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3. The Royal Marsden Hospital Bone-Marrow Transplantation Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977; 2:242-4.

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5. Karlsson S, Humphries RK, Gluzman Y, Nienhuis AW. Transfer of genes into hemopoietic cells using recombinant DNA viruses [abstract]. Blood 1984; 64(Suppl 1):58a.

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Symposium
IDARUBICIN:
NEW PHARMACOLOGICAL INSIGHTS
AND CLINICAL APPLICATIONS
Taormina, Italy, October 1997

Chairmen: Prof. Sergio Amadori, Prof. Mario Lazzarino

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PHARMACOLOGY OF CHRONIC ORAL DAILY ADMINISTRATION OF IDARUBICIN

GIUSEPPE TOFFOLI,° ROBERTO SORIO,* PAOLA AITA,° DIANA CRIVELLARI,* GIUSEPPE CORONA,°
GIOIA RIMONDI,* ALESSANDRA BEARZ,* FRANCO STOCCO,# ISABEL ROBIEUX,* MAURO BOIOCCHI°

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ABSTRACT

Idarubicin (4-demethoxydaunorubicin) (IDA) is a daunorubicin analogue with substantial activity in hematologic malignancies and solid tumors. Among several reasons, IDA is of interest because of its main metabolite derivative, the C-13 alcohol analogue, idarubicinol (IDOL). Previous studies have suggested that IDOL, unlike other anthracycline metabolic derivatives, possesses a striking growth-inhibitory activity in tumor cell lines. This suggests that IDOL, like IDA could be useful in circumventing MDR. IDA is bioavailable in an oral dosage form. After oral administration of IDA to the patients, the concentration of IDOL quickly exceeds that of IDA and is retained in the plasma for a longer period. Hence, administration of IDA to cancer patients results in a much greater overall exposure of the tumor to IDOL than to the parent compound. At the Oncology Center (CRO) in Aviano we performed a dose-finding and pharmacokinetic (PK) study of chronic daily oral IDA with intrapatient escalation in patients with metastatic breast cancer (MBC). All the patients were pretreated with anthracyclines (the cumulative dose was 530 mg and 264 mg, respectively, for epirubicin and DOX) and had at admittance a PS ≤ 2 and a left ventricular ejection fraction >50%. IDA (1 mg capsules) was administered orally twice a day for 21 days every two weeks. Treatment was continued at escalating doses until progression or intolerance. Twenty-five patients were enrolled. MTD has not yet been reached and clinical results are reported in Table 1. Treatment was well-tolerated in all but one patient (300 ANC at day 28). Three patients had tox G3 ANC for more than three weeks after 3, 6, and 7 mg doses, respectively. Two of them stopped chemotherapy after 1 cycle and 1 patient stopped after 2 cycles (6 mg doses). Despite previous treatments with anthracyclines (the mean cumulative dose before entering the study was 530 and 264 mg, respectively, for epirubicin and DOX) no cardiotoxicity due to IDA treatment was observed. This trial demonstrates the feasibility of chronic daily IDA administration. At the dosage reported, treatment was generally well tolerated. The PK findings (high IDOL concentrations) and the unexpected G4 myelotoxicity in patients with the highest IDOL plasma concentrations suggest that IDOL is clinically relevant.

Key words: chemotherapy, idarubicin, idarubicinol, oral administration

Anthracyclines have proven to be among the most effective drugs in the therapy of malignant diseases. However, their clinical use is restricted by acute bone marrow toxicity and chronic cumulative cardiotoxicity; moreover, the development of drug resistance is an important factor limiting the use of anthracyclines.[1] A number of analogs of daunorubicin (DAU) and its 14-hydroxy derivative, doxorubicin (DOX), have been synthesized with the aim of reducing side effects and overcoming drug resistance to anthracyclines.

Idarubicin (4-demethoxydaunorubicin) (IDA) is a DAU analog with substantial activity in hematologic malignancies and solid tumors.[2] The therapeutic index of IDA in experimental animals and its clinical efficacy in acute myelogenous leukemia are more favorable than DAU,[3,4] probably because of its pharmacological characteristics and intracellular action mechanism. IDA has greater growth inhibitory activity in experimental human tumors,[5] possesses superior activity in DNA damage,[6] and is less cardiotoxic than DAU.[7] *In vitro* studies indicate that it is more effective than the parent compound in tumor cell lines with the multidrug resistant

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(MDR) phenotype.[8,9] We previously demonstrated that in MDR LoVo human cell lines, the P-glycoprotein (P-gp) localized in the subcellular structures affected DAU, but not IDA nuclear/cytoplasmic distribution; this may explain why MDR cell lines were less resistant to IDA than to DAU.[10]

IDA is of further interest because of its main metabolite derivative, the C-13 alcohol analog, idarubicinol (IDOL). Conversion of anthracyclines to their alcohol metabolites via ketoreductase metabolism is generally regarded as an inactivation pathway on the basis of the relatively lower activity of the corresponding alcohol metabolites. Previous studies have suggested that IDOL, unlike other anthracycline metabolic derivatives, possesses a striking growth inhibitory activity in tumor cell lines.[11] Moreover, IDOL is effective in MDR cell lines.[12,13] This suggests that IDOL, like IDA could be useful in circumventing MDR.

IDA is bioavailable in an oral dosage form. After oral administration of IDA to the patients, the concentration of IDOL quickly exceeds that of IDA and is retained in the plasma for a longer period.[14] Hence, administration of IDA to cancer patients results in a much greater overall exposure of the tumor to IDOL than to the parent compound. There are several advantages to an oral dosage form. Currently, anthracyclines must be administered by the i.v. route, which requires an office, hospital, or clinical visit. An oral dosage form would improve the ease of administration, eliminate the costs associated with i.v. therapy, eliminate the potential for tissue damage from drug extravasation, and possibly improve patient compliance. However, there is another important aspect deriving from the use of oral IDA, i.e., the possibility of investigating new schedules for this drug. There is now a growing interest in the use of prolonged infusion schedules or chronic daily administration rather than single dose regimens[15] as a possible way to improve the therapeutic index of chemotherapy. One simple pharmacokinetic goal of this approach would be the reduction of potentially toxic peak concentrations while maintaining the overall drug exposure (i.e., the area under the curve of plasma concentration versus time; AUC or C x T). Moreover, the degree of resistance tends to be lower with continuous vs drug exposure in model systems.[16] The high half-life of IDA (10-33 h) and its active metabolite IDOL (36-64 h) are favorable features for an oral chronic daily administration of the drug since these pharmachokinetic characteristics avoid swayings in plasma drug concentrations, thus simulating a prolonged i.v. infusion and allowing tumor cells to be continuosly exposed to the drug.

At the Oncology Center (CRO) in Aviano we performed a dose-finding and pharmacokinetic (PK) study of chronic daily oral IDA with intrapatient escalation in patients with metastatic breast cancer (MBC). All the patients were pretreated with anthracyclines (cumulative dose was 530 mg and 264 mg, respectively, for epirubicin and DOX) and had at admittance a PS ≤ 2 and a left ventricular ejection fraction >50%. IDA (1 mg capsules) was administered orally twice a day for 21 days every two weeks. Treatment was continued at escalating doses until progression or intolerance. If hematologic toxicity grade 0, 1, or 2 was observed, the dose was escalated by 1 mg step; for G3 toxicity, the same dose was to be continued. The maximum tolerated dose (MTD) was defined as 2 episodes of G4 hematological toxicity, or febrile neutropenia, or G3 hematological toxicity.

IDA and IDOL were measured in plasma by HPLC and PK was studied at steady state using non-compartmental equations.

Twenty-five patients were enrolled. The maximum tolerated dose of MTD has not been yet reached and clinical results are reported in Table 1. Treatment was well-tolerated in all but one patient (300 ANC at day 28). Three patients had toxicity G3 ANC for more than three weeks after 3, 6, and 7 mg doses, respectively. Two of them stopped chemotherapy after 1 cycle and 1 patient stopped after 2 cycles (6 mg doses). Despite previous treatments with anthracyclines (the mean cumulative dose before entering the study was 530 and 264 mg, respectively, for epirubicin and DOX), no cardiotoxicity due to IDA treatment was observed. The patient who had G4 neutropenia also had a peculiar PK profile with a very high systemic exposure to IDOL [6.7 times higher than that of other patients at the same dose level (p <0.01)].

PK results were: half-life 21±11 hr, and 45±18 hr for IDA and IDOL, respectively; apparent systemic clearance was 598±311 L/hr for IDA and 83±38 L/h for IDOL. IDA distribution volume ($Vd_{app}IDA$) was 15400±5350 L and $Vd_{app}IDOL$ was 5100±2600 L. Mean IDOL/IDA ratio was 11.

In conclusion, this trial demonstrates the feasibility of chronic daily IDA administration. At the dosage reported, treatment was generally well toler-

Table 1. Escalating doses and hematological toxicity (# pts - WHO grade neutropenia).

| | *Daily dose* | | | | | | | |
	2 mg	*3 mg*	*4 mg*	*5 mg*	*6 mg*	*7 mg*	*8 mg*	*9 mg*
# new (total) pts	7 (7)	4(9)	1(6)	4(6)	3(7)	5(9)	1(4)	0(2)
Toxicity	7(G0)	1(G4)	1(G2)	1(G2)	2(G3)	3(G3)	1(G3)	1(G3)
# cycles with PK	7	9	6	4	11	9	4	2

Pts, patients; PK, pharmacokinetics.

ated. The PK findings (high IDOL concentrations) and the unexpected G4 myelotoxicity in patients with the highest IDOL plasma concentrations suggest that IDOL is clinically relevant.

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LOW-DOSE LONG-TERM ORAL IDARUBICIN IN MAINTENANCE TREATMENT OF ELDERLY ACUTE MYELOID LEUKEMIA

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Abstract

Background and Objective. Low-dose long-term oral IDA may play a role in maintainance treatment of elderly patients with AML; in fact, continuous exposure to IDA and IDAol could be efficacious in the disease control possibly inducing cell-differentiation and/or apoptosis.

Methods. We enrolled 25 previous responder patients in standard induction therapy to receive maintenance oral IDA 5 mg daily on days 1-14 at 2-week intervals for at least 6 months. We also evaluated the cell-cycle and apoptosis in leukemic cells from patients after IDA administration and, as a control, from HL60 lines exposed to IDA and IDAol *in vitro*.

