Fludarabine and cytarabine as continuous sequential infusion for elderly patients with acute myeloid leukemia

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Background and Objectives. A phase II study was conducted to investigate the effects of a therapeutic program based on the combination of fludarabine and cytarabine (ARA-C) administered as a sequential continuous infusion in untreated elderly patients with acute myeloid leukemia (AML).

Design and Methods. Sixty-three patients with non-M3 AML, median age 69 years (range 61-81), were accrued. Twenty-four patients (38%) had AML secondary to myelodysplastic syndrome. Fludarabine and ARA-C were administered as a continuous sequential infusion for 72 and 96 hours, respectively, after a loading dose. Patients achieving complete remission (CR) were intended to receive an additional course, followed by autologous stem cell transplantation (ASCT).

Results. Overall, 42 patients (67%) achieved CR. There were 10 induction deaths (16%), while 11 patients were refractory (17%). Among those achieving a remission, 35 patients (83%) received the planned consolidation course and 29 underwent mobilization of $CD34^+$ cells into the peripheral blood for collection, which was successful in 23 (79%). Overall, 17 patients (27% of the whole population) received ASCT. The median overall and disease-free survival were both 10 months.

Interpretations and Conclusions. Patients with an intermediate karyotype and those receiving ASCT had a significantly better clinical outcome. Results in terms of CR achievement, $CD34^+$ cell collection and ASCT feasibility. A longer follow up is needed in order to evaluate the actual benefit on long-term survival.

Key words: acute myeloid leukemia, elderly patients, continuous infusion, fludarabine, cytarabine

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Current therapeutic strategies for the treatment of acute myeloid leukemia (AML), including intensive induction followed by consolidation with chemotherapy and/or stem cell transplantation, result in long-term disease free survival in about 40% of young/adult patients.[1-3] However, AML is a disease affecting predominantly elderly individuals, with a median age at diagnosis of 65-70 years and, as compared to younger adults or children, older AML patients have a poorer prognosis.[4-7] Apart from host-related factors, such as concomitant diseases and an inferior capacity to withstand the side-effects of chemotherapy, disease-related factors including more frequent unfavorable cytogenetics, antecedent hematologic disorders and high levels of multidrug resistance protein account for the unsatisfactory outcome of the disease in the elderly.[4-9] New approaches are, therefore, strongly needed in order to improve therapeutic results. The efficacy of the combination of fludarabine with intermediate dose cytarabine (ARA-C) is dependent on the synergy between the two agents.[10] In addition, granulocyte colony-stimulating factor (G-CSF) is thought to potentiate the effects of ARA-C by enhancing its incorporation into DNA.[11] Accordingly, different authors have investigated the potential utility of the combination fludarabine + ARA-C + G-CSF +/- anthracyclines as treatment for poor-risk AML or advanced myelodysplastic syndromes (MDS) with encouraging results.[12-18] Promising results have also been reported in both children and adults following administration of fludarabine and ARA-C as a continuous sequential infusion in refractory, relapsing or poor-risk newly diagnosed AML patients.[19-20] Such a schedule enables the administration of high-dose therapy without increasing the non-hematologic

toxicity, an approach that would be particularly useful in older patients.[21]

In a preliminary study on 20 AML patients aged over 60 years, we previously demonstrated that continuous sequential infusion of fludarabine + ARA-C was feasible with a promising complete remission (CR) rate of 70%.[22] Here we report the disease characteristics and treatment results from a larger series of 63 patients affected by AML with a median age of 69 years, treated with induction/consolidation therapy based on continuous sequential infusion of fludarabine + ARA-C. In addition, CD34-positive ($CD34^+$) cell mobilization and collection as well as overall feasibility of autologous stem cell transplantation (ASCT) was investigated. All the 20 patients accrued in the pilot study are included in the present series.

Design and Methods

Inclusion criteria

Patients older than 60 years with a morphologic diagnosis of AML according to FAB criteria,[23] with the exception of acute promyelocytic leukemia, were accrued into the study. In all cases, diagnosis was confirmed by immunophenotypic analysis after flow cytometry examination as previously described.[24] Cytogenetic analysis was performed by a direct method using RHG banding on a minimum of 20 fully evaluable metaphases, and the prognostic categorization was defined according to MRC criteria.[25] Patients with AML arising after a previously diagnosed MDS or with AML secondary to prior chemotherapy or radiotherapy for other malignancies were also included. According to WHO criteria,[26] patients were required to have a performance status (PS) of 0-3. Criteria for exclusion included severe organ dysfunction not related to AML or left ventricular ejection fraction (LVEF) less than 40% as measured by echocardiography. Written informed consent was obtained in all cases after the local ethical committees' approval of the study protocol. All cases of AML in patients aged over 60 years diagnosed at the two participating institutions during the period of the study were registered in order to assess the ratio between patients observed and patients actually accrued into the trial.

