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Sequential continuous infusion of fludarabine and cytarabine associated with liposomal daunorubicin (DaunoXome®) (FLAD) in primary refractory or relapsed adult acute myeloid leukemia patients

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Abstract A large proportion of adult patients with acute myeloid leukemia (AML) relapse after treatment, and some of them are resistant to primary induction chemotherapy. Sixty-one patients from seven hematological centers with poor-risk AML, primary refractory ($n=16$), or relapsed ($n=45$) were treated with a salvage regimen, including fludarabine (2 days) and cytarabine (3 days) in a sequential continuous infusion, associated with liposomal daunorubicin (3 days) (FLAD). Complete response rate was 44% and 56% for refractory and relapsed patients, respectively, with an overall response rate of 52% (32 of 61). Twenty-two patients (36%) were resistant

to the salvage therapy. Seven patients (12%) died early during chemotherapy, four of them because of sepsis. Nineteen patients in complete remission (CR) underwent a stem-cell transplant (SCT) procedure: five autologous, nine from a HL-A identical sibling, and five from HL-A matched unrelated donors. Post-treatment aplasia and mucositis were major toxicities. Twenty patients (62.5%) relapsed after this treatment in a median of 7.3 months; ten patients relapsed after a SCT procedure. Nine patients are alive and disease free; three of them were rescued after a further cytotoxic treatment. The FLAD regimen proved to be an effective and well-tolerated treatment, with acceptable toxicity in this group of high-risk patients. A better response rate was obtained in the subgroup of relapsed patients, compared to patients treated for refractory disease. More than half (five of nine) of long-surviving patients are those who were submitted to a transplant procedure; thus, the main indication for FLAD seems to be to try to induce a rapid CR with minimum toxicity in order to perform a transplant as soon as possible.

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Keywords Refractory acute myeloid leukemia · Relapsed acute myeloid leukemia · Salvage treatment · DaunoXome · FLAD

Introduction

Although intensive chemotherapy, often including a stem-cell transplant (SCT), is currently used in the treatment of

acute myeloid leukemia (AML), a large proportion of patients relapse in their bone marrow or in extra-medullary sites, cure rate ranging from 30% to 40% [1, 2]. On the other hand, a minority of cases fail to achieve complete remission (CR) after a standard first line treatment. Age, cytogenetics, MDR-1 expression, previous or concomitant myelodysplasia, and first CR duration are the major prognostic factors influencing the outcome [3–7]. Primary refractory patients, and those who relapse early (i.e., first CR duration <6 months) cannot receive a transplant procedure, which is the best option with curative intent. The main objective in treating this group of poor prognosis patients is to adopt a salvage regimen having acceptable toxicity and capable of inducing high rate of complete remission, thus enabling patients to receive SCT.

Various salvage regimens, often including fludarabine associated with high-dose cytarabine (HDARAC) with or without an anthracycline, have been employed in this setting of AML patients. HDARAC increases intracellular availability of the active metabolite (Ara-CTP) and can rescue patients with refractory or relapsed leukemia; however, no survival advantage was found between poor-risk AML patients treated with HDARAC-containing regimens and those treated with investigational therapies [1]. Recent evidences suggest that fludarabine acts synergistically with cytarabine to increase intracellular Ara-CTP, if given soon before or simultaneously with cytarabine [8–10]. Such regimens (FLAG, Flag-Ida, FLANG) have been employed in several studies for treating AML in elderly patients and in those with a poor-risk disease, with CR rate ranging from 30% to 60% [11–17]. Unfortunately, toxicity is often relevant, and response duration is usually short, precluding most patients from benefiting of a transplant procedure.

A formulation of daunorubicin encapsulated in liposomal particles (liposomal daunorubicin, DaunoXome®) has an interesting pharmacokinetic profile: increased delivery to leukemic cells, decreased susceptibility to the membrane efflux pump, and reduced extra-hematological toxicity [18–23]. We have combined HDARAC, fludarabine and DaunoXome® in a salvage regimen to be applied in a multicenter study for rescuing primary refractory and relapsed AML adult patients. For the latter drug, we scheduled three escalating dose levels, each one to be administered to a small cohort of patients. The aims of this trial were (1) to induce CR in primary refractory patients or a further remission in relapsed patients, (2) to evaluate the toxicity of a high-dose chemotherapy, including liposomal daunorubicin, (3) to evaluate the dose-limiting toxicity of DaunoXome® when associated with a sequential continuous infusion of fludarabine and HDARAC, and (4) to test the feasibility of post-remission consolidation by a SCT procedure.