Results. Long-term long-dose IDA was well-tolerated. Neutrophil and platelet count never below under 1×10^9/L and 50×10^9/L respectively in CR patients, and no infectious complications were encountered. Non-hematological toxicity was also acceptable: easily controlled nausea and vomiting, non-recorded diarrhea or mucositis were reported. The convenience of oral administration contributed to excellent compliance. DNA analysis performed *in vivo* after IDA and IDAol exposure showed an increase of G_2/M cell frequencies and evidence of sub-G_1 peak.

Interpretation and Conclusions. In conclusion, long-term low doses of oral IDA would appear valuable as a maintenance regimen for elderly patients. Our results seem to confirm the preliminary hypothesis that IDA + IDAol induce an increase of apoptosis in leukemic cells.

Key words: elderly, AML, idarubicin, apoptosis

Acute myeloid leukemia (AML) is seen at all ages, however, more than half of the patients with AML are over 60 years old.[1,2] Despite the increase of AML with age, the optimum regimen for elderly patients is still undefined; in fact, modern therapeutic approaches do not significantly benefit these patients. Over 65% of adults of under 60 years of age achieve complete remission (CR), and survival rates of 35% are commonly obtained, while only 50% of older patients obtain a CR with chemotherapy and only 10% of them have a disease-free survival of beyond 4 years after diagnosis.[3] The reason for unsatisfactory results is associated with the underlying biology of AML in older patients, the adverse cytogenetic characteristics, prior myelodysplasia, phenotypic features, MDR1 over-expression, and BCL-2 positivity.[4-7]

These considerations represent the rationale for offering new therapeutic approaches to older patients with AML. The choice of treatment for elderly patients depends on the balance of the toxicity and the quality of life. Increased treatment-related toxicity complicates chemotherapy, therefore most studies on AML in the elderly suggest a less intensive induction therapy to reduce toxicity.[8]

However, a large fraction of AML patients achieve CR relapse; this disease progression depends on two factors: acquired tumor cell drug resistance and tumor re-growth.[9] Recently, Shiller *et al.* reported a longer leukemia-free survival in patients treated with high dose ARA-C and autologous stem cell transplantation than in those treated with standard doses.[10]

On the other hand, maintenance treatment could play a role in elderly patients with AML, considering that they often cannot be treated with an intensive first line therapy. The choice of post-induction therapy should be a balance between control of leukemia and quality of life.

Idarubicin (IDA), which can be administered orally, is an active anthracycline in AML. Its metabolite, idarubicinol (IDAol), is also active and is formed in much larger amounts after oral administration. Patients over 60 seem to have an impaired IDAol elimination, so that the exposure to IDAol is prolonged in comparison to younger patients treated at the same dosage.[11]

Oral IDA could play a role in maintenance treatment of elderly patients at low doses and for long-term therapy. In fact, continuous exposure to IDA

and idarubicinol could be efficacious in disease control because of its effects on low-rate proliferation residual leukemic cells, induce an apoptotic cell death, beside a minor dependence of IDA/IDAol on multidrug resistance-mediated MDR1 genes.

On this basis, we have evaluated the feasibility of oral administration of Idarubicin at the dose of 3 mg/m²/day for 14 days consecutively every 4 weeks up to disease progression in elderly AML patients in complete or partial remission after the first conventional scheduled therapy containing ARA-C+VP16+ IDA/Mitox. Moreover, we have studied the apoptosis responses to this therapeutic agent *in vitro* and *in vivo* in myeloid cells collected from patients in partial remission.

Materials and Methods

Twenty-five elderly patients with myeloid leukemia, 5 of them with secondary AML to myeloidysplastic syndrome, with a median age of 70 years (range 60-83), M/F ratio 17/8, responsive to first line treatment, 8 in CR and 17 in PR, were enrolled in this study (Table 1) .

Induction and consolidation treatment consisted of ARA-C 100 mg/m² × 5 days-VP16 100 mg/m² × 3 days and IDA or Mitox at a dosage of 8 mg/m² and 12 mg/m², respectively, for 3 days; response to therapy was assessed according to the *Cancer and Leukemia B Group Criteria*.[12]

The maintenance treatment consisted of oral IDA 3 mg/m²/day for 14 days every 4 weeks until relapse or disease progression occurred.

Fresh and frozen cells samples were obtained from all elderly patients included in this study at 3 different times: before beginning IDA administration, during treatment at the 7th day and at the 14th day for the studies *in vivo*. As an *in vitro* control we used cell line HL60. These samples were Ficoll purified and contained approximately 1×10^7 cells each; part of the cells were used for cytogenetic analysis.

Ficoll-separated cells were suspended at a concentration of 2×10^6/mL in minimal essential medium (MEM) supplemented with 20% FCS and cultured for 16-18 hours in plastic flasks at 37°C in a humidified atmosphere containing 5% CO_2, with IDA at the dosage of 0.001 µg/mL and or with IDAol at the dosage of 0.0012 µg/mL corresponding to the concentration of 1 ng/mL *in vivo*.

They were assayed for cell-cycle and apoptosis at 16-20 hours post-treatment.

These cells were incubated with propidium iodide for DNA stain for 30 minutes and analyzed by cytometric assay with the FACScan by CellFIT program (BD); 10.000-50.000 cells of each sample were analyzed: cells frequencies in cell-cycle compartments are expressed as percentage of the number within the complete cell-cycle.

Apoptosis cell frequencies are expressed as per-

Table 1. Clinical and biological characteristics of elderly patients with AML.

	CR	PR
N. of patients	8	17
Median age	66	74
Range	60-70	60-83
Sex M/F	6/2	11/6
FAB		
M0	1	1
M1	2	5
M2	4	7
M4	1	4
Secondary	2	3
Hepatomegaly	1	8
LDH U/L		
median	408	441
range	275-683	382-1238
Favorable karyotype	5	3
Adverse karyotype	—	6

Table 2. Hematological toxicity in a) and transfusional requirement in b) in patients in CR and PR.

a)	CR		PR	
	n° pts	WHO	n° pts	WHO
WBC	3-8	1-2	10/17	1-3
Hb	3-8	1	12/17	2-4
PLT	2/8	2	12/17	1-4

b)	CR	PR
RBC Units	—	2 (0-4)
PLT Units	—	12 (0-16)

centage of the total cell number.

Cells with sub-G_1 DNA content were scored as apoptotic according to previous studies.[6]

Drugs effects on apoptosis and necrosis were determined morphologically by fluorescent microscopy after labelling with acridine orange and ethidium bromide as described by Duke and Cohen.[13]

Surface markers were analyzed by flow cytometry as previously described with antibodies of the following specificities: CD13, CD14, CD15, CD33, CD34; while BCL-2 expression was analysed using the antihuman BCL-2 clone antiserum (DAKO) after permeabilization by Permeaphix (Ortho). The staining was considered positive when 20% more cells than in the control were stained.

Table 3. Hematological toxicity in a) and transfusional requirement in b) in patients in CR and PR.

	CR		PR	
	n° pts	WHO	n° pts	WHO
Alopecia	8	0	17	0
Cardiac	8	0	17	0
Hepatic	8	0	17	0
Nausea/vomiting	1	1	3	2
Diarrhea	8	0	17	0

Table 4. Results in a) patients in complete remission; in b) patients in partial remission.

a)	Disease-Free Survival	Overall Survival
Median	8	13.5
Range	4-16	5-40

b)	Progression-Free Survival	Overall Survival
Median	5	8.5
Range	3-19	3-24

Results

In CR patients, only mild to moderate hematological toxicity was registered (Tables 2 and 3).

No transfusion requirement occurred in CR patients while in PR patients, a median of 2 U of RBC and 12 U of PLT were transfused. No major bleeding or documented infection occurred.

Mild nausea and vomiting that were easily controlled were noted in 3 PR patients.

Table 4 shows the clinical results. In patients in CR, DFS and OS were 8 and 13.5 months, respectively; OS and PFS in those patients in PR were 5 and 8.5 months, respectively. DNA analysis showed a sub G_1 peak both *in vivo* (only in patients with PR) and in *in vitro* samples. Moreover, we observed an increase in G_2+M cell frequencies (Figures 1-6). An apoptotic morphology assay confirmed these results. For all patients in PR, the leukemic cells were BCL-2 positive, furthermore, no change of BCL-2 expression emerged during treatment.

Discussion

Age is a major adverse prognostic factor in AML in elderly patients, both in the achievement of CR and OS, because these patients frequently have a poor performance status and other biological features such as hepatomegaly, elevated serum urea and the presence of cytogenetic abnormalities.[14]