Treatment design

Fludarabine was administered at a loading dose of 10 mg/m^2 over 15 min on day 0 followed 6.5 hours later by continuous infusion of 20 mg/m^2/24 hours for 72 hours (days 0-2); ARA-C was given at a loading dose of 390 mg/m^2 3.5 hours after the fludarabine and then as a continuous infusion over 96 hours at 1440 mg/m^2/24 hours (days 0-3). G-CSF was added on day +15 at a dose of 5 μg/kg. A second identical course was planned for patients obtaining a partial response (PR), defined as less than 5% blasts in peripheral blood and less than 30% blasts in the bone marrow. Patients achieving morphologic CR, defined as less than 5% blasts in the bone marrow, normal blood count and differential and absence of extramedullary leukemia,[27] were intended to receive an additional identical course of continuous infusion fludarabine + ARA-C as consolidation therapy. Patients with bone marrow blast cells less than 5% and incomplete hematopoietic recovery were defined as having CRi.[27] However, after the first 20 patients consolidation was shortened by reducing both the duration of fludarabine and the ARA-C infusions to two and three days, respectively, because of excessive toxicity (*see Results*). Following consolidation, G-CSF was given at a dose of 10 μg/kg from day 15 with the aim of shortening neutropenia and mobilizing $CD34^+$ cells. All patients in whom collection of $CD34^+$ cells was successful (≥2x10^6/kg) were intended to receive ASCT with a conditioning regimen consisting of high dose continuous infusion idarubicin plus busulphan, as previously described.[28] A PS <3, absence of active infection and/or severe organ damage were required for the patients to be considered for ASCT. Toxicity was recorded according to WHO criteria.[26] Prophylaxis against infection consisted of oral ciprofloxacin and oral fluconazole, while no antiviral prophylaxis was given. Indications for antibiotic therapy included fever >38°C with a leukocyte count <1×10^9/L, as well as signs or symptoms of infection. Intravenous fluconazole was used for proven candidiasis infection, while amphotericin B was given for aspergillosis or other invasive fungal infections when suspected (fever persisting for more than 7 days while on treatment with broad-spectrum antibiotics) or documented. In all patients red cell concentrates were given to maintain the hemoglobin level >8 g/dL, while platelet concentrates were administered to keep the platelet value > 10x10^9/L. All transfused blood products were depleted of leukocytes to minimize the risk of transfusional graft-versus-host disease. Disease-free survival (DFS) was defined as the time from CR achievement to relapse or death from any cause, overall survival (OS) as the time from diagnosis until death from any cause. DFS and OS were calculated by the Kaplan–Meier method.[29] Differences in the distribution of individual parameters among subsets of patients were analyzed using χ^2 or Student's t-tests. The *p* value for all statistical comparisons was two-tailed. Multivariate analysis was performed by a Cox proportional hazard regression model. Finally, the log-rank test was applied to calculate the significance of differences between survival curves. Differences were considered to be statistically significant when the *p* value was <0.05.

Results

Patients' characteristics and accrual

Between December 2001 and April 2004, a total of 122 patients with non-M3 AML older than 60 years were diagnosed at the two institutions involved in the trial. Among these, 59 patients (48%), whose median age was 74 years (range 64-88), were considered ineligible for intensive treatment. The most frequent reasons for exclusion were severe co-morbidity and/or a poor PS. Less frequently, lack of family or social support and refusal accounted for the therapeutic choice. According to age, the rate of inclusion into the trial was 77% and 35% for patients aged 61-70 years and >70 years, respectively ($p<0.001$).

Overall, 63 patients (52%) were considered eligible for the trial and all received the induction therapy. The median age of these patients was 69 years (range 61-81). In 24 patients (38%) a previously diagnosed MDS preceded the onset of AML; among these, MDS was secondary to chemo- or radiotherapy for another malignancy in 3 patients (2 multiple myeloma, 1 breast cancer). Cytogenetic analysis was successful in 54 out of 63 cases (86%). Among these, a normal karyotype was found in 31 patients (57%) and a complex karyotype or other unfavorable chromosomal abnormalities in 23 (43%); no patient had AML with t(8;21) or inv(16). Thirty-seven patients (59%) were aged 61-70 years, while 26 (41%) were older than 70 years. The main characteristics of the 63 patients considered eligible for the trial are summarized in Table 1.

Table 1. Characteristics of the patients.

# patients	63
Sex M/F	30/33
Median age (range)	69 (61-81)
Performance status	
0-1	35 (56%)
2	22 (35%)
3	6 (9%)
Age 61-70/Age >70	37/26 (59%/41%)
WBC at diagnosis ($\times 10^9$/L)(range)	8,6 (0,7-276)
FAB	
M1	17 (27%)
M2	21 (33%)
M4	18 (29%)
M5	6 (9%)
M7	1 (2%)
Cytogenetics	
Intermediate	31 (49%)
Unfavorable	23 (37%)
No metaphases	9 (14%)
Previous MDS	
Yes	24 (38%)
No	39 (62%)

Table 2. Therapeutic results.

Response to induction	
CR	42 (67%)
Death	10 (16%)
Refractory	11 (17%)
Consolidation	
Yes	35
No	7
Death after consolidation	6 (17%)
Mobilization	
Yes	23 (79%)
No	6 (21%)
CD34+ cells ($\times 10^6$/kg)	7,56 (2.1-60.3)
ASCT	
Yes	17
No	6
Transplant related mortality	0 (0%)
N. of relapses	
Overall	17/42 (40%)
After ASCT	5/17 (29%)

Treatment results and toxicity

The therapeutic results are shown in Table 2. Overall, 42 patients achieved CR (67%), all following one course of continuous infusion fludarabine + ARA-C. Among these, two were classified as achieving CRi, because of incomplete platelet recovery, not needing platelet transfusion. There were 10 induction deaths (16%), while 11 patients showed primary refractoriness to treatment (17%). Data on hematopoietic recovery and load of supportive therapy are summarized in Table 3. The median number of days to neutrophil >1.0×10^9/L and platelet >20×10^9/L recovery was 19 (range 7-34) and 19 (range 9-38), respectively. Patients needed a median of 3 platelet units (range 1-19) and 7 blood units (range 1-38). Most patients experienced severe pancytopenia requiring broad spectrum empiric antibiotic therapy. Induction deaths were due to infectious episodes which occurred during the aplastic phase (n=8) and cerebral hemorrhage (n=2). WHO >2 extrahematologic toxicity consisted of increases in liver enzymes (3 patients), an increase in liver enzymes plus serum bilirubin (1 patient) and diarrhea (7 patients). In addition, three patients developed a tumor lysis syndrome, 2 with severe hypocalcemia. In all cases, tumor lysis syndrome resolved after hyperhydration and correction of metabolic abnormalities. No neurologic toxicity was observed. The main toxicity was due to infections: 17

Table 4. Influence of different parameters at diagnosis on CR achievement.

