It has been postulated, however, that IL-2 might be more effective in the treatment of minimal residual disease after intensive chemotherapy (33–35). In this study, we investigated the feasibility and safety of administering low-dose IL-2, with and without intermediate pulse dose IL-2, as postremission therapy after intensive induction and consolidation chemotherapy to elderly AML patients. This group of AML patients ≥60 years is known to be less tolerant of therapy and to have a poor outcome (36). To our knowledge, a study assessing the safety of IL-2 in elderly AML patients after achievement of CR has not been reported.

Our results indicate that IL-2 administration after intensive

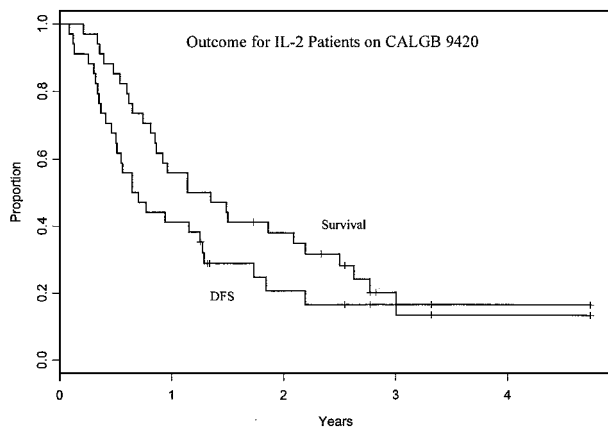


Fig. 4 DFS and OS of patients treated with the IL-2 regimen.

induction and consolidation is well tolerated by patients with AML ages ≥ 60 years. Whereas the majority of patients experienced toxicity, this was mild in most instances and easily managed with simple supportive measures. The addition of pulses of intermediate-dose IL-2 to the low-dose regimen to activate the *in vivo*-expanded NK cells increased the frequency and severity of nonhematological and hematological toxicity, although this was reversible and manageable. No life-threatening events occurred during either prolonged low-dose IL-2 therapy or intermediate pulse dose therapy. Fatigue and malaise, almost entirely grade 1–2, were the most common side effects experienced by almost all patients. In only 2 patients receiving low-dose IL-2 only, the severity of fatigue was sufficient to remove the patients from study. Other side effects did not prevent any patient from continuing therapy. However, hematological toxicity, particularly anemia and thrombocytopenia, was significant with each occurring in $>70\%$ of patients receiving either low-dose IL-2 only or low-dose IL-2 with intermediate pulse dose therapy. Neutropenia was less frequent. Although myelosuppression was more common than previously reported in less extensively pretreated patients (37), it was mostly mild and rapidly reversible on interruption or attenuation of IL-2 therapy. Indeed, grade 3–4 neutropenia and thrombocytopenia resulted in the discontinuation of therapy in only 2 patients, both in the intermediate pulse dose cohort. The increased frequency of myelosuppression may be related to decreased marrow reserve because of recent intensive induction and consolidation chemotherapy and/or the older age of our patients. Otherwise, the toxicity profile was similar to that seen in a younger group of metastatic cancer patients treated with the same low-dose IL-2 and intermediate pulse dose IL-2 regimen (37).

There are very few reports of IL-2 postremission therapy in AML patients, and the reported doses and schedules of IL-2 have varied. Cortes *et al.* (38) recently reported their experience with a low-dose IL-2 schedule of 4.5×10^5 unit/m²/day by continuous i.v. infusion for 12 weeks, with weekly bolus injection of 1×10^6 units/m² IL-2 starting on day 8. This schedule resulted in similar toxicity to that seen our study, with fever, chills, local skin reactions, and thrombocytopenia being the most common side effects. Unlike these investigators, however,

we found more severe anemia and lymphopenia, which may be attributable to the higher doses of IL-2 used in our study. However, despite the lower doses of IL-2 used and the younger median age of patients treated, 7 of 18 patients required interruption of IL-2 therapy in the study by Cortes *et al.* (38). The low-dose IL-2 schedules used by these investigators (38) and by us, however, have been much better tolerated than higher dose schedules used in other studies where hemodynamic and metabolic complications have been severe. For example, McDonald *et al.* (39) treated 9 AML patients in first CR with high-dose (3×10^6 units/m²/day) IL-2 given for 5–45 days intermittently. The schedule was poorly tolerated, with a mean tolerated dose of 57% of the planned dose. In 14 patients ages ≤ 55 years with relapsed AML, treatment with repeated 5-day cycles of escalating high-dose IL-2 (up to 18×10^6 units/m²/day) followed by maintenance therapy with 4×10^6 units/m²/day of IL-2 was associated with marked toxicity (20). Hypotension, fever, and vomiting of grade ≥ 3 occurred in 90% of patients, and grade ≥ 3 oliguria was seen in all patients. In addition, 43% of patients developed documented infections during high-dose IL-2 therapy (20). Similarly, in a Phase II study of high-dose IL-2 (16 – 24×10^6 units/m²/day) in patients with relapsed leukemia, significant toxicity was observed (21). Grade 4 thrombocytopenia was seen in 84% of patients, and in 10 of 49 patients treated, hemodynamic and metabolic toxicities led to discontinuation of treatment.