Materials and methods

Eligibility criteria Adult patients older than 16 years, with primary refractory or relapsed AML, with exclusion of promyelocytic leukemia, were included in this study. Absence of heart, liver, or kidney damage, as well as no active infection, were required for inclusion in the trial. Adequate heart function was assessed by echocardiography (left ventricular ejection fraction $\geq 50\%$) and electrocardiogram. Written informed consent was obtained from all patients.

Disease evaluation Morphologic leukemia subtypes were evaluated on bone-marrow smears according to standard procedures, using the revised FAB criteria [24] and according to the more recent WHO classification [25]. The disease status was assessed by bone-marrow cytology and flow cytometry analysis (FACSCalibur, Becton Dickinson). In addition, in several patients, gp170 expression was evaluated by incubating blasts with the MRK16 antibody (Kamiya Biochemical LTD) followed by flow cytometry analysis.

Cytogenetics Cytogenetic analysis was performed at diagnosis on 24-h cultured preparations of bone-marrow cells without stimulation. GTG-banded chromosomes were classified according to the International System for Human Cytogenetic Nomenclature [26]. At least ten metaphases were scored.

Treatment plan The salvage regimen consisted of a loading dose of fludarabine (10 mg/m^2) administered intravenously over 15 min, followed after 3 h by cytarabine (390 mg/m^2) for 3 h on day 1, then by fludarabine (25 mg/m^2) administered intravenously on days 1 and 2, which was associated with cytarabine (1.9 g/m^2) on days 1 and 3 and both administered by continuous infusion over 24 h. DaunoXome was diluted in 250 ml of 5% dextrose and administered i.v. as 1-h infusion on days 2, 3, and 4, scheduled with a dose escalation as follows: the first group received 60 mg/m^2 (level 1), the second group received 80 mg/m^2 (level 2), and the third group was planned to receive 100 mg/m^2 (level 3). Each group was scheduled to consist of four patients from each participating institution.

Patients who achieved CR or partial remission (PR) were consolidated with a second FLAD course, in which liposomal daunorubicin was administered for two doses on days 2 and 3. Additional consolidation courses could be administered to patients in CR or PR while waiting for a SCT procedure. The salvage regimen is shown in Fig. 1.

All responder patients aged less than 55 years underwent an allogeneic SCT according to donor availability (patients

Fludarabine 10 mg/m² i.v. 15' short infusion <i>followed after 3 hr by</i>	d 1
Cytarabine 390 mg/m² i.v. 3 hr infusion <i>followed by</i>	d 1
Fludarabine 25 mg/m² i.v. 24 hr continuous infusion	d 1, 2
Cytarabine 1.9 g/ m² i.v. 24 hr continuous infusion	d 1, 2, 3
DaunoXome 60 mg/ m² level 1 i.v. 1 hr infusion 80 mg/ m² level 2 100 mg/ m² level 3	d 2, 3, 4

Fig. 1 The FLAD regimen

with a HL-A identical sibling, who had achieved their first CR after the salvage protocol, underwent allogeneic SCT from identical sibling), while patients in second CR (with no identical sibling) were recruited for an unrelated marrow donor search. Auto-transplant was performed in patients aged 55 or older, and in some cases, by medical decision. Stem-cell transplants were performed at the various institutions according to the Center's own transplantation protocol, with conditioning regimens and GVHD prophylaxis given according to each institution's policy.

Concomitant medications Granulocyte colony stimulating factor (filgrastim or lenograstim at the dosage of 300 or 260 µg/day, respectively) was administered in patients with persisting neutropenia after the 20th day from the first day of therapy or in patients with neutropenia and proven infection until neutrophil recovery. Antiemetics, antibiotics, and antimycotics were also allowed according to clinical practice guidelines. Corticosteroids were used to control fever and chills during liposomal daunorubicin infusion. Packed red cells and platelets transfusions were used when hemoglobin was <8 g/dL and platelet count was below 10 × 10⁹/L, respectively.