	CR rate	*p value*
Cytogenetics		
intermediate	77%	0.08
unfavorable	52%	
Age		
≤ 70 years	70%	0.59
> 70 years	61%	
Previously diagnosed MDS		
yes	61%	0.41
no	75%	
WBC count at diagnosis	≤	
≤ 50x10^9/L	72%	0.10
> 50×10^9/L	46%	

Table 3. Hematopoietic recovery and load of supportive treatment.

Median days to ANC>1.0×10^9/L (range)	19 (7-34)
Median days to Plt>20×10^9/L (range)	19 (9-38)
No. of RBC units, median (range)	7 (1-38)
No. of Plt transfusions, median (range)	3 (1-19)
Median days of fever (range)	6 (1-19)
Median days of iv antibiotics (range)	15 (4-60)
Median days of hospitalisation (range)	25 (13-65)

Data refer to induction therapy.

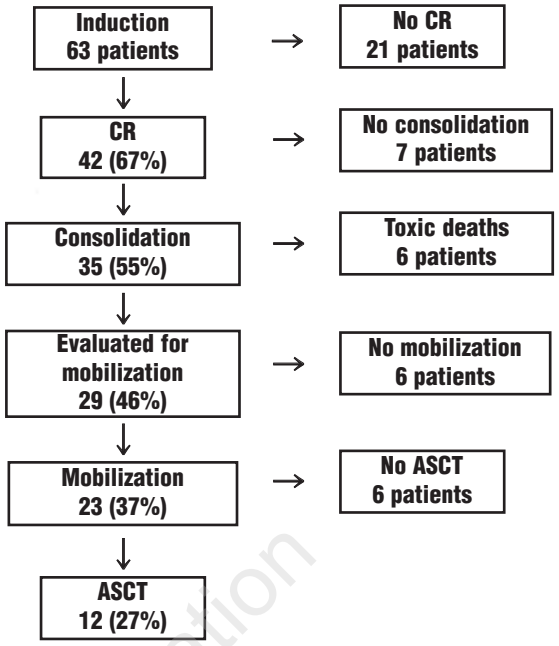

Figure 1. Flow chart showing the progressive reduction of patients from diagnosis to ASCT.

patients (27%) experienced documented infections (13 of bacterial and 4 of fungal origin), accounting for death in 8 cases. Fever of unknown origin (FUO) occurred in 37 patients, while 8 subjects did not experience fever at all. The median number of days with fever >38.5°C and intravenous antimicrobial agents was 6 (range 1-19) and 15 (range 4-60), respectively. The median time spent in hospital for induction treatment was 25 days (range 13–65).

Table 4 summarizes the influence of different parameters on the achievement of CR. Cytogenetic findings at diagnosis did exert a border line effect (77% CR for patients with intermediate karyotype as compared to 52% for those with adverse karyotype, p=0.08). On the other hand, white blood cell (WBC) count at presentation higher or lower than 50×10^9/L, age less or more than 70 years and the presence or not of a previously diagnosed MDS did not significantly affect CR achievement. Of the 42 patients who achieved a remission, 35 (83%) received the planned consolidation course, while in 7 cases therapy was discontinued due to loss from follow-up (n=1), unresolved infection (n=2), persistent thrombocytopenia (n=2), a cerebral hemorrhage which occurred after the CR had been obtained (n=1) and extrahematologic toxicity (n=1). In addition, while adopting consolidation therapy identical to the induction, 6 patients died in first CR, 4 from infection in the post-therapeutic aplastic phase (2 bacterial and 2 fungal) and 2 from myocardial infarction apparently unrelated to previous chemotherapy. No infectious or hemorrhagic deaths were observed after reduction of the consolidation course from four to three days of therapy. Twenty-nine patients were monitored for the mobilization of CD34$^+$ cells, collection being successful in 23/29 (79%). The median age of mobilizers was 67 years (range 61-77); of note, 9 out of them (39%) had secondary AML and 9 (39%) were aged over 70 years. There was no difference in CD34$^+$ collection between patients receiving consolidation with three or four days of chemotherapy (p=0.78). The median number of CD34$^+$ cells collected was 7.56×10^6/kg (range 2.1-60.3), the median number of aphereses was 2 (range 1-3). Overall, 17 patients (27% of the total patient population, 40% among those who had a remission, and 74% of mobilizers) received ASCT, after a median time from CR achievement of 3 months (range 2-4). The median age of autografted patients was 67 years (range 61-77). Reasons for not autografting the 6 patients who did have successful CD34$^+$ collection were early relapse (n=2), which occurred in both cases 2 months after CR achievement, refusal (n=1), infection (n=2), and severe uncontrolled diabetes resulting in ischemic gangrene of the left foot (n=1). Of note, both early relapsing patients had a complex karyotype; in addition, one had AML secondary to previous chemotherapy for multiple myeloma and one had developed AML secondary to MDS after an orthotopic liver transplant. At the time of writing 24 patients are alive: 18 are in continuous CR