In fact, only 10% of patients will survive free of leukemia beyond 4 years after diagnosis. The choice
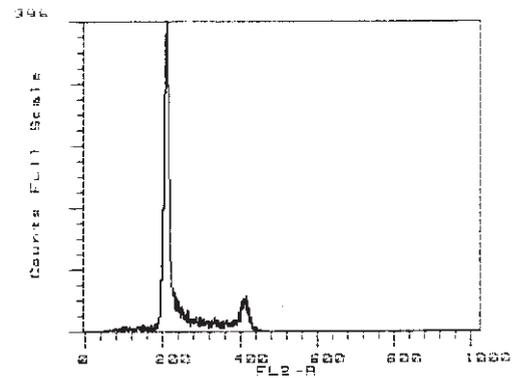
Figure 1. DNA analysis of HL60 cells after 24-hour culture (control) showing a G2+M cell frequencies of 5.5%.
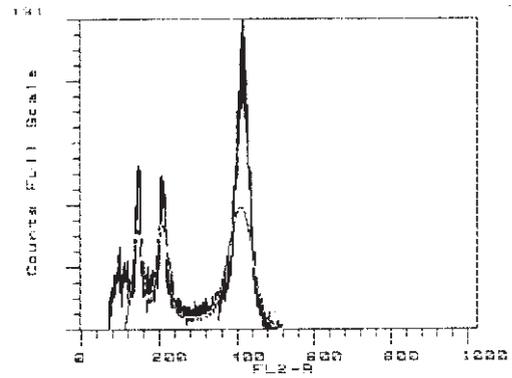
Figure 2. DNA analysis of HL60 cells after 24-hour exposure to IDA/IDAol, showing a sub-G1 peak (13.4%), and an increase of G2+M cell frequencies (48%).
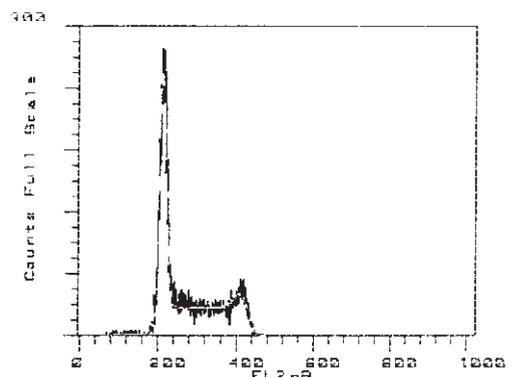
Figure 3. DNA analysis of HL60 cells after 48-hours culture (control) showing a G2+M cell frequencies of 14.9%.
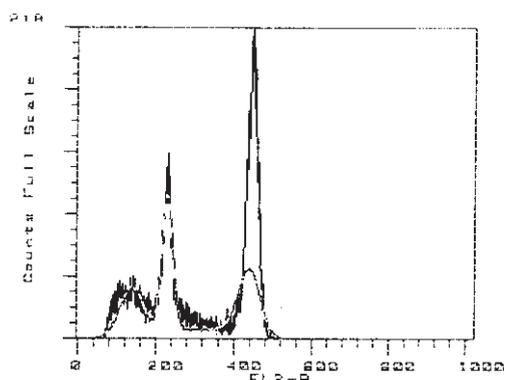
Figure 4. DNA analysis of HL60 cells after 48-hour colture with IDA/IDAol, showing a sub-G1 peak (26.2%), and a G2+M cell frequenciesof 28.8%.
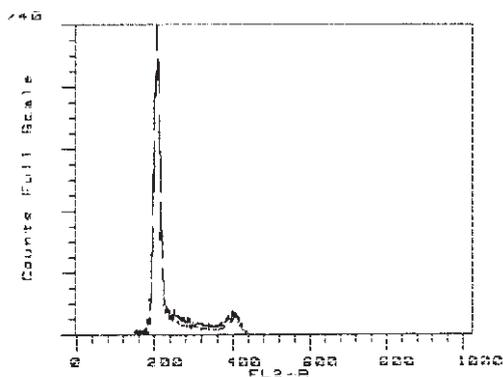
Figure 5. DNA analysis at day 0 of leukemia cells showing a G2+M cell frequencies of 6.5%.

Figure 6. DNA analysis at day 7 of leukemia cells from patients treated with IDA 3 mg/m^2/d shoiwing an increase of G2+M cell frequencies (42%) and a sub-G1 peak of 17.8%. Similar results were found at day 14 analysis.

of treatment for elderly patients depends on the balance between toxicity and quality of life. Most studies about AML in patients over 60 suggest less intensive induction therapy to reduce toxicity. Moreover, a large percentage of patients who achieve a CR have a relapse. Maintenance treatment could play an important role in these patients who often cannot be treated with an intensive first-line therapy. In fact, the post-induction therapy by continuous administration of low doses of IDA for a long term should warrant a control of the residual leukemic cells (inducing an apoptotic cell death). Recently, studies by cytometric assay report a variable response to treatment-induced apoptotic AML cells after exposure to several drugs (ARA-C, DNR).[9]

Our results show an increase in apoptosis in the leukemic cells after IDA and IDAol *in vitro* and *in vivo* exposure only in those samples exposed to IDA and

IDAol at the same time. Moreover, we observed an increase in G$_2$+M cell frequencies (Figures 1-6).

On the other hand, Ara-C gives similar results, while only an increase of G$_2$+M cell frequencies is reported after DNR exposure.[9]

Regarding the BCL-2 positive cells and the prospective apoptosis correlation, we found over-expression in many AML cells of patients in PR, yet these were correlated to treatment-induced apoptosis frequencies. Furthermore, only cells from PR patients showed high BCL-2 positive cells, suggesting that tumor drug resistance was more frequent in these case. Our clinical results showed a very low hematological and extrahematological toxicity, and a good compliance with this schedule of treatment. The preliminary biological study results seem to confirm the hypothesis that long term exposure to low doses of IDA induces increase of apoptosis in leukemic cells.

In conclusion, long-term low dose oral IDA could represent a choice for maintenance treatment of elderly AML patients in CR, even if more extensive and randomized trials are necessary for evaluating the impact of DSF and OS.

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IDARUBICIN AND CYTOSINE ARABINOSIDE IN THE INDUCTION AND MAINTENANCE THERAPY OF HIGH-RISK MYELODYSPLASTIC SYNDROMES

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Abstract

Background and Objective. Recently, the results of a few pilot studies have shown the efficacy of the association of idarubicin (IDA) and cytosine arabinoside (Ara-C), already successfully employed in acute myeloid leukemia (AML), for remission induction in patients with myelodysplastic syndrome (MDS). We set out to evaluate in a multicenter study the efficacy and tolerability of an intensive therapy with IDA and Ara-C in patients with RAEB and RAEB-t, the rate and duration of CR and the overall survival in adults treated with full doses and in the elderly treated with lower doses; furthermore, we investigated the efficacy of low-dose maintenance chemotherapy.

Methods. Pretreated adult patients with *de novo* RAEB and RAEB-t, meeting at least one of the following criteria, were included: neutrophils $< 0.5 \times 10^9$/L or moderate neutropenia with infectious episodes, platelets $< 30 \times 10^9$/L or moderate thrombocytopenia but with bleeding symptoms, transfusion > 4 red cell units/months, rapid increase of bone marrow blasts. Induction treatment consisted of a cycle with IDA and Ara-C. Adult patients less than 65 years old were treated with the following doses: Ara-C 1 g/m²/day i.v. 6-hour infusion, on days 1-4, IDA 10 mg/m²/day i.v., on days 1-3.

Elderly patients (≥ 65 yrs) were treated with lower doses: Ara-C 1 g/m²/day 6 hours infusion, on days 1 and 2, IDA 10 mg/m²/day i.v., on days 1 and 2. Responders followed a consolidation course identical to induction.

Results. From February 1994 to February 1997, 25 patients were enrolled, 20 males and 5 females aged between 22 and 76, 10 were ≥ 65 years old, 7 had RAEB and 18 had RAEB-t. Twelve cases (48%) achieved complete remission (CR), 7 cases (28%) achieved partial remission, 4 patients were resistant and two patients (8%) died during the aplastic phase. A significantly higher CR rate was found in younger patients (p = 0.036), while gender, FAB subtype, presence of Auer rods, cytogenetic findings, and the interval from diagnosis to treatment did not significantly influence CR achievement.

Interpretation and Conclusions. Our results show that in *de novo* RAEB and RAEB-t, the employed treatment with IDA and Ara-C is associated with satisfactory frequency of response with acceptable toxicity.

Key words: acute myeloid leukemia, chemotherapy, idarubicin, cytosine arabinoside

Prognosis in patients with myelodysplastic syndrome (MDS) and a high percentage of bone marrow blasts is very poor: according to the reports of the largest numbers of cases published in the last decade, median survival rates of patients with refractory anemia with excess of blasts (RAEB) and RAEB in transformation (RAEB-t) are 13 and 6 months, respectively.[1]

Lack of randomized clinical trials does not allow for the definition of guidelines for therapy of RAEB and RAEB-t; furthermore, the relative efficacy of the various treatment approaches is not clear, nor are their advantages in comparison with supportive therapy alone. The only strategy capable of prolonging survival significantly is allogeneic or autologous bone marrow transplantation.[2] For patients aged between 55 and 65, intensive chemotherapy is the most efficacious treatment. In fact, complete remission (CR) can be reached in about 60% of cases; it lasts, however, only about 10 months and less than 10% of cases are disease-free at 3 years.[3,4]

Recently, the results of a few pilot studies have shown the efficacy of the association of idarubicin (IDA) and cytosine arabinoside (Ara-C), already successfully employed in acute myeloid leukemia (AML), for remission induction in patients with MDS.[5-9] The lower cardiotoxicity of IDA compared to other anthracyclines, and a reduction of Ara-C doses made the treatment feasible and well tolerated even in elderly patients.

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We set out to evaluate in a multicenter study the efficacy and tolerability of an intensive therapy with IDA and Ara-C in patients with RAEB and RAEB-t, the rate and duration of CR and the overall survival in adults treated with full doses and in the elderly treated with lower doses; furthermore, we investigated the efficacy of low-dose maintenance chemotherapy.

Patients and Methods

Patients

Pretreated adult patients with *de novo* RAEB and RAEB-t meeting at least one of the following criteria, were included: neutrophils $<0.5\times10^9$/L or moderate neutropenia with infectious episodes, platelets $< 30\times10^9$ or moderate thrombocytopenia but with bleeding symptoms, transfusion > 4 red cell units/months, rapid increase of bone marrow blasts.

Criteria for exclusion were the following: presence of documented infection, asymptomatic disease with stable hematological values, performance status > 2 according to the WHO scale, severe heart failure and/or severe arrhythmias, inadequate liver or renal function, and patients already treated by intensive chemotherapy and/or radiotherapy for previous neoplasms.

Informed consent was obtained from all patients.

Design of the protocol

Induction treatment consisted of a cycle with IDA and Ara-C; adult patients less than 65 years old were treated with the following doses: Ara-C 1 g/m^2/day i.v. 6-hour infusion, on days 1-4, IDA 10 mg/m^2/day i.v., on days 1-3; elderly patients (≥ 65 yrs) were treated with lower doses: Ara-C 1 g/m^2/day i.v. 6-hour infusion, on days 1, 2, IDA 10 mg/m^2/day i.v., on days 1 and 2. Responders followed a consolidation course identical to induction. Patients in CR after consolidation regimen had to be treated for 2 years with maintenance therapy consisting of alternate cycles of Ara-C 10 mg/m^2 s.c. every 12 hours for 10 days a month, and IDA 15 mg/m^2/day p.o. for 3 days a month.

Complete remission was defined as normocellular bone marrow with less than 5% blasts and at least 1.0×10^9/L neutrophils and 100×10^9/L platelets in the peripheral blood. Partial remission (PR) was defined as a 50% or more reduction of blasts in a normocellular marrow.

Statistics

Actuarial curves were calculated according to the method of Kaplan-Meier. The differences between curves were statistically tested using the two-tailed log-rank test. Comparisons of some parameters for their prognostic value were performed with the Fisher exact test. The duration of survival was calculated from the date of starting of treatment until death. For patients who achieved CR, the disease-free survival (DFS) was calculated from the date of first CR to the date of relapse, death in CR or the most recent follow-up.