Toxicity All patients were carefully monitored during the salvage chemotherapy until hematological recovery. All events were graded according to WHO toxicity criteria and registered on the patient clinical record.

Response evaluation Hematological response to salvage chemotherapy was evaluated by bone-marrow cytology on day 28, or delayed for 1 or 2 weeks until blood count recovery. Patients who achieved CR (bone-marrow blasts <5%) were scheduled to receive one or more consolidation courses, followed by a SCT procedure, while patients in PR (bone-marrow blasts between 5% and 20%) were consid-

ered for an additional salvage course. Patients showing leukemia persistence went off-study.

Statistical methods Disease-free survival (DFS) was calculated from the date of CR to the date of relapse, death, or last follow-up. Overall survival (OS) was defined as the time from inclusion in the study to death or last follow-up. Survival distributions were estimated by the method of Kaplan–Meier. Statistical differences in survival distributions were evaluated with the log-rank test. A two-tailed *p* value of 0.05 or less was considered statistically significant. Data analysis was performed using the STATISTICA 7.0 software.

Results

Patient population From December 2000 to December 2006, 61 consecutive patients from seven institutions (see Appendix) entered the study. There were 35 men and 26 women, with a median age of 43 years (range, 21–72). FAB and WHO subtypes are shown in Table 1. Sixteen patients entered the trial because of leukemia persistence after first line induction therapy (eight of 16 patients had received more than one course of induction therapy), while 45 had had medullary leukemia relapse. For both refractory and relapsed patients, FACS analysis performed on bone-marrow leukemic cells confirmed the immunophenotype

Table 1 Patient characteristics

	Refractory	Relapsed
<i>N</i>	16	45
Age, median (range)	43 (26–81)	43 (19–72)
Males/females	10/6	25/20
FAB subtypes		
M0	–	1
M1/M2	5/3	16/8
M4/M4eo	4/1	10/3
M5a/M5b	1/2	5/2
WHO subtypes		
AML with recurrent genetic abnormalities		
11q23 abnormalities	–	2
<i>t</i> (8;21)(q22;q22)	–	1
<i>inv</i> (16)(p13q22)	–	1
AML with dysplasia ≥50% of cells in 2 or more lineage	3	1
AML, not otherwise categorized		
Minimally differentiated	–	1
Without maturation	3	15
With maturation	3	6
Myelomonocytic leukemia	4	11
Monoblastic/monocytic leukemia	1/2	5/2

Table 2 Cytogenetic data ($n=49$)

	Number
Normal karyotype	31
Hyperploidy	6
+M	1
+8	2
+18	1
+22	1
66, XX	1
Hypoploidy	3
-5	1
-7	1
-20	1
Translocations	8
<i>t</i> (9;22)	2
<i>t</i> (8;21)	1
<i>t</i> (8;15)	1
<i>t</i> (3; 3)	1
<i>t</i> (2;14)	1
<i>t</i> (9;11)	1
<i>t</i> (15;19)	1
Others	6
del 2	1
der 7	1
del 17	1
inv 7	1
inv 16	1
iso 11	1

One patient showed both inv16 and trisomy 8. One patient showed both del 17 and trisomy 18. One patient showed both del 5 and iso 11. One patient showed both del 7 and der 7. One patient showed both trisomy 8 and +M.

+ Additional chromosome, - missing chromosome, *del* deleted chromosome, *der* derivated chromosome, *inv* inverted chromosome, *iso* isochromosome, +M additional unidentified genetic material

assessed at diagnosis. Multiple drug-resistance expression was evaluated in 30 cases, showing a low expression of gp-170, ranging from 3% to 7%. Fifty-seven patients had received as front-line treatment a standard three-drug regimen, including daunorubicin, or idarubicin, associated with standard or high dose of cytarabine and etoposide according to the EORTC/GIMEMA AML 10 or AML 12 multicenter trials. One patient had received liposomal daunorubicin in association with cytarabine, and three patients had already been treated with the association of fludarabine and HDARAC. Thirty-one patients (51% of the whole study population) had relapsed after a SCT procedure, 26 after auto-transplant, and five after allogeneic marrow transplant (one of them had received also one course of DLI). For relapsed patients, the median duration of the first CR was 7 months (range, 2–69 months).