In this study, we initially used a low maintenance dose of IL-2 of 1×10^6 units/m²/day given by s.c. injection for 90 days. This was based on demonstration by Caligiuri *et al.* (40) in a Phase I study of IL-2 therapy in patients with advanced malignancy that the maximum-tolerated dose of IL-2 was 1.25×10^6 units/m²/day. At this dose, serum IL-2 concentrations between 10–100 pmol are achieved, which are sufficient to saturate high-affinity IL-2 receptors found on a normally small population ($\sim 10\%$) of circulating NK cells (CD56^{bright} CD16^{dim}). Saturation of high-affinity IL-2 receptors by low-dose IL-2 resulted in the *in vivo* selective expansion of CD56^{bright} CD16^{dim} NK cells (23–25, 40). However, these expanded NK cells are not activated and required higher concentrations of IL-2 to activate intermediate-affinity IL-2 receptors to lyse NK-resistant tumor cells (23). Therefore, our protocol was modified to incorporate intermediate pulse dose IL-2 therapy to achieve *in vivo* IL-2 levels that could engage a significant proportion of intermediate-affinity receptors on the expanded NK cell population to augment their cytotoxicity. In a previous study of patients with advanced cancer, this regimen resulted in significant total CD56⁺ NK cell ($796 \pm 210\%$) and CD56^{bright} cell ($3247 \pm 1382\%$) expansion with low-dose IL-2 treatment (26). With intermediate-dose pulsing, serum IL-2 levels exceeded 100 pmol with *in vivo* activation of the expanded cells as evidenced by significantly higher IFN- γ production by PBMC after pulse (26). In addition, as we have demonstrated in 4 patients in this study, fresh PBMC isolated after intermediate-dose pulse therapy showed significantly higher cytolytic activity against the NK-resistant COLO 205 tumor target, compared with prepulse PBMC, while having a similar percentage of CD56⁺ NK cells.

The efficacy of the IL-2 regimens used in our study in prolonging remission or preventing relapse in patients with

AML is currently unknown. In the study by Cortes *et al.* (38), who used a similar approach in AML patients in first CR, a comparison of CR duration and survival of IL-2 treated with that of historical controls not treated with IL-2 suggested a benefit from IL-2 therapy. However, this result must be interpreted with caution given the inherent limitations associated with comparison to historical controls, and definitive conclusions await the results of ongoing randomized studies.

An interesting finding in our study is the demonstration of transcripts for several NKG2D ligands, MICA and ULBP-1 and 2, in primary leukemic blasts but not in normal BM-purified CD34⁺ cells or PBMC. High MICA and MICB expression has been demonstrated by many human epithelial tumors (31, 41) and more recently on the JA3 and Raji leukemic cell lines (31), although expression by primary myeloid leukemic blasts had not been reported. Our results show that MICA and MICB expression is also seen in blasts from a proportion of primary human AMLs and is not restricted only to primary human epithelial tumors. A limitation of our finding is that surface expression of these ligands was not studied. However, the presence of 54 MICA and 16 MICB alleles makes study of these molecules on the cell surface by monoclonal antibodies difficult because it is not currently known to what extent the currently available antibodies can detect all these alleles. In contrast to the MIC molecules, transcripts of the ULBP were more frequently expressed, with all our AML cases expressing at least ULBP-3. As with the MIC ligands, surface expression of ULBPs could not be assured from detection of their transcripts. Indeed, in one study, tissue expression of ULBP mRNA did not always correlate with surface expression as detected by monoclonal antibodies, suggesting that surface expression of the ULBPs may be regulated at a posttranscriptional level (14). Furthermore, as MICB and ULBP-3 transcripts were found in samples of normal blood, BM, and CD34⁺ enriched cells, we cannot exclude the possibility that their demonstration in our AML cases may be because of contaminating nonleukemic cells. Therefore, further study of activating ligand expression on the surface of leukemic cells as monoclonal antibody reagents become available is required. The observation that transcripts of some of the activating ligands (MICB and ULBP-3) is also expressed in normal blood and BM cells suggests the possibility that these ligands may not be as important as MICA, ULBP-1 and ULBP-2, and RL4 for the recognition and selective killing of leukemic cells by autologous NK cells. Such a difference may be related to potential differences in the affinity of different ligands to NKG2D. Alternatively, the selective killing of leukemic cells by autologous NK cells may be explained by a dissociation between mRNA expression and surface expression of ligand in normal cells, such that while normal cells express MICB and ULBP-3 mRNA, surface expression may be low or lacking. This requires additional investigation and is currently being studied by our group. Indeed, elucidation of the relative importance of the different activating ligands in triggering NK cells is relevant because AML blasts have recently been demonstrated to express normal levels of MHC class I molecules (42), which could be expected to engage inhibitory receptors on autologous NK cells. The study of the relevance of activating ligand surface expression may assist in the selection of AML cases that have high ligand

expression and, therefore, may be most suitable for IL-2-based therapy.

In conclusion, our study has demonstrated that IL-2 postremission therapy, particularly low-dose IL-2 to expand NK cells, with intermittent high-dose bolus therapy to activate these expanded cells, is well tolerated in elderly AML patients. The efficacy of this regimen is currently being tested in a randomized study of patients ≥ 60 years in first CR (CALBG 9720).

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