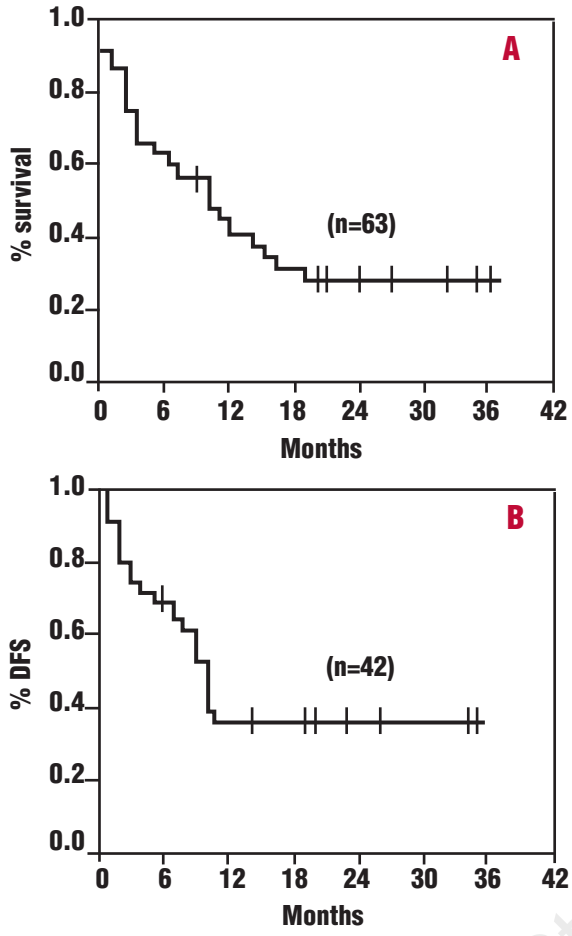

Figure 2. Median overall survival (panel A) and disease-free survival (panel B) of the whole patient population.

after a median follow-up for surviving patients of 12 months (range 6-36), and 6 have relapsed disease. Figure 1 shows the progressive loss of patients from diagnosis to ASCT. The median survival for the whole population of patients was 10 months and the median DFS for patients achieving CR was 10 months (Figure 2). According to cytogenetics at diagnosis, overall survival was significantly better in patients with an intermediate risk karyotype as compared to those with an unfavorable karyotype, the median survival being 16 and 6 months (p=0.02), respectively, as shown in Figure 3. In contrast, the presence of antecedent MDS and age more or less than 70 years had a negligible impact on survival (Figure 4). As far as concerns WBC count lower or higher than 50×10^9/L at diagnosis, there was a trend toward statistical significance when median survival was evaluated (11 vs. 6 months, respectively, p=0.09); however, long-term survival was only achieved in patients without hyperleukocytosis at diagnosis (Figure 5). In the multivariate analysis, performed by taking into account age below or above 70 years, WBC count

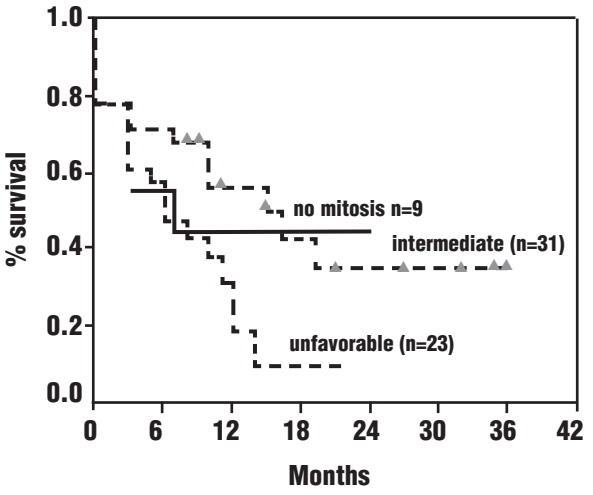

Figure 3. Overall survival by cytogenetics at diagnosis: the difference between intermediate karyotype (n=31 patients) vs. unfavourable karyotype (n=23 patients) was statistically significant (p=0.02).

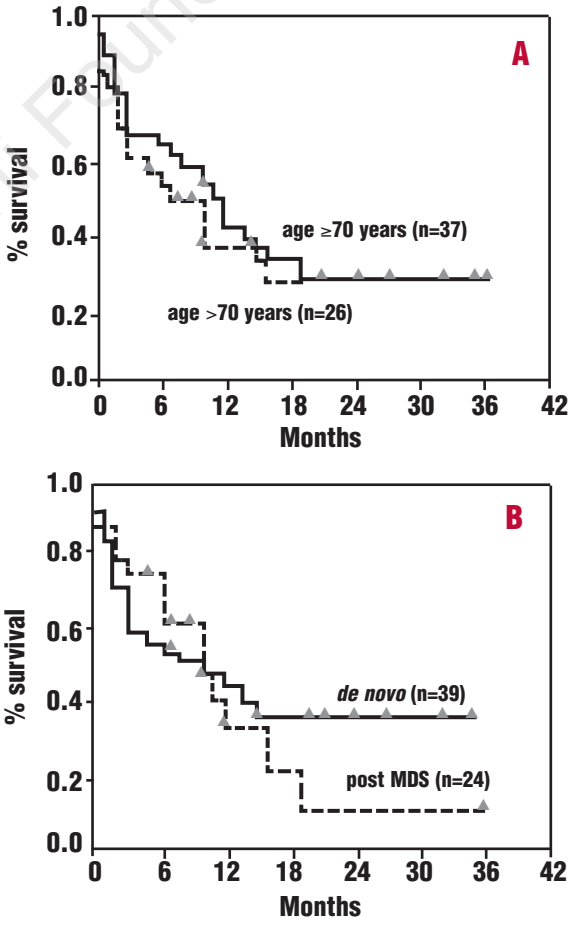

Figure 4. Panel A: overall survival by age more or less than 70 years (p=0.53). Panel B: overall survival by presence of previously diagnosed MDS (p=0.67).