Cytogenetics Evaluable metaphases were obtained at diagnosis in 49 of 61 patients. A normal karyotype was

observed in 31 cases. Various chromosome translocations were present in eight patients, two of them showing the Ph chromosome [*t*(9;22)]. Hyper- and hypodiploidy were detected in six and three patients, respectively. Other anomalies were observed in six cases, as shown in Table 2.

DaunoXome® dose escalation study With DaunoXome used at 80 mg/m² (i.e., second dose level), the toxicity was judged unacceptable. Indeed, four patients from two different centers treated with the salvage regimen, including the second dose level, experienced severe neutropenia (ANC < 0.1 × 10⁹/L) lasting more than 30 days, associated with severe mucositis (grade IV WHO) and requiring total parenteral nutrition for several days. For these reasons, the protocol writing committee decided to stop the dose escalation program and fixed the DaunoXome dose at 60 mg/m² for all subsequent patients entering this study.

Response evaluation At hematological recovery after salvage therapy, CR was documented in seven of 16 patients (44%) with refractory leukemia and in 25 of 45 patients (56%) with leukemia relapse, with an overall response rate (ORR) of 52% (32 of 61). Twenty-two (36%) patients showed leukemia persistence, and seven patients (12%) died during chemotherapy: four because of infection, two because of disease progression, and one because of cerebral hemorrhage. These data are summarized in Table 3. Twenty-three patients received one or more courses of consolidation therapy, with a median of two courses. Hematological relapse was observed in 20 of 32 cases (62.5%), with a median of 7.3 months (range, 0.6–54.1 months) from CR. Eighteen patients had medullary relapse, whereas two patients experienced extra-hematological relapse: one in the left breast and the other in the left leg soft tissue. As for hematological response, no statistical difference was observed when patients were stratified for age (≤40 years vs >40 years). When patients were stratified according to their first CR duration (≤12 vs >12 months), a better CR rate (68%) was observed in the group of patients with a first CR >12 months ($p=0.0002$; Fig. 2). As for relapse after a transplant procedure, when this group of patients was stratified according to time from SCT (≤6 vs >6 months), a trend of better response was observed for patients who relapsed >6 months after the transplant.

Table 3 Response to salvage treatment

	Refractory	Relapsed	Total
<i>n</i>	16	45	61
CR	7 (44%)	25 (56%)	32 (52%)
Leukemia persistence	8 (50%)	14 (31%)	22 (36%)
Early deaths	1 (6%)	6 (13%)	7 (12%)

Table 4 Toxicity after the salvage regimen

	Days to recovery, median	Grade (WHO)	Number
Hematological toxicity			
ANC > 0.5 × 10 ⁹ /L	21		
PLT > 20 × 10 ⁹ /L	24		
Extra-hematological toxicity			
Heart function			
		1	3
		2	2
Hepatic			
		1	8
		2	4
		3	3
Oral			
		1	4
		2	11
		3	4
		4	6
Enteric			
		1	7
		2	7
		3	1
FUO			34
Sepsis			6

Acute toxicity The main toxicity was myelosuppression. Median time to granulocyte (>0.5 × 10⁹/L) and platelet (>20 × 10⁹/L) recovery was 21 and 24 days, respectively. Fever (>38°C) for a median of 4 days (range, 1–20) occurred in 40 patients: six patients had positive blood cultures, while 34 had fever of unknown origin. Blood sepsis was fatal in four out of the six patients. Heart, liver, and enteric toxicity were negligible.