Results

From February 1994 to February 1997, 25 patients were enrolled, 20 males and 5 females aged between 22 and 76, 10 were ≥ 65 years old, 7 had RAEB and 18 had RAEB-t. Their clinical and hematological features are summarized in Table 1. In 5 cases, treatment was started more than 3 months after diagnosis.

Twelve cases (48%), 2 RAEB and 10 RAEB-t, 2 ≥ 65 years old achieved CR; 7 cases (28%), 5 ≥ 65 years old achieved PR; 4 patients (16%), 2 ≥ 65 years old were resistant to therapy (Table 2). A significantly higher CR rate was found in younger patients (p=0.036), while gender, FAB subtype,

Table 1. Characteristics of the patients.

Total patients	25
M/F	20/5
Age	
median (range)	61 (22-76)
≥ 65 yrs	10
AREB/AREB-t	7/18
Interval from diagnosis	
< 3 months	20
≥ 3 months	5
Karyotype	
normal	9
abnormal	12
Hb g/L	
median (range)	8.8 (4.5-12.8)
Neutrophils x 10^9/L	
median (range)	0.95 (0.01-6.49)
Platelets x 10^9/L	
median (range)	46 (8-260)

Table 2. Response to therapy.

	Total	< 65 years	(65 years
Patients	25	15	10
Complete remission	12 (48%)	10 (67%)	2 (20%)
Partial remission	7 (28%)	2 (13%)	5 (50%)
No remission			
Death in aplasia	2 (8%)	1 (7%)	1 (10%)
Resistant	4 (16%)	2 (13%)	2 (20%)
Median survival (months)	12	21	5

Table 3. Toxicity of chemotherapy (> grade 1 WHO).

	Induction course	Consolidation course	Maintenance course
No. of evaluable cases	25	18	11
Infection/FUO	11/13	3/5	0/2
Hemorrhage	9	6	0
Gastrointestinal	5	2	0
Hepatic	2	2	1
Cardiovascular	3	1	0
Alopecia	9	5	1

Table 4. Supportive therapy.

	Induction course	Consolidation course
Platelets units (mean±se)		
Apheresis	3.1±1.6	1.2±0.7
Random	20.5±8.4	11.5±3.8
Erythocyte unit (mean±se)	9.4±1.7	8.0±1.9

presence of Auer rods, cytogenetic findings, and the interval from diagnosis to treatment did not significantly influence CR achievement. Two patients (8%) died during the aplastic phase, one from sepsis and one from cerebral hemorrhage, aged 57 and 66, respectively. Complications of chemotherapy as well as supportive therapy are summarized in Tables 3 and 4.

Among CR patients, median times to recovery of neutrophils (1×10^9/L) and platelets (100×10^9/L) were, respectively, 21 (range 16-24) and 18 (range 14-28) days. No severe complications occurred during consolidation and maintenance courses.

The median duration of DFS was 18 months. The median survival duration of patients achieving CR was 21 months, while it was only 5 months in the PR and resistant patients (p=0.002); median survival for the whole population was 12 months (Figure 1). Survival duration was significantly (p=0.043) longer in patients with normal karyotypes (median 21 months) compared to patients with cytogenetic anomalies (median 11 months). Eight patients are still living, 7 of whom are in continuous CR that has lasted for more than 19 months in 3 cases.

Discussion

Since the early eighties, according to the schedules commonly used for acute leukemia, intensive chemotherapy has been employed for the treatment of high risk MDS, with the aim of eradicating the pathological clone, allowing for the recovery of normal hematopoiesis.[10,11] Lack of randomized trials, however, does not yet allow for the evaluation of the real efficacy of this kind of treatment, nor of its advantages in comparison with supportive therapy alone. Furthermore, the interpretation of the results of the studies already published is difficult for other reasons: groups of patients are small, FAB subtype is not specified, both primary and secondary cases, almost always in leukemic progression as well, are included.[3,4]
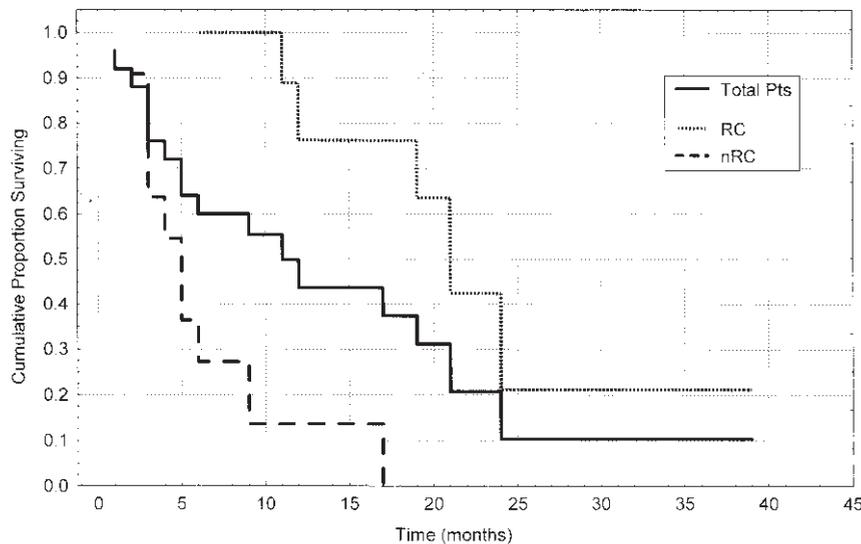

Figure 1. Actuarial survival curves of all patients, of patients achieving CR and of patients not achieving CR.

Generally, combinations of anthracyclines and Ara-C at standard or high doses, with or without thioguanine, have been used.[5,6,8,12-14] CR rates were variable, ranging from 15% to 80%, but they were usually lower than in *de novo* acute leukemia.[7,8,11,14,15] The most important reasons for this phenomenon are the generally older age of patients with MDS, as well as peculiar biological characteristics of the dysplastic clone, namely, the increased expression of chemoresistance proteins and the higher incidence of complex cytogenetic anomalies.[16,17] In fact, if such prognostic factors are matched, the results of treatment are similar in *de novo* AML and in MDS.[17]

Cytopenias are long with high incidence of toxic deaths (14-25%), generally due to infections. The duration of remission as well as survival, ranging respectively from 6 to 12 months and from 9 to 18 months, is shorter than in *de novo* AML.[8,10,14,18,19]

We performed a prospective multicenter study of intensive chemotherapy for patients with RAEB and RAEB-t, in good clinical conditions, with serious or symptomatic cytopenia. Our protocol consisted of induction and consolidation regimens with IDA and Ara-C and of maintenance cycles with the same drugs at low doses. Idarubicin was used in consideration of its lower cardiotoxicity compared to other anthracyclines, and of its pervious documented efficacy against blasts expressing high drug resistance.

Our results show that in *de novo* RAEB and RAEB–t, this combination regimen is associated with a satisfactory frequency of response with acceptable toxicity. In fact, 76% of our patients achieved CR or PR, while the therapy-related mortality was rather low. Only a complete response, however, significantly influenced the duration of survival. These data support the results of other recent studies;[8,14,18,19] in patients less than 65 years old, the CR rate (67%) reported by us was even higher than those described by other authors using similar regimens, but excluding elderly patients. Therefore, intensive chemotherapy is certainly indicated in young patients with high risk MDS, although these are only a minority of the whole MDS patient population.[20] Probably, the use of full dose therapy in the elderly as well may increase their rather low CR rate, since no other prognostic factors were found to influence the achievement of remission.

Finally, although the short follow-up period does not allow for definitive conclusions, the fact that the CR duration of our patients was slightly longer than those reported by other authors suggests that maintenance therapy may prolong the period of CR and survival. It remains to be seen whether autologous stem cell transplantation can improve the outcome of CR in MDS patients as it does in AML patients.[21] A limiting factor for this procedure, however, might be a low number of normal residual stem cells.

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IDARUBICIN IN INDUCTION TREATMENT OF ACUTE MYELOID LEUKEMIA IN THE ELDERLY

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ABSTRACT

Background and Objective. The best approach to treatment of acute myeloid leukemia (AML) in elderly patients remains controversial. Intensive chemotherapy is the treatment of choice in selected patients, but age related changes might affect the pharmacokinetics of antineoplastic agents resulting in enhanced toxicity. We report our experience in elderly patients treated with idarubicin at attenuated doses plus cytarabine and etoposide.

Methods. Sixty-six AML patients, median age 66, with progressive disease and high tumor burden received idarubicin 8 mg/sqm i.v. d 1,3,5; cytarabine 200 mg/sqm by continuos i.v. infusion d 1-7; etoposide 60 mg/sqm i.v. d 1-5. A second course with the same drugs was planned irrespective of complete remission (CR) achievement. No consolidation was given; 44% had a documented preexisting myelodysplasia, 45% presented with fever. Promyelocytic leukemias were excluded.

Results. Thirty-five patients (53%) achieved CR and 9 PR for an overall response rate of 67%. Nine of them (13%) died early or during the aplastic phase. Preexisting myelodysplasia had no significant impact on CR achievement. Resistant disease was associated with CD7 phenotype and unfavorable karyotype. Overall survival and disease free survival were 14 and 13 months, respectively. The maior toxicity consisted of infectious complications (WHO > 2 in 24% of patients). Six patients died for infection, 2 for heart failure, 1 for pulmonary embolism.

Interpretation and Conclusions. This induction regimen with attenuated doses of idarubicin is feasible and effective, but long-term survival remains an unresolved problem. Alternative post remission approaches are advisable in the aim of improving the remission duration.

Key words: acute myeloid leukemia, chemotherapy, idarubicin

Since acute myeloid leukemia (AML) in the elderly is intrinsically more chemoresistant,[1] an intensive treatment would be the best choice when toxicity related to intensive chemotherapy can be tolerated. Selecting criteria are needed and treatment should be tailored case by case, according to disease and host related variables. Patients with slowly progressing disease, hypocellular bone marrow or low blast count can as well respond to a low-dose chemotherapy.[2] In contrast, an aggressive treatment must be applied to AML cases with unfavorable biological characteristics, frequently associated with a multi-drug resistant phenotype. Standard induction treatments are based on cytarabine (ara-C) plus an antracycline. The availability of the new antracycline idarubicin (IDA), less involved in multi-drug resistance[3,4] and superior to daunorubicin in randomized trials would result in better response rate. However, conventional dosages have been regarded as too toxic in the elderly.[5] We demonstrated that aged patients have an impaired elimination of IDA active metabolite idarubicinol and therefore they should receive IDA at attenuated doses.[6] Topic of this report are 66 AML patients aged 60 years or older treated with an induction protocol based on IDA plus ara-C and etoposide.