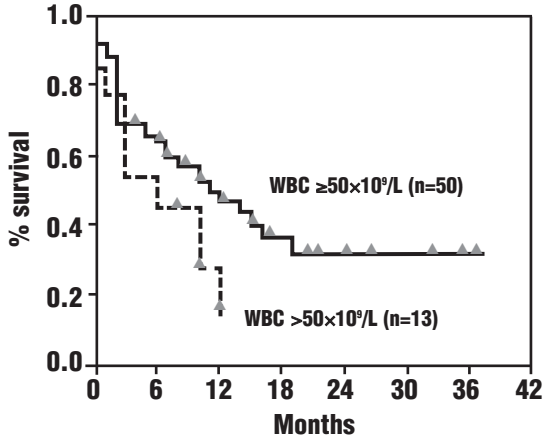

Figure 5. Overall survival by number of WBC more or less than 50×10^9/L at diagnosis (p=0.09).

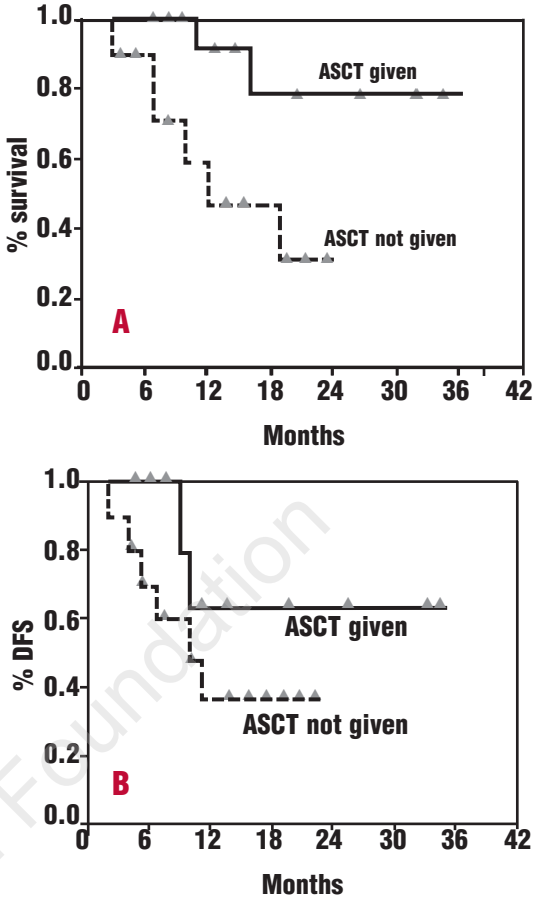

Figure 6. Overall survival (panel A) and disease–free survival (panel B) of the mobilized patients (n=27): ASCT given (n=17) vs. no further consolidation (n=10) (p=0.01 for OS and p=0.08 for DFS)

at presentation (> or < 50×10^9/L), presence of previous MDS and cytogenetic findings, adverse karyotype was the only parameter significantly related to either OS or DFS duration (p=0.02 and 0.04, respectively). The impact of ASCT on the clinical outcome was evaluated within the group of patients (n=27) who survived after consolidation and were monitored for mobilization of $CD34^+$ cells, excluding the 2 patients not autografted because of early relapse. Patients receiving ASCT (n=17) had significantly better OS and DFS as compared to the group which did not undergo autografting (n=10) (median not reached vs 12 months, p=0.01 for OS, and median not reached vs 10 months, p=0.08 for DFS, respectively, as shown in Figure 6). Five patients relapsed after ASCT; of note, four of them had a normal karyotype and one had a complex karyotype.

Discussion

The optimal therapeutic approach for elderly patients with AML is still matter of debate.[30-32] An ideal therapy would result in a high CR rate, without hampering the possibility of peripheral blood stem cells (PBSC) mobilization or the feasibility of stem cell transplantation procedures, given that both autologous and allogeneic stem cell transplants have been proven useful in this setting.[33-37] However, although results reported for patients managed with supportive therapy and/or hydroxyurea are poor, a relevant selection of patients still occurs in clinical practice. In leukemia trials from NCI-sponsored cooperative groups, less than 10% of patients were older than 65 years.[38] In the present study, age over 70 years resulted in the exclusion of the majority of patients from intensive therapeutic programs (35% accrual rate for patients older than 70 years as opposed to 77% in those aged 61-70, p<0.001). Clearly, overly optimistic results reported in the literature are a consequence of the above selection and refer to selected patients with AML rather than AML in the elderly.[1,6,7] Accordingly, the ratio of patients diagnosed to patients actually included in a therapeutic program should be reported and taken into account in order to enable a proper evaluation of therapeutic results in any clinical study focusing on elderly patients with acute leukemia.[1]

The combination of fludarabine plus ARA-C offers the possibility of delivering high-dose chemotherapy, with less toxicity than associated with conventional high-dose ARA-C-based regimens. In a recent study on elderly AML patients, in which conventional fludarabine, cytarabine and G-CSF (FLAG) was compared to intermediate dose ARA-C plus G-CSF, an impressively high CR rate with negligible extrahematologic toxicity was reported after FLAG, even though in the absence of a significant survival advantage.[39] In the present study we investigated the toxicity and efficacy of a modified FLAG schedule, based on the continuous sequential infusion of fludarabine + ARA-C after a loading dose of both drugs. Doses and schedules were adopted on the