The main extra-hematological toxicity was stomatitis, occurring as grade II–III in 14 patients, while grade IV was

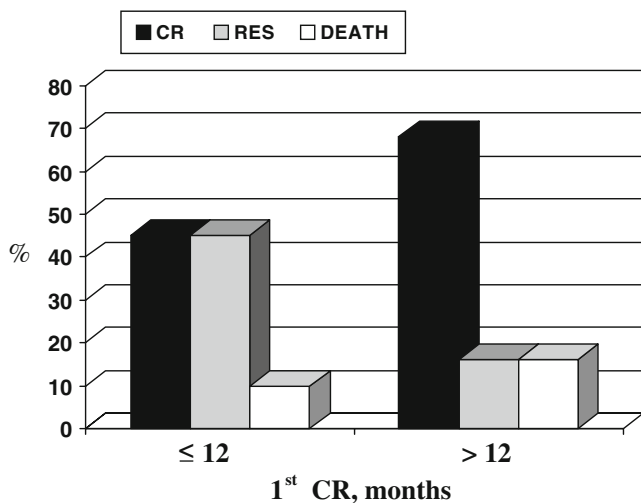


Fig. 2 Response rate according to first CR duration

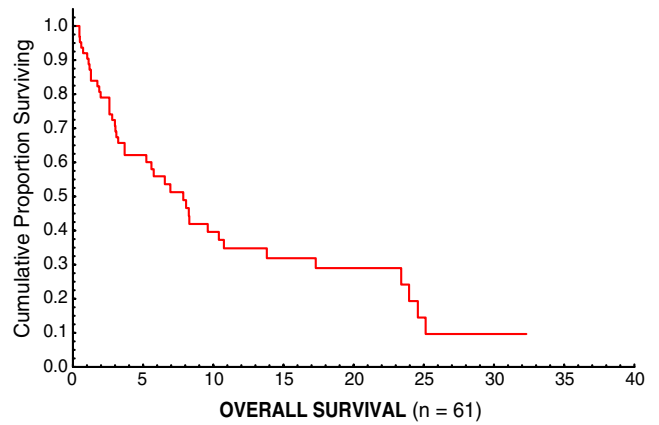


Fig. 3 Overall survival, months

observed in all patients (*n*=4) who had been treated with the salvage regimen containing the second dose level (i.e., 80 mg/m²) of liposomal daunorubicin. Thus, in our hands, stomatitis represented the dose-limiting toxicity in this study. The data on toxicity are summarized in Table 4.

Stem-cell transplant Nineteen patients, 31% of the whole patient population (59% of those achieving CR), underwent a stem-cell transplant according to age and donor availability. Nine patients received a transplant from an HL-A identical sibling and five patients from a unrelated marrow donor; five patients were auto-transplanted, four from peripheral blood stem cells and one from bone marrow. Ten patients relapsed after the SCT procedure (four after auto-transplant, three after a HL-A identical sibling, and three after an unrelated donor transplant), with a median of 6.6 months. Among the transplanted patients, five are alive and are in continuous remission.

Survival analysis Median OS was 5.8 months (range, 0.5–67.3; Fig. 3) for the whole population; it was 12.8 months

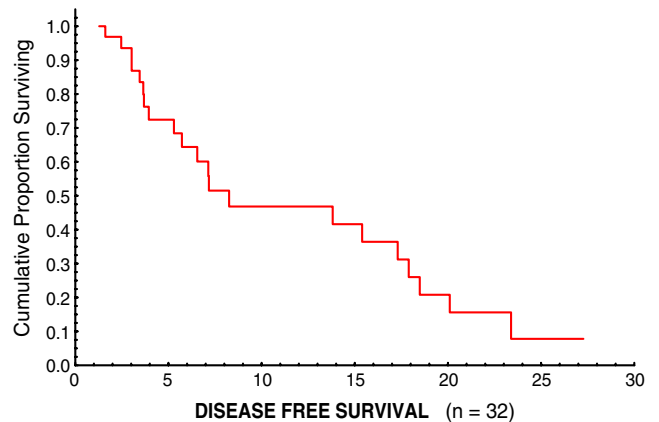


Fig. 4 Disease-free survival, months

in therapy responders, and only 2 months for resistants to salvage treatment. Median DFS was 7.3 months (range, 0.6–54.1; Fig. 4). Three patients died in CR: one from acute GVHD, one from pulmonary aspergillosis, and one from heart failure. Nine patients are alive, six of them in CCR after salvage, and three were rescued with additional cytotoxic treatment after a further relapse.