Patient and Methods

Between January 1992 and December 1996, 140 patients aged sixty or more with previously untreated AML were referred to our Institution. Median age was 67 years (range 60-82). Seven patients with acute promyelocytic leukemia were enrolled in a specific protocol with all-trans retinoic acid. Twenty-two patients presenting with hypoplastic or oligoblastic leukemia were designed to receive three courses of ara-C 20 mg/sqm/d subcutaneously for three weeks (LDara-C). Only supportive care or palliative chemotherapy was delivered to 45 patients due to severe concomitant non hematological disorders or patient's refusal. Sixty-six patients with hypercellular bone marrow or monocytic involvement and rapidly accumulating leukemia related problems were allocated to conventional chemotherapy and are the topic of this report. There was no upper age limit to entry in the

study and patients with AML secondary to a pre-existing myelodysplastic syndrome or myeloproliferative disease (AHD) or to a previous chemo-radiotherapy (t-AML) were eligible. Those presenting with fever due to documented infection, received chemotherapy after a short antibiotic treatment, irrespective of infection resolution. An absolute exclusion criteria was a severe heart failure. Details on the patients clinical characteristics are outlined in Table 1. The diagnosis of AML was defined according to French-American-British (FAB) criteria[7] and trilineage dysplasia of bone marrow was recorded as proposed by Brito-Babapulle.[8] Phenotype was determined at diagnosis on bone marrow or peripheral blood specimens by direct immunofluorescence and a FACScan flow cytometer. A wide panel of lineage associated antigens was applied. Determinations were gated and single or double parameters of at least 10.000 cells analyzed. By convention, a case was considered positive if more than 20% of blasts expressed the antigen. Chromosome analysis was performed on bone marrow cells after 24-hour culture. Cells were G-banded with Wright stain and twenty-five metaphases were karyotyped according to the *International System for Human Cytogenetic Nomenclature*.[9] The karyotypic findings were classified as low-risk (t8;21, inv16 or t16;16, del20q, 5q– only) intermediate (normal, +8, 12p abnormality, hypo/hyperdiploid), high risk (complex or >3 abnormalities, –7/7q–).

Induction treatment consisted of two cycles: the first with IDA 8 mg/sqm i.v. on days 1,3 and 5; ara-C 200 mg/sqm by continuous i.v. infusion on days 1-7 and etoposide 60 mg/sqm i.v. on days 1-5; the second was a five-day course at the same schedule but with IDA on days 1 and 3, ara-C and etoposide on days 1-5. Subsequently, patients who achieved partial remission (PR) or complete remission (CR) were designed to receive three 21 days courses of LDara-C as maintenance therapy.

CR required normal peripheral blood counts with no evidence of extramedullary disease and <5% of blasts in a normocellular bone marrow, lasting more than one month; PR was defined as 5-25% of bone marrow blasts with normal peripheral blood counts or moderate pancytopenia and less than 5% of circulating blast cells. Treatment failures were registered as either induction deaths (ID) if patients died early or during marrow aplasia before CR could be ascertained, or resistant disease (RD) if criteria for PR were not fulfilled.

Cardiac toxicity was assessed by clinical and electrocardiographic examination for signs of failure or rhythm abnormalities. The others extrahematological toxicities were recorded according to the WHO grading system.[10]

The duration of disease-free survival (DFS) was measured from the day CR was documented to day

Table 1. Patient characteristics.

No. of patients	66
M/F	29/37
Age median (range)	66 (60-77)
WBC x 10^9/L median (range)	58 (1.8-295)
Plt x 10^9/L median (range)	69 (15-227)
Hgb g/L median (range)	87 (42-135)
LDH UI median (range)	1161 (240-12500)
Fever (>38°C) at diagnosis	30 (45%)
Documented infections	10 (15%)
Secondary leukemias	29 (44%)
AR	12
AREB	6
MF	2
t-AML	9
FAB	
M0	4
M1	15
M2	13
M4	22
M5	8
M6	2
M7	2
Trilineage displasia (*de novo* AML)	16/37 (43%)
immunophenotype (55 evaluated)	
CD34+	34 (62%)
CD7+	11 (20%)
CD14+	21 (37%)
cytogenetic (25 performed)	
no metaphases	7
low risk	1
intermediate risk	11
high risk	6

of relapse or last follow-up; patients who died without relapse were censored at the date of death. The duration of overall survival (OS) was measured from the time of initial therapy to date of death or last follow-up. The DFS and OS curves were drawn according to the Kaplan-Meier method. Differences among subgroups in the CR rate were compared using the Chi-square test.

Results

Among the 66 patients who received IDA plus ara-C and etoposide, 35 (53%) achieved CR, 23 (66%) after the first course. Nine were PR, for an overall response rate of 67% . Twenty-two were treatment failures: 13 (20%) RD and 9 (13%) ID (6 for sepsis, 2 for heart failure, 1 for pulmonary embolism). CR rate was 59% in *de novo* AML, 45 and 44% in cases with previous AHD and t-AML, respectively. Neither WBC count, LDH level, trilineage dysplasia nor fever at diagnosis were significant for the achievement of CR.

Phenotype was determined in 55 patients: 34 (62%) were positive for the the CD34 stem cell antigen; 11 were CD7 positive and they all coexpressed

CD34; 21 were CD14 positive and 10 coexpressed CD34. Only the CD7 positivity was significantly associated with chemoresistance since 7/11 (64%) had RD.

A successful karyotype was available in 18 of the 25 evaluated cases: among the 6 patients with unfavorable karyotype, one achieved CR lasting 19 months, 4 had RD, and one died early.

Of the 57 patients who survived the first cycle, 38 (67%) received the second planned course according to the protocol schedule, 19 did not. Five patients with absolute chemoresistance after the first course were assigned to salvage regimens but none of them achieved a response. Fourteen did not receive further aggressive chemotherapy, 6 for refusal and 8 for medical decision due to the toxicity or poor tolerance of the first course. Eight of these 14 patients were responders (4 CR, 4 PR) and went through to maintenance with LDara-C, the remaining 6 received only supportive or palliative chemotherapy.

Common side effects were nausea and vomiting, easily managed by i.v. antiemetics. All patients experienced profound granulocytopenia (< 0.2 PMN$\times10^9$/L) and thrombocytopenia (platelets < 20$\times10^9$/L). Mean days from the end of therapy to PMN >1.5$\times10^9$/L were 19.1 (range 11-30), to platelets >30$\times10^9$/L were 17.2 (range 10-30). Transfusion support was constantly required, with a mean of 7.1 packed RBCs and 17.2 platelets from random donor per cycle. The major toxicity consisted of infectious complications. At the study entry, 30 patients were febrile and 10 of them had a documented infection. During the induction phase, severe infections (WHO grade > 2) occurred in 16 patients. Six of them died early or while aplastic (1 for a *Candida tropicalis* sepsis, 1 *Pseudomonas*

aeruginosa sepsis and 4 for pneumonia). Overall, days with fever >38°C were 3.4 per course. Two patients died for heart failure and 1 for pulmonary embolia: they all had a severe infectious complication at the time of death. Hepatic toxicity grade 3 was observed only in 1 patient. No other significant extrahematological toxicities were registered.

At the time of the analysis (May 30, 1997), 16 patients are alive and 50 have died. The projected median survival of the whole group is 14 months, with < 10% of patients alive after 3 years (Figure 1). One patient died in CR for myocardial infarction 1 month after the second course. Nine patients are in first CR, 26 relapsed. The median DFS was 13 months, with 20% of responding patients projected in continuous CR at 3 years (Figure 2).

Discussion

The unfavorable outcome of AML in the elderly is related both to the poor tolerance to chemotherapy and to the biological features of the disease. In a number of recent studies more than twenty per cent of patients failed to achieve a response due to resistance to chemotherapy.[11,12] A conventional aggressive treatment should be the best choice but its value is still questioned due to the high incidence of ID. However, when lower dose chemotherapy is applied, a higher incidence of RD is observed without a reduction of ID.[13] The purely supportive approach results not only in unfavorable survival but also in frequent hospitalization and poor quality of life.[14] Sixty per cent or more of CR are reported with conventional induction chemotherapy but these results could be biased by patient's selection.[15] Most of the hematologists agree that the majority of elderly patients would benefit from chemotherapy performed directly following diagno-
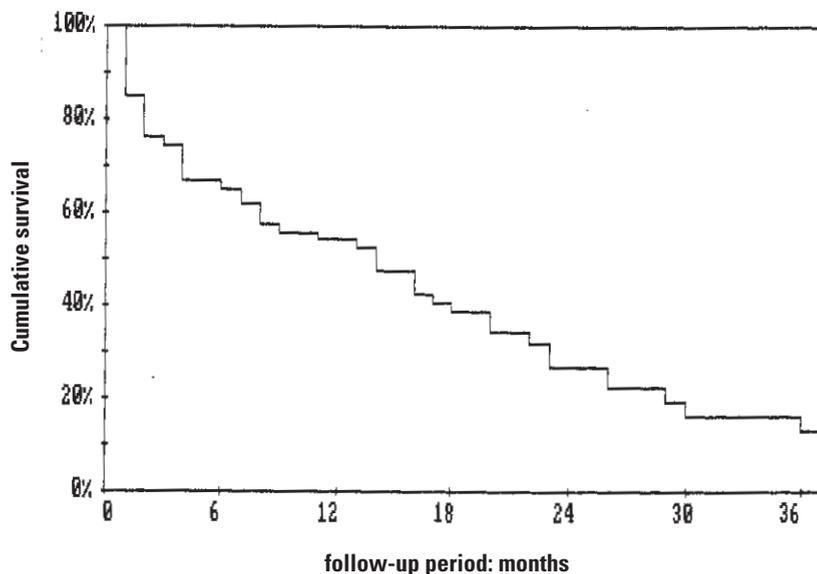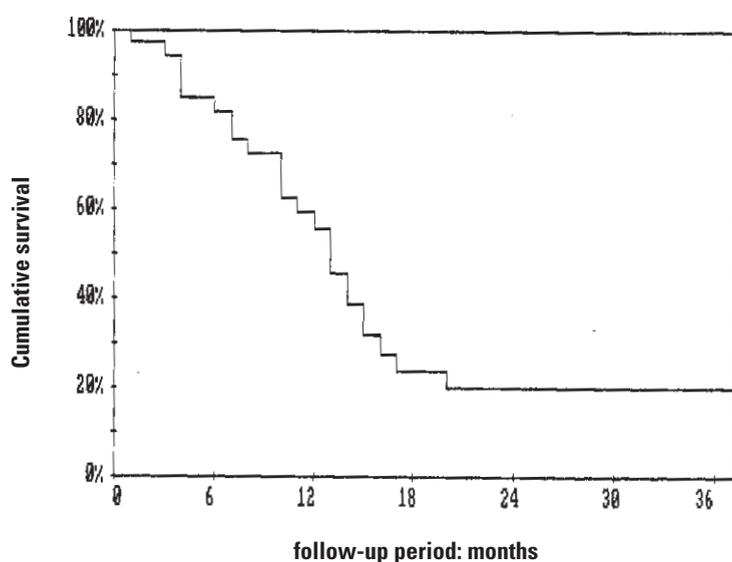