basis of previous studies demonstrating that this approach was effective and well tolerated in relapsing, refractory and newly diagnosed poor risk AML in children and young/adult patients.[19-21] Overall, a CR rate of 67% was achieved and this result is remarkable considering that a substantial fraction of the patients (38%) had secondary AML, which represents a main adverse prognostic parameter itself.[40-42] In addition, there was no difference in CR rate, percentage of refractory cases and, notably, toxic death rate between patients aged less or more than 70 years, suggesting that continuous sequential infusion of fludarabine + ARA-C is effective and well tolerated also in very elderly AML patients. The absence of cardiac toxicity in this study, possibly due to the exclusion of anthracyclines from both induction and consolidation, would candidate our regimen as a particularly useful therapy in this age category. Nonetheless, it is conceivable that favorable results achieved in the cohort of patients aged > 70 years are at least partially due to the considerable selection operated within this age cohort. However, the overall induction death rate (16%) observed in our series is comparable to that reported following conventional induction therapy and due to a similar rate of severe infectious complications. Previous MDS was not found to have prognostic relevance in this series as far as CR rate and survival were concerned. In this regard two considerations should be made: first, a substantial proportion of patients with apparently *de novo* disease presented with trilineage dysplastic abnormalities, therefore we cannot exclude that in some cases a previously undiagnosed MDS may have preceded the onset of leukemia. Secondly, multilineage dysplasia is not, *per se*, an adverse prognostic factor unless associated with unfavorable cytogenetics.[12,43] As a matter of fact, patients with an unfavorable karyotype had a lower CR rate and a significantly shorter survival as compared to those with an intermediate risk karyotype (Figure 3); this finding is in keeping with previous data demonstrating an unsatisfactory outcome of patients with unfavorable karyotypes in AML as well as in advanced MDS after fludarabine/cytarabine based induction/consolidation therapy.[12-15,44] Toxicity due to consolidation was very considerable when a course identical to induction was adopted. Conceivably, myelosuppression and immunosuppression due to a 4-day combination of fludarabine plus ARA-C was too toxic for a population of patients with a median age of 69 years. Accordingly, after reduction of consolidation from 4 to 3 days of therapy, no toxic death was observed and, notably, the less intensive treatment did not result in a lower rate of peripheral blood stem cell mobilization (*data not shown*). Among the 42 patients achieving CR and the 35 who actually received consolidation therapy, 29 were evaluated for stem cell mobilization; early relapse and toxicity were the more frequent reasons accounting for withdrawal from the study. Overall, mobilization was successful in 79% of patients; this result compares favorably with other series including younger patients, in which anthracycline based chemotherapy was adopted in either induction or consolidation.[45-49]

Concern has been raised regarding the ability to mobilize sufficient stem cells into the periphery for autografting after the use of purine analogs in AML as well as in lymphoproliferative disorders.[50-53] However, after conventional FLAG therapy, we previously reported a 66% mobilization rate in a series of 44 patients with a median age of 61 years affected by *de novo* AML with trilineage dysplastic abnormalities;[12] in addition, the possibility that fludarabine given as continuous infusion could cause less stem cell damage and therefore less impairment of mobilization capacity should be taken into account. So far, few studies have specifically addressed the feasibility of ASCT in elderly patients with AML. Montillo *et al.*[54] and, more recently, Oriol *et al.*,[55] used induction/consolidation with conventional anthracycline-based chemotherapy and reported rates of ASCT feasibility of, respectively, 6% and 12% among all patients accrued. Furthermore, in Oriol's study, patients with secondary AML, included in our series, were considered as not eligible. In the present series, 27% of patients did actually receive ASCT in the absence of transplant-related death suggesting that continuous infusion of sequential fludarabine + ARA-C is superior, in terms of reduction of non-hematologic toxicity and increase of ASCT feasibility, as compared to conventional anthracycline-based treatment. As in Oriol's study, in our series the patients who actually underwent ASCT had a significantly longer survival and DFS than did those receiving no further therapy after consolidation, suggesting that every effort should be made to lead patients to transplantation procedures, given the particularly dismal outcome of relapsed patients.[56] Clearly, it should be considered that autografted patients are highly selected for best response to induction, consolidation and mobilization as well as for minor non-hematologic toxicity. In conclusion, these data demonstrate the efficacy of continuous sequential infusion of fludarabine plus ARA-C in elderly patients with AML. In particular, results in terms of CR achievement, PBSC collection and ASCT feasibility are encouraging. Finally, while data on median OS and DFS seem not to differ from those in other series of elderly AML patients treated with intensive chemotherapy, a longer follow-up is needed in order to evaluate the real benefit to long-term survival.

FF: conceived the protocol and drafted the paper; MD, CC, GM, BP, PD: were responsible for the clinical management of the patients; CC and SP: collected data; FF and SP did the statistical analysis; SP and AD critically reviewed the paper.