Discussion

In adults with AML, while remission rate has improved in the last decade, leukemic relapse remains the main cause of treatment failure. Indeed, in spite of the availability of several effective cytotoxic drugs and of various intensive regimens, including SCT, the outcome of refractory or relapsed AML patients is still disappointing [1]. Because of the synergistic activity of fludarabine with cytarabine, this combination has been recently employed to rescue patients with relapsed AML [9]. While it is well known that high-dose cytarabine is able to overcome leukemic cell resistance, the efficacy of anthracyclines in salvage regimens, in combination with the mentioned drugs, is not yet established. Furthermore, patients with relapsed or primary refractory AML were frequently proven to be resistant to various cytotoxic drugs because of the emergence of multiple-drug resistant clones showing high expression of the gp-170 molecule and/or of alternative mechanisms of resistance, mainly affecting leukemic cell susceptibility to anthracyclines [5].

Several preclinical and clinical studies have shown that liposomally encapsulated daunorubicin has a peculiar pharmacokinetic and pharmacodynamic profile, with improved leukemic cell delivery, reduced susceptibility to gp-170 membrane efflux pump, and reduced toxicity [18, 27, 28].

Taking into account these data, we designed this multicenter trial for salvage treatment of adult patients with primary refractory or relapsed AML, which included the combination of sequential continuous infusion of fludarabine and HDARAC combined with liposomal daunorubicin.

The observed ORR (52%) can be considered excellent in this subset of patients, which included those with primary refractory disease after standard or high-dose Ara-C induction (44% CR), even if the best response rate was obtained in the group of relapsed patients. Similar results have been recently published by Clavio et al. [29] using a salvage treatment with the same drugs as in this study but without continuous infusion in a variety of patients with poor-risk acute leukemias (ALL, AML resistant or relapsed, de novo AML in elderly people).

SCT procedures (autologous or allogeneic transplant) were performed in a high proportion of patients enrolled in this study, allowing them to attempt leukemia eradication; this opportunity was also given to a few patients with a HL-A identical sibling, who had not achieved CR after front-line induction treatment.

As foreseeable for relapsed patients, the response rate to salvage therapy correlated with the duration of the first CR. Indeed, when we stratified the patients according to first CR duration into two groups (≤ 12 and >12 months), we observed a better outcome for patients with first CR duration >12 months.

We also performed a dose-finding study to evaluate the maximum tolerated dose (MTD) of DaunoXome[®], planning three escalating doses in the context of the intensive three-drug regimen. Although the number of patients was too small to draw any definitive conclusion, the cumulative dose of 180 mg/m² (60 mg/m² for 3 days) showed the best efficacy/toxicity ratio. Indeed, when we used the dose scheduled for the second level (80 mg/m² for 3 days), we observed severe stomatitis and unacceptable prolonged neutropenia in four patients. The MTD of DaunoXome[®] is reported to be much higher when used as a single cytotoxic agent [21].

On the whole, the association of DaunoXome cumulative dose 180 mg/m² with HDARAC and fludarabine caused acceptable hematological and extra-hematological toxicity, with early deaths occurring only in few cases. It is to be noticed that no patient showed severe heart or gut toxicity.

In conclusion, the association of fludarabine, high-dose cytarabine, and DaunoXome[®] proved to be effective in inducing high CR rate in poor-risk AML patients, without the occurrence of significant toxicity. This salvage regimen allowed to perform a SCT procedure in a high proportion of patients in CR. High relapse rate remains the major cause of unsatisfactory outcome in these patients, pointing out to the need of more eradicating chemotherapeutic and/or conditioning regimens.

Appendix: Participating centers and number (#) of patients enrolled

Università Federico II, Napoli (Prof. Bruno Rotoli) #30
A.O.R.N. Cardarelli, XIX, Napoli (Dr. Felicetto Ferrara) #17
Ospedale Civile, Avellino (Dr. Nicola Cantore) #7
A.O.R.N. Cardarelli, TERE, Napoli (Dr. Vincenzo Mettivier) #2
Ospedale S. Giovanni Bosco, Napoli (Dr. Eustachio Miraglia) #2
Ospedale S. Gennaro, Napoli (Dr. Lucia Mastrullo) #2
Ospedale Civile, Arienzo (Caserta) (Dr. David Pagnini) #1

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