Figure 1. Overall survival.

sis and prognostic score systems based on criteria readily available at the time of presentation have been proposed.[16,17] Furthermore, models predicting overall survival rather than remission rate may be more appropriate in the elderly. We previously reported that LDara-C could be a useful option in patients who lack clinical features predictive for resistance to low-dose chemotherapy, i.e. an hypercellular marrow or a monocytic involvement.[18] A low bone marrow cellularity has a favorable impact on the achievement of CR and duration of response when LDara-C therapy is applied.[2] Lower remission rates are reported in FAB subtypes M4/M5 suggesting that the clinical effect of LDara-C may be related to cell lineage.[19] Thus, we designed to receive LDara-C those patients presenting with hypoplastic or oligoblastic leukemia without a monocytic involvement. Ten out of 22 (45%) achieved a CR without the hazards of aggressive chemotherapy, with a median DFS of 14 months. Conventional chemotherapy was restricted to AML cases with high leukemia burden, probably unresponsive to low-dose chemotherapy. Unlike in other studies,[11,12] an AHD, t-AML or infections at diagnosis were not criteria of exclusion. However, 45 patients could not receive adequate chemotherapy due to a very poor performance status or a severe heart failure. Thus, only about 60% of elderly AML patients referred to our institution and judged to necessitate standard chemotherapy, could be treated.

The 53% CR rate achieved in the 66 patients treated with IDA, ara-C and etoposide is comparable with results of other studies (ref. #20-32; Table 2). We found no significant differences in CR rate between *de novo* AML, those preceded by an AHD and t-AML (59% vs 45% vs 44%). It has been shown that AML in the elderly present the characteristics of the secondary AML even in most *de novo* cases.[1] In our series, 43% of the apparently *de novo* cases presented trilineage dysplasia. Furthermore, some patients recovered with dysplastic hematopoiesis, indicating that in older age group there may be patients presumed to have *de novo* AML who previously had an unrecognized myelodysplastic syndrome.

A significant association between elevated serum LDH and poor outcome was outlined by Ferrara[25] while others reported that prolonged remission was linked to elevated serum LDH and higher peripheral blood counts at diagnosis.[26] We could not demonstrate an impact on CR rate of serum LDH but patients with LDH < 800 UI have a better survival (465 vs 227 days, p=0.09).

While the CD34 expression failed to predict the outcome, CD7 was strongly associated with resistant disease: out of the 13 RD, 7 were registered among the 11 CD7 positive cases (p < 0.05). A successful cytogenetic study was available only in a minority of cases: as expected, the incidence of favorable karyotypes was negligible. Among the 6 patients with complex or unfavorable aberrations, only one achieved CR and 4 had RD.

In our study, 9 patients achieved a PR and, although it is conventionally regarded as a treatment failure, in this series of elderly patients the return to a nearly normal peripheral blood counts allowed a satisfying quality of life without supportive therapy until florid leukemic relapse occurred. Applying a global quality of life score, Bow *et al.*[26] reported that an improvement not different from that of the remitters could be observed in patients achieving PR.

Toxicity of induction treatment was mainly due to myelosuppression, bacterial and fungal infec-

Table 2. Results of induction chemotherapy in elderly AML patients.

Authors (year)	references	No pts	Age (median)	AHD	Complete remission %	Resistant leukemia %	OS (months)	DFS (months)
Baudard (1994)	15	238	n.r.	yes	33	45	3.5	9.7
Ruutu (1994)	20	25	72	yes	60	36	10	7
Jehn (1994)	21	32	68	yes	50	18	n.r.	7
Bigelow (1995)	13	31	66	no	42	42	8	n.r.
Dombret (1995)	12	173	68	no	59	41	n.r.	n.r.
Frenette (1995)	22	19	67	yes	37	17	n.r.	9.5
Stone (1995)	11	388	69	no	53	22	9	9
Ferrara (1996)	23	72	67	n.r.	57	30	9	12
Bow (1996)	24	67	68	n.r.	55	33	9.2	8.4
Pignon (1996)	25	233	59	no	66	184	12	17
Clavio (1996)	26	30	n.r.	yes	63	n.r.	n.r.	n.r.
Stasi (1996)	17	92	67	no	52	19	n.r.	9
Maslak (1996)	27	26	66	yes	58	34	8	10
Montillo (1996)	28	43	67	yes	49	39	8	11
Feldman (1997)	29	54	70	yes	50	50	8	6
present study		66	66	yes	53	47	14	13

AHD= antecedent haematologic disorder; OS= overall survival; DFS= disease free survival

tions representing the major source of morbidity and mortality. Colony stimulating factors were not routinely delivered, since randomized studies with GM-CSF[11,23] or G-CSF[12] failed to demonstrate any impact on ID rate. Severe heart failure was recorded in 2 patients and both have died with a concomitant infectious complication. The overall survival and remission duration are in keeping with those reported by others (Table 2) but less than 10% of patients are projected to be alive and disease free at 3 years. Long-term survival remains an unresolved problem in elderly patients with AML and the choice of post-induction therapy may be crucial. Results of high-dose ARA-C have been disappointing[22] and it has been shown that a unique consolidation course may be as effective as more prolonged treatments.[33] The duration of hospitalization for our patients completing the induction protocol was nearly two months. An intensive consolidation regimen would request further hospitalization and could result in unacceptable toxicity in front of any improvement of DFS. Thus we decided to deliver a maximum of two courses of intensive chemotherapy, irrespective whether CR was achieved after the first or the second course. The *maintenance* treatment with LDara-C was chosen for the suggested efficacy in prolong DFS[34] with negligible extramedullary toxicity. Since also in the present study the majority of relapsed or refractory patients either failed subsequent salvage therapy or did not receive any, it can be concluded that the major impact on survival comes from the initial treatment, not from salvage therapy.

In conclusion, the balance between the benefits and risks of intensive chemotherapy is particularly delicate in older patients. The best therapeutic choice is not the most aggressive but the best balanced. Our regimen with IDA at the dose of 8 mg/sqm for 3 days instead of conventional 10-12 mg/sqm is feasible and effective. The dosage attenuation does not result in a reduced exposure to the drug, since the idarubicinol elimination is impaired in patients over sixty.[6] Furthermore, there is some clinical evidence suggesting that 12 mg/sqm for 3 days is probably too toxic for most elderly patients.[36] It's unlikely that far more better results can be achieved by conventional chemotherapy. Alternative approaches are advisable to overcome the intrinsic chemoresistance of AML of the elderly. MDR revertants are currently studied in clinical trials and a greater understanding of drug pharmacodynamics has lead to the use of novel chemotherapeutic regimens. The efficacy and tolerability of fludarabine, ara-C and G-CSF (FLAG) in MDS/AML has been confirmed in clinical studies.[28] Addition of IDA to FLAG regimen[35] may further improve clinical results.

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A SINGLE HIGH DOSE OF IDARUBICIN COMBINED WITH HIGH-DOSE ARA-C (MSKCC ALL-3 PROTOCOL) IN ADULT AND PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA. EXPERIENCE AT THE UNIVERSITY "LA SAPIENZA" OF ROME

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ABSTRACT

Background and Objective. The anthracycline analogue idarubicin, either alone or in combination with other antineoplastic drugs, has shown antileukemic activity in relapsed and refractory acute lymphoblastic leukemia (ALL). In an attempt to minimize the non-hematologic toxicity and obtain a potent antileukemic effect, MSKCC activated a pilot study in previously treated adult ALL, using HD-ARA-C combined with idarubicin administered as a single high-dose infusion. We herein report our experience with a series of pediatric and adult high risk ALL and NHL patients treated with the protocol above, which confirms its feasibility, response rate and individual compliance.

Methods. In a clinical phase II study the combination of a single high dose (HD) idarubicin and HD cytosine-arabinoside (ARA-C), as designed at the Memorial Sloan Kettering Cancer Center, was applied to 70 adults and children with refractory or early relapse acute lymphoblastic leukemia (ALL) and T-cell lymphoblastic non-Hodgkin's lymphoma (NHL). Therapy consisted of HD-ARA-C 3 g/m^2/d on days 1-5, idarubicin 40 mg/m^2 on day 3, prophylactic intrathecal methotrexate on days 1 and 4, and G-CSF 5 mg/kg/d s.c. from day 7 to hematopoietic reconstitution (PMN $> 0.5 \times 10^9$/L).

Results. Fifty-five of the 70 patients (78%) achieved complete remission (CR), four died in aplasia due to infection and 11 were non-responders. Recovery of blood counts occurred at a median of 21 days from the start of treatment. Non-hematologic side effects were extremely limited and consisted predominantly of infections.

Interpretation and Conclusions. In view of the highly unfavorable series of patients selected, this study confirms the feasibility and antileukemic activity of the HD-idarubicin + HD- ARA-C combination in patients with refractory and early relapse ALL and NHL. The excellent tolerance to this regimen does not preclude bone marrow trasplantation as post-remission treatment.