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References

1. Ferrara F. Unanswered questions in acute myeloid leukaemia. Lancet Oncol 2004;5:443-50.
2. Smith M, Barnett M, Bassan R, Gatta G, Tondini C, Kern W. Adult acute myeloid leukaemia. Crit Rev Oncol Hematol 2004;50:197-222.
3. Schiffer CA. Hematopoietic growth factors and the future of therapeutic research on acute myeloid leukemia. New Engl J Med 2003;349:727-9.
4. Ries LAG, Eisner MP, Kosary CL, Editors. SEER Cancer Statistics Review, 1973-1998. National Cancer Institute, Bethesda, MD, USA. 2001.
5. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA Cancer J Clin 2003; 53:5-26.
6. Pinto A, Zagonel V, Ferrara F. Acute myeloid leukaemia in the elderly: biology and therapeutic strategies. Crit Rev Oncol Hematol 2001;39:275-87.
7. Ferrara F, Mirto S, Zagonel V, Pinto A. Acute myeloid leukemia in the elderly: a critical review of therapeutic approaches and appraisal of results of therapy. Leuk Lymphoma 1998;29: 375-82.
8. Leith CP, Kopecky KJ, Godwin J, McConnell T, Slovak ML, Chen IM, et al. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood 1997; 89:3323-9.
9. Wahlin A, Markevarn B, Golovleva I, Nilsson M. Prognostic significance of risk group stratification in elderly patients with acute myeloid leukaemia. Br J Haematol 2001;115: 25-33.
10. Gandhi V, Estey E, Keating MJ, Plunkett W. Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 1993;11:116–24.
11. Tafuri A, Andreef M. Kinetic rationale for cytokine induced recruitment of myeloblastic leukemia followed by cycle-specific chemotherapy in vitro. Leukemia 1990;4:826–34.
12. Ferrara F, Palmieri S, Pocali B, Pollio F, Viola A, Annunziata S, et al. De novo acute myeloid leukemia with multilineage dysplasia: treatment results and prognostic evaluation from a series of 44 patients treated with fludarabine, cytarabine and G-CSF (FLAG). Eur J Haematol 2002;68:203-9.
13. Jackson G, Taylor P, Smith GM, Marcus R, Smith A, Chu P, et al. A multicentre open, non-comparative phase II study of a combination of fludarabine phosphate, cytarabine and granulocyte colony stimulating factor in relapsed and refractory acute myeloid leukaemia and de novo refractory anaemia with excess of blasts in transformation. Br J Haematol 2001;112:127-37.
14. Ferrara F, Melillo L, Montillo M, Leoni F, Pinto A, Mele G, et al. Fludarabine, cytarabine, and G-CSF (FLAG) for the treatment of acute myeloid leukemia relapsing after autologous stem cell transplantation. Ann Hematol 1999; 78: 380–4.
15. Ferrara F, Leoni F, Pinto A, Mirto S, Morra E, Zagonel V, et al. Fludarabine, cytarabine, and granulocyte-colony stimulating factor for the treatment of high risk myelodysplastic syndromes. Cancer 1999;86:2006–13.
16. Jackson GH. Use of fludarabine in the treatment of acute myeloid leukaemia. Hematology J 2004; 5 Suppl 1: S62–S7.
17. Clavio M, Gatto S, Beltrami G, Cerri R, Carrara P, Pierri I, et al. First line therapy with fludarabine combinations in 42 patients with either post myelodysplastic syndrome or therapy related acute myeloid leukemia. Leuk Lymphoma 2001;40:305–13.
18. Pastore D, Specchia G, Carluccio P, Liso A, Mestice A, Rizzi R, et al. FLAG-IDA in the treatment of refractory/relapsed acute myeloid leukemia: single-center experience. Ann Hematol 2003;82:231–5.
19. Dinndorf PA, Avramis VI, Wiersma S, Krailo MD, Liu-Mares W, Seibel NL, et al. Phase I/II study of idarubicin given with continuous infusion fludarabine followed by continuous infusion cytarabine in children with acute leukemia: a report from the Children's Cancer Group. J Clin Oncol 1997;15: 2780-5.
20. Vidarsson B, Abonour R, Williams EC, Woodson RD, Turman NJ, Kim K, et al. Fludarabine and cytarabine as a sequential infusion regimen for treatment of adults with recurrent, refractory or poor prognosis acute leukemia. Leuk Lymphoma 2001;41:321-31.
21. Avramis VI, Wiersma S, Krailo MD, Ramilo-Torno LV, Sharpe A, Liu-Mares W, et al. Pharmacokinetic and pharmacodynamic studies of fludarabine and cytosine arabinoside administered as loading boluses followed by continuous infusions after a phase I/II study in pediatric patients with relapsed leukemias. The Children's Cancer Group. Clin Cancer Res 1998;4:45-52.
22. Ferrara F, Mele G, Califano C, Palmieri S, Pocali B, Danise P, et al. Fludarabine and cytarabine as continuous sequential infusion for elderly patients with acute myeloid leukemia. Blood 2002; 100:344a[abstract].
23. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985;103: 620–5.
24. Ferrara F, Morabito F, Martino B, Specchia G, Liso V, Nobile F, et al. CD56 expression is an indicator of poor clinical outcome in patients with acute promyelocytic leukemia treated with simultaneous all-transretinoic acid and chemotherapy. J Clin Oncol 2000;18:1295–300.
25. Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood 2001;98:1312-20.
26. WHO Handbook for Reporting Results of Cancer Treatment. Vol. 38 World Health Organization. Geneva; 1979.
27. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukaemia. J Clin Oncol 2003; 21:4642-9.
28. Ferrara F, Palmieri S, Annunziata M, Viola A, Pocali B, Califano C, et al. Continuous infusion idarubicin and oral busulfan as conditioning for patients with acute myeloid leukemia aged over 60 years undergoing autologous stem cell transplantation. Bone Marrow Transplant 2004;34:573-6.
29. Kaplan EL, Meier P. Non-parametric estimation from incomplete observation. J Am Stat Assoc 1958;53: 457–81.
30. Sekeres MA, Stone RM, Zahrieh D, Neuberg D, Morrison V, De Angelo DJ, et al. Decision-making and quality of life in older adults with acute myeloid leukaemia or advanced myelodysplastic syndrome. Leukemia 2004;18:809-16.
31. Ferrara F, Annunziata M, Copia C, Magrin S, Mele G, Mirto S. Therapeutic options and treatment results for patients over 75 years of age with acute myeloid leukemia. Haematologica 1998;83:126–31.
32. Vey N, Coso D, Bardou VJ, Stoppa AM, Braud AC, Bouabdallah R, et al. The benefit of induction chemotherapy in patients age > 75 years. A retrospective study of 110 patients from a single institution. Cancer 2004;101: 325-31.
33. Gorin NC, Labopin M, Pichard P, Sierra J, Fiere D, Rio B, et al. Feasibility and recent improvement of autologous stem cell transplantation for acute myelocytic leukaemia in patients over 60 years of age: importance of the source of stem cells. Br J Haematol 2000;110:887-93.
34. Ferrara F, Venditti A, Carella Jr AM, Cantore N, Buccisano F, Tamburini A, et al. Autologous stem cell transplantation for patients with acute myeloid leukaemia aged over 60 years. Eur J Haematol 2002;69: 200–4.
35. Massenkeil G, Nagy M, Lawang M, Rosen O, Genvresse I, Geserick G, et al. Arnold R. Reduced intensity conditioning and prophylactic DLI can cure patients with high-risk acute leukaemias if complete donor chimerism can be achieved. Bone Marrow Transplant 2003;31:339-45.
36. Wong R, Giralt SA, Martin T, Couriel DR, Anagnostopoulos A, Hosing C, et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. Blood 2003:102:3052-9.
37. Taussig DC, Davies AJ, Cavenagh JD, Oakervee H, Syndercombe-Court D, Kelsey S, et al. Durable remissions of myelodysplastic syndrome and acute myeloid leukemia after reduced-intensity allografting. J Clin Oncol 2003; 15: 3060-5.
38. Rathnasabapathy R, Lancet J. Management of acute myelogenous leukemia in the elderly. Cancer Control 2003;10: 469-77.
39. Ossenkoppele GJ, Graveland WJ, Sonneveld P, Daenen SM, Biesma DH, Verdonck LF, et al. The value of flu-