Key words: acute lymphoblastic leukemia, chemotherapy, cytosine arabinoside, idarubicin, relapse

The anthracycline analog idarubicin, either alone or in combination with other antineoplastic drugs, has shown antileukemic activity in relapsed and refractory acute lymphoblastic leukemia (ALL). Three previous Italian multicenter studies (ALL R85; ALL R87; ALL R93) used standard dose idarubicin, administered daily or every other day, in association with intermediate or high dose (HD) cytosine-arabinoside (ARA-C) as reinduction therapy for relapsed or refractory adult and pediatric ALL patients.[1,2] In our experience, these combinations showed antileukemic activity but caused severe gastrointestinal toxicity frequently associated to serious and persistent infections. At the Memorial Sloan Kettering Cancer Center (MSKCC), New York, idarubicin has been used as once a week treatment or in continuous infusion in adult relapse ALL.[3] Both protocols showed significant mucosal toxicity (limiting dose intensity) suggesting that protracted low level exposure to idarubicin may contribute to mucosal toxicity. Idarubicinol, the active metabolite of idarubicin, in fact has a very long half-life (> 48 hours).

In an attempt to minimize the non-hematologic toxicity and obtain a potent antileukemic effect, MSKCC activated a pilot study in previously treated adult ALL, using HD-ARA-C combined with idarubicin administered as a single high dose infusion. A phase I study with an escalating dose of idarubicin was first carried out to determine the phase II dose. An idarubicin dose of 40 mg/m^2 was well tolerated and effective in relapsed and refractory adult ALL patients.[4]

We herein report our experience with a series of

pediatric and adult high risk ALL and NHL patients treated with the protocol above, which confirms its feasibility, response rate and individual compliance.

Patients and Methods

The current series includes patients with refractory or early relapse ALL. Patients with lymphoid blastic crisis (BC) of chronic myelogenous leukemia (CML) and hematological relapse of T-lymphoblastic non-Hodgkin's lymphoma (NHL) were also included. Patients were elegible for this regimen when the following pre-requisites were fulfilled: 1) age under 60 years; 2) non-mature B-ALL; 3) primary resistance ALL to intensive first line treatment (AIEOP-BFM as a therapy for children, GIMEMA-5-drug induction for adults); 4) early first ALL or T-lymphoblastic NHL hematological relapse (adults: first remission < 24 months; children: first remission < 30 months); 5) second or subsequent hematologic relapse; 6) lymphoid BC of CML.

Prior to therapy, patients gave their consent after having been advised about the investigational nature of the study, as well as of the potential risks.

The protocol (Figure 1) consisted of ARA-C 3 g/m^2/d intravenously (i.v.) over a 3 hour infusion for five days, idarubicin 40 mg/m^2 i.v. at day 3 and prophylactic intrathecal methotrexate (MTX dose per age) at day 1 and 4. G-CSF (Lenograstim) was subcutaneously (s.c.) administered at the dose of 5 mg/kg/d starting on day 7 (48 hrs after the last dose of ARA-C) and continuing until the granulocyte count exceeded 0.5×10^9/L for two consecutive days. Prednisone was also given at a dose of 0.5 mg/kg/d during treatment. For the prophylaxis of HD-ARA-C-induced photophobia and conjunctivi-tis, all patients received glucocorticoid eyedrops every 8 hours starting before the first dose and continuing for 48 hrs after the last dose of HD-ARA-C.

Post-remission therapy was not planned, but nonetheless, allogeneic bone marrow transplant (BMT) from an HLA identical sibling or an unrelated volunteer donor or with umbilical cord blood stem cells was mandatory in responsive patients. Toxicity was defined according to the World Health Organization (WHO) grading system.[5] Evaluation of the antileukemic efficacy was based on CALGB criteria.[6,7]

Results

From January 1995 to April 1997, 70 patients (47 males and 23 females) were enrolled in this study. Their pre-therapeutic characteristics are summarized in Table 1. Forty-four patients were children (age < 15 years) and 26 were adults (age > 15 years). The immunophenotype was of B-cell lineage in 43, of T-cell lineage in 19 and hybrid (My+) in 8. Fifty-one patients were in first and eight were in second bone marrow relapse; 10 were refractory to intensive first line therapy and 1 patient presented a lymphoid BC of CML. The median duration of initial remission for patients treated in first relapse was 11 months (3-30 months). All 44 children had received standard first line treatment according to the AIEOP 88-91-95 BFM-like protocols,[8] consisting of an 8-week induction with prednisone, vincristine (VCR), daunorubicin and L-asparaginase for the first 4 weeks followed by cyclophosphamide, ARA-C and 6-mercaptopurine (6-MP) for the subsequent 4 weeks. Induction was followed by 9 courses of multidrug

INDUCTION SCHEMA

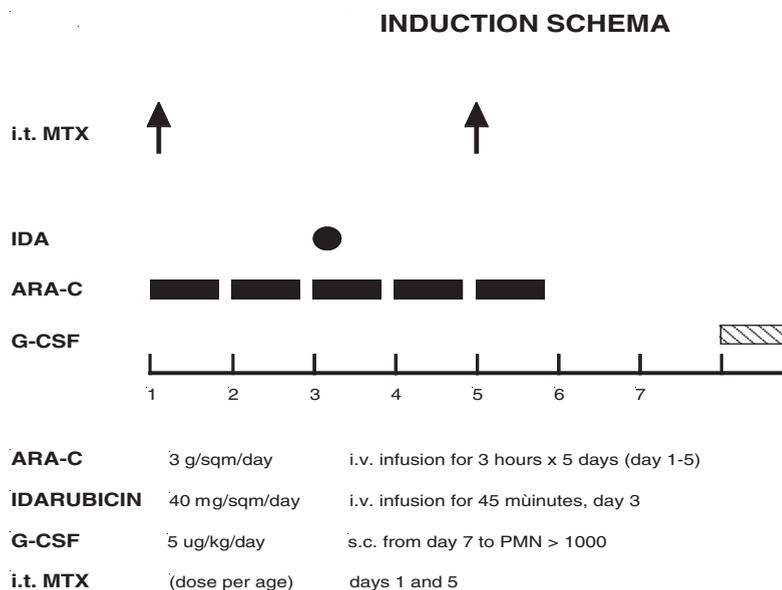|		
ARA-C	3 g/sqm/day	i.v. infusion for 3 hours x 5 days (day 1-5)
IDARUBICIN	40 mg/sqm/day	i.v. infusion for 45 mùinutes, day 3
G-CSF	5 ug/kg/day	s.c. from day 7 to PMN > 1000
i.t. MTX	(dose per age)	days 1 and 5

Figure 1. MSKCC ALL-3 protocol: induction schema.

chemotherapy (including HD-MTX, ARA-C and L-asparaginase) for high risk patients (9 children) and by HD-MTX consolidation, VCR, adriamycin, dexamethasone reinduction and standard MTX, 6-MP maintenance for standard risk patients (6 children). One child treated for his second relapse was reinduced with 2 blocks of the BFM-REZ 85 protocol[9] and a weekly standard dose of idarubicin and VCR. Front-line therapy for the 26 adult patients was as follows: the GIMEMA 0288 protocol[10,11] in 3; the GIMEMA 0394 pilot protocol[12] (8-week induction: standard dose ARA-C, etoposide, idarubicin combination followed by dexamethasone, VCR, L-asparaginase) in 12; Verona ALL protocol (high-dose anthracyclin) in 3; Stanford or LSA2-L2 protocols in 6 cases initially diagnosed as T-lymphoblastic NHL and hydroxyurea plus interferon-α in the patient with BC-CML.

Fifty-five of the 70 patients (78%) achieved complete remission (CR). In 11 non-responders (16%), leukemia recovered after marrow aplasia; four patients died during the aplastic phase from sepsis. Thirty-seven of the 44 children achieved CR (84%), while 18 of the 26 adults (69%) were complete responders (Table 2).

Treatment induced profound myelosuppression in all patients; G-CSF was administered to all patients, as planned. The median time to granulocytes $> 0.5 \times 10^9/L$ and to thrombocytes $> 50 \times 10^9/L$ was 17 days (8-36 days) and 22 days (5-41 days) from the start of treatment, respectively.

A summary of the extra-hematological side effects is reported in Table 3. Infection was the most frequent complication; twenty grade III-IV infective episodes were observed. Forty-eight febrile episodes occurred during neutropenia: bacterial infections were documented in 36 cases (12 sepsis; 4 fatal) and fungal in 8 (1 sepsis). Twelve patients experienced gastrointestinal toxicity including oral mucositis and diarrhea (WHO $\geq$ II). No severe cardiac events were observed after one induction cycle; one heavily pre-treated child experienced a dilatative miocardiopathy which precluded a post-remission bone marrow transplant following a second identical induction course, administered as a post-remission phase. No therapy-related pulmonary toxicity was observed. A transient cerebellar toxicity was observed in one child a few days after high-dose ARA-C infusion.

Fourty-nine of the 55 responders are evaluable for the follow-up. Three patients died in CR from infections; 2 were withdrawn for consolidation therapy-related toxicity; 12 relapsed at a median time of 4 months and 32 were considered eligible for bone marrow transplant. Twenty-one underwent an allogeneic BMT (10 from an HLA identical sibling, 4 from an unrelated donor and 7 with umbilical cord blood cells) and 3 received autologous BMT. Sixteen transplanted patients are cur-

rently living in CR with a median time from trasplant of 7 months (range 3-12 months); one died from transplant-related toxicity and 7 relapsed at a median of 6 months from transplant.

The other 8 responding patients received different chemotherapeutic maintenance treatments: four patients are in CR and in a waiting list for an unrelated BMT; four relapsed at a median time of 6 months while the search for an unrelated volunteer bone marrow donor was still ongoing.

Table 1. Patients characteristics.

	Total	Age < 15 yrs	Age > 15 yrs
Number of patients	70	44	26
Sex: M/F	47/23	29/15	18/8
Age			
median (years)	10	4.11	20
range	0.10-44	0.10-15	15.5-44
Phenotype			
B-lineage (Ph+)	43 (5)	31 (2)	12 (3)
T-lineage	19	9	10
Hybrid	8	4	4
Disease status			
primary refractory	10	4	6
2nd relapse	8	6	2
1st relapse	52	34	18
Previous treatments			
BFM-like protocols	42	42	-
GIMEMA 0394	12	-	12
GIMEMA 0288	3	-	3
GIMEMA "Verona"	5	2	3
"Stanford" - LSA$_2$-L$_2$	6	-	6
Others	2	-	2

Table 2. Induction results.

	Total	Age < 15 yrs	Age > 15 yrs
Number of patients	70	44	26
Complete Remissions	55 (78%)	37 (84%)	18 (69%)
Induction deaths	4 (6%)	3	1
Resistants	11 (16%)	4	7

Table 3. Extrahematologic toxicity.

	WHO=3	WHO=4
Oral mucositis	5	2
Diarrhea	6	3
Hepatic	3	2
Cardiac	1	0
Neurologic	1	–
Renal	3	–

Discussion

The efficacy of idarubicin associated to HD-ARA-C has been demonstrated in high risk ALL patients[1,2,3,13] and previously reported in this journal.[14,15] In the previous Italian multicenter experience, the two drugs were utilized in combination, at different doses, and in timing of administration, as reinduction therapy in refractory or relapse ALL (ALL-R-85, -87, -93 protocols). ARA-C was given at high/intermediate dose (3 g/m^2/12 hrs or 1 g/m^2/d x 6 doses) and idarubicin was administered following ARA-C infusion at a standard dose (12 or 10 or 5 mg/m^2/d), either daily or every other day. The ALL-R-85 and -87 studies included both children and adults with pretreated ALL, while in the ALL-R-93 protocol only children were enrolled. A total of 88, 147 and 80 patients, respectively, were treated with these three regimens. The combination of ARA-C and idarubicin showed antileukemic efficacy in all three protocols with CR rates of 59, 66 and 84%, respectively. Children had a better induction response as compared to adults, with CR rates ranging between 68 and 84% compared to 54-55% for adults. However, in all three studies gastrointestinal toxicity with mucositis and diarrhea, associated to serious bacterial and fungal infections, was the main side effect. Most of the complete responders suffered from severe or persistent infections precluding further intensive post-remisson treatment. Only 40-50% of responders resulted elegible for BMT. Similar observations on idarubicin + HD-ARA-C efficacy and toxicity have been witnessed at MSKCC in adult pretreated ALL patients.[3] With the aim of minimizing the extra-hematological toxicity, the current MSKCC (ALL-3) protocol uses HD-ARA-C combined with idarubicin administered as a single high dose (40 mg/m^2) in pretreated adult ALL.[4]

In agreement with MSKCC investigators, we applied the ALL-3 induction regimen to our high-risk ALL patients including adults and children. Seventy patients were treated and 55 (78%) achieved CR. The protocol was restricted exclusively to patients who were refractory to intensive first-line therapy, or to patients who were heavily pretreated and relapsed after a very brief initial remission (median CR length 11 months). Taking these prerequisites into account, which led to the selection of a highly unfavorable group of ALL, the CR rate of 78% clearly indicates the high efficacy of HD-idarubicin and HD-ARA-C regimen. These data can be compared with our previous trials, using the HD-ARA-C and idarubicin combination; again, children had a significantly better induction response compared to adults: 84% and 69%, respectively. Despite the intensive previous therapy, including drugs administered at high doses, the extra-hematological side effects were acceptable. Infection was the most frequent complication occurring in 44 cases; gastrointestinal toxicity was less frequent but severe, compared to that observed in the previous Italian multicenter studies. The majority of evaluable responder patients (32/49) were considered eligible for aggressive post-remission management, including allogeneic BMT.

The feasibility and the efficacy of this regimen suggest its application in cooperative studies, for the treatment of adults and children with refractory and relapse ALL. A multicenter study is necessary to delineate the incidence and the duration of response.

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IDARUBICIN IN LOW-GRADE NON-HODGKIN'S LYMPHOMAS

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ABSTRACT

The majority of responses produced in patients with low-grade lymphomas are unique among non-Hodgkin's lymphomas (NHL), and even with a more intensive chemotherapy regimen, they are only partial; the very few complete responses which are induced are usually of short duration and do not influence overall survival. There is, therefore, a need for new approaches to the management of low-grade NHLs. Studies are currently in progress to assess the potential benefits in the treatment of NHL offered by new drugs, including fludarabine, idarubicin and 2-chlorodeoxyadenosine. In order to evaluate Idarubicin in combination with purine analogs, we used a combination of fludarabine and idarubicin, called the FLU-ID regimen, to treat 10 patients with recurrent low-grade NHL. Of the 10 patients, 2 (20%) achieved complete response, 5 (50%) partial response, and the remaining 3 showed no benefit from the treatment. The 2 CR patients are still in remission after 12 and 14 months, respectively. The median duration of overall survival of all patients was 18 months. These results indicate the efficacy of the FLU-ID regimen in inducing a good remission rate with moderate side effects in recurrent low-grade NHL. On the basis of this pilot study, we planned a cooperative randomized trial for untreated patients.

Key words: non-Hodgkin lymphomas, idarubicin

Low-grade lymphomas are unique among non-Hodgkin's lymphomas (NHL) because in the early stages of disease most patients do not require treatment, and when treatment becomes necessary, they can be maintained on non-aggressive therapy, such as single alkylating agents, for many years. However, the majority of responses produced in these patients, even with more intensive chemotherapy regimens, are only partial, and the very few complete responses which are induced are usually of short duration and do not influence overall survival. There is, therefore, a need for new approaches to the management of low-grade NHLs. Idarubicina has been proven to be effective in different hematological neoplasms.[1-4] Studies are currently in progress to assess the potential benefits in the treatment of NHL offered by new drugs, including fludarabine, idarubicin and 2-chlorodeoxyadenosine.

Idarubicin, a derivative of daunorubicin, has been shown to be well-tolerated and effective in the treatment of low-grade NHL, producing response rates of up to 65% in various clinical studies.[5-8] Since this anthracycline can be administered orally as well as intravenously, it is an excellent candidate for the treatment of selected groups of patients such as the elderly and those with indolent diseases not requiring aggressive intervention therapy.

Case *et al.*[6] conducted a phase II study to assess the efficacy and toxicity of oral idarubicin in 46 patients with previously treated low-grade NHL. Each course of treatment consisted of a total of 45 mg/sqm of idarubicin, administered orally on three consecutive days, with intervals of three weeks between treatment courses. Complete remissions were observed in 10 patients (22%) and partial responses were seen in 16 (36%) patients, resulting in an overall tumor response rate of 58%. Idarubicin was generally well-tolerated, and no patients withdrew from the study due to treatment toxicity. Mild hematological toxicity was observed and was dose-limiting at higher doses of idarubicin. The major non-hematological symptom was nausea/vomiting which was experienced by 50% of the patients. Clinically relevant cardiac toxicity was not observed. Significant but mild changes in the resting cardiac ejection fraction were detected in four patients.

Low-grade NHLs are commonly treated by single alkylating agents such as chlorambucil. The advent of the new oral cytotoxic anthracycline, Idarubicin, has prompted an assessment of its efficacy within current treatment regimens in the management of patients with low-grade NHL. The identification of discrete prognostic groups of patients with low grade NHL has allowed an assessment of the efficacy of the treatment with respect to the prognostic risk status of patients. Proctor *et al.*[8] defined a treatment protocol (CID) for untreated patients with low-grade NHL as described below:

- chlorambucil 20 mg/sqm/day, days 1-3
- idarubicin 10 mg/sqm/day, days 1-3
- dexamethasone 4 mg bd., days 1-5

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given for 6 courses.

The rationale behind the treatment regimen was to expose the tumor, over a prolonged period of time, to the alkylating activity of chlorambucil, the cytotoxic and apoptotic activity of Idarubicin, and the apoptotic effect of dexamethasone. Under this regimen the lymphoma received sustained exposure to anti-tumour therapy for 3-5 days, with an additional therapeutic effect provided by the active metabolite of idarubicin, idarubicinol, which remains in the bloodstream for up to seven days.[9]

The median age of the population treated was 54 years: 24 females and 38 male patients. 79% were stage IV, 76% had follicular histology and the remaining patients had other low-grade histological groups. The overall response rate was 77%; the combined groups of patients achieving CR was 50% with patients achieving GPR and PR of 27%. The remaining 23% of the patients showed responses lower than PR. In this phase II study, the overall response rate for those with follicular histology was 88%.

Concerning toxicity, the majority of patients (35-50%) showed either mild (grade I and grade II, according to the WHO grading system) or no treatment-related toxicity throughout the six cycles of therapy. Only a small number of patients (1%) showed grade IV toxicity during the fifth course of treatment, although none of these patients required hospitalization for management of their symptoms. Overall, the CID treatment was well-tolerated, particularly in elderly patients, with dyspepsia being reported as the major adverse event; no patient suffered from alopecia.

As a consequence of the encouraging results from the phase II study, a phase III study has been undertaken to evaluate the efficacy of CID at the doses indicated above, in comparison with equivalent doses of chlorambucil and dexamethasone (CD) therapy in patients with low-grade NHL. These patients will undergo six courses of induction therapy with CID or CD at intervals of three weeks. Following determination of the tumor response rates and administration of radiotherapy to treat initial areas of bulky disease, patients will be randomized to receive one of two consolidation/maintenance therapies: no further therapy or α-interferon will be administered.

In order to evaluate Idarubicin in combination with purine analogs, we used a combination of Fludarabine and Idarubicin, called the FLU-ID regimen, to treat 10 patients with recurrent low-grade NHL.[10] The FLU-ID regimen was as follows: fludarabine 25 mg/sqm i.v. on days 1 to 3 and idarubicin 12 mg/sqm i.v. on day 1. Of the 10 patients, 2 (20%) achieved complete response, 5 (50%) partial response, and the remaining 3 showed no benefit from the treatment. The 2 CR patients are still in remission after 12 and 14 months, respectively. The

median duration of overall survival of all patients was 18 months. The major toxic effects observed were neutropenia (40%) and infections and/or febrile episodes (15%); no fatalities occurred due to the side effects of the drug. These results indicate the efficacy of the FLU-ID regimen in inducing a good remission rate with moderate side effects in recurrent low-grade NHL.

On the basis of this pilot study, we planned a cooperative randomized trial for untreated patients with these considerations:

1) use, at diagnosis, of the *International Prognostic Index* to stratify patients before treatment to deliver a risk-based therapy;
2) introduction of fludarabine in first-line treatment because of its activity in slow proliferating and resting cells through the apoptotic way, and its promising activity in previously treated low-grade NHL;
3) introduction of a fludarabine-idarubicin (FLU-ID) combination-containing regimen, because, among the typical drugs, idarubicin has shown interesting therapeutic results while reducing cardiotoxicity and alopecia;
4) use of a MACOP-B-like regimen, the CIVOP-B (cyclophosphamide, idarubicin, vincristine, prednisone, VP-16, and bleomycin) protocol, as conventional therapy for high-risk patients;
5) the role of α-interferon as maintenance therapy in prolonging disease-free survival. This study has been ongoing since September 1995 and, so far, 205 patients have been enrolled.

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