darabine in addition to ARA-C and G-CSF in the treatment of patients with high-risk myelodysplastic syndromes andAML in elderly patients. Blood 2004;103:2908-13.
40. Estey EH. Prognostic factors in acute myeloid leukemia. Leukemia 2001;15: 670-2.
41. Lowenberg B. Prognostic factors in acute myeloid leukaemia. Best Pract Res Clin Haematol 2001;14:65-75.
42. Ferrara F, Palmieri S, Mele G. Prognostic factors and therapeutic options for relapsed or refractory acute myeloid leukaemia. Haematologica 2004; 89:998-1008.
43. Haferlach T, Schoch C, Loffler H, Gassmann W, Kern W, Schnittger S, et al. Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML Cooperative Group studies. J Clin Oncol 2003;21:256-65.
44. Estey EH, Thall PF, Pierce S, Cortes J, Beran M, Kantarjian H, et al. Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin +/- all trans retinoic acid +/- granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndromes. Blood 1999;93: 2478–84.
45. Bruserud O, Tjonnfjord G, Gjertsen BT, Foss B, Ernst P. New strategies in the treatment of acute myelogenous leukemia: mobilization and transplantation of autologous peripheral blood stem cells in adult patients. Stem Cells 2000;18: 343-51.
46. Ewing JC, Robertson JD, Kell WJ, Burnett AK, Ryder D, Chang J, et al. Autologous peripheral blood stem cell transplantation in first remission adult acute myeloid leukaemia - an intention to treat analysis and comparison of outcome using a predictive model based on the MRC AML10 cohort. Hematology 2003;8:83-90.
47. Pastore D, Specchia G, Mestice A, Liso A, Pannunzio A, Carluccio P, et al. Good and poor CD34$^+$ cells mobilization in acute leukemia: analysis of factors affecting the yield of progenitor cells. Bone Marrow Transplant 2004; 33:1083-7.
48. Carral A, de la Rubia J, Martin G, Molla S, Martinez J, Sanz GF, et al. Factors influencing the collection of peripheral blood stem cells in patients with acute myeloblastic leukemia and non-myeloid malignancies. Leuk Res 2003;27:5-12.
49. Keating S, Suciu S, de Witte T, Zittoun R, Mandelli F, Belhabri A, et al. The stem cell mobilizing capacity of patients with acute myeloid leukemia in complete remission correlates with relapse risk: results of the EORTC-GIMEMA AML-10 trial. Leukemia 2003;17:60-7.
50. Visani G, Lemoli RM, Tosi P, Martinelli G, Testoni N, Ricci P, et al. Fludarabine-containing regimens severely impair peripheral blood stem cells mobilization and collection in acute myeloid leukaemia patients. Br J Haematol 1999; 105:775-9.
51. Robak T. Purine nucleoside analogues in the treatment of myeloid leukemias. Leuk Lymphoma 2003;44:391–409.
52. Tournilhac O, Cazin B, Lepretre S, Divine M, Maloum K, Delmer A, et al. Impact of frontline fludarabine and cyclophosphamide combined treatment on peripheral blood stem cell mobilization in B-cell chronic lymphocytic leukemia. Blood 2004;103:363-5.
53. Montillo M, Tedeschi A, Rossi V, Cairoli R, Pungolino E, Intropido L, et al. Successful CD34+ cell mobilization by intermediate-dose Ara-C in chronic lymphocytic leukemia patients treated with sequential fludarabine and Campath-1H. Leukemia 2004;18:57-62.
54. Montillo M, Tedeschi A, Pagano L, Venditti A, Ferrara F, Fabris P, et al. Feasibility of peripheral blood stem cell rescue as intensification in elderly patients with acute myelocytic leukaemia: a pilot study from the GIMEMA group. Br J Haematol 2000; 111:334-7.Q1.
55. Oriol A, Ribera JM, Esteve J, Guardia R, Brunet S, Bueno J, et al. Feasibility and results of autologous stem cell transplantation in de novo acute myeloid leukemia in patients over 60 years old. Results of the CETLAM AML-99 protocol. Haematologica 2004;89:791-800.
56. Ferrara F, Morabito F, Latagliata R, Martino B, Annunziata M, Oliva E, et al. Aggressive salvage treatment is not appropriate for the majority of elderly patients with acute myeloid leukaemia relapsing after first complete remission. Haematologica 2000;86:814-20.