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Autografting

Double reinforcement with fludarabine/high-dose cytarabine enhances the impact of autologous stem cell transplantation in acute myeloid leukemia patients

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Summary:

Reinforced chemotherapy based on a double high-dose consolidation regimen could be a different way to enhance *in vivo* purging prior to autologous stem cell transplantation (auto-SCT) in acute myeloid leukemia (AML). We investigated the impact on outcome of auto-SCT after two different strategies of early intensification performed after an identical induction regimen in adult patients with AML. Between January 1993 and December 1998, 140 consecutive AML patients were enrolled in a program consisting of an identical anthracycline-based induction (ICE) and two different consolidation regimens: one cycle, cytarabine-based (single-NOVIA: 91 patients); two cycles, fludarabine-based (double-FLAN: 49 patients). Seventy out of 91 patients received single-NOVIA consolidation: 60 underwent a transplantation procedure (allogeneic bone marrow transplantation (allo-BMT):16 patients; auto-SCT: 44). Thirty-five out of 49 patients received double-FLAN consolidation: 31 underwent a transplantation procedure (allo-BMT: 10; auto-SCT: 21). The double consolidation regimen was well-tolerated with only minor side-effects. Median follow-up observation time for surviving patients was 38 months (range, 17–71) for the double-FLAN consolidation group and 70 months (range: 48–93) for the single-NOVIA consolidation group. Among the patients who received auto-SCT, the double consolidation strategy produced a superior disease-free survival curve at 36 months (78.6% (95%CI: 59.4–97.8) *vs* 47.7% (95%CI: 33–62.4)) compared with the single-NOVIA group. This difference was confirmed when the patients were analyzed for intention to treat ($P = 0.04$). In addition, the double-FLAN consolidation group showed a superior overall survival and lower relapse rate ($P = 0.02$). We conclude that the double-FLAN reinforcement strategy is safe and enhances the clinical impact of auto-SCT for AML patients in first complete remission. It may provide specific clinical benefit for patients undergoing auto-SCT. *Bone Marrow Transplantation* (2001) **27,** 829–835.

Keywords: myeloid leukemia; autologous transplantation; fludarabine; stem cells

Standard induction/consolidation chemotherapy in acute myeloid leukemia (AML) results in a median remission duration of 15 months, with a 5-year disease-free survival (DFS) of less than 30%.[1–3] Consolidation chemotherapy programs based on autologous stem cell transplantation (ASCT) for patients lacking an HLA-matched donor have been widely used.[4,5] The relative advantages and disadvantages of autologous bone marrow transplantation (ABMT) and peripheral blood stem cell transplantation (PBSCT) are still under evaluation.[6] Despite encouraging results from randomized trials showing a clinical benefit for AML patients undergoing ASCT in first complete remission (CR), relapse remains the most frequent cause of treatment failure.[7,8] Thus, intensification of induction/consolidation chemotherapy, mainly based on high-dose cytarabine, has been suggested to improve *in vivo* purging prior to auto-SCT.[9–11] Combination chemotherapy regimens based on fludarabine and high-dose cytarabine, such as FLAG or FLAN, are known to be effective in myeloproliferative disorders.[12–14]

Reinforced chemotherapy mainly based on a double high-dose consolidation regimen is a new way to enhance *in vivo* purging prior to auto-SCT and has not been previously reported. Bearing this in mind, we planned a comparison of an early intensified double consolidation regimen, using two cycles of FLAN, with a single consolidation therapy, based on high-dose cytarabine and mitoxantrone, both administered after the same induction cycle (ICE).

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Patients and methods

Patients

From January 1993 to December 1998, 140 adults with newly diagnosed AML were consecutively treated in our institution. AML was diagnosed according to the French–American–British (FAB) criteria.[15] Acute myeloid leukemia was considered to be *de novo* when no documented hematological abnormality had been identified more than 2 months before diagnosis.

Induction therapy

All 140 patients received a remission induction cycle (ICE) consisting of: idarubicin 10 mg/m^2 i.v. push per day on days 1, 3, 5; cytosine arabinoside 100 mg/m^2/day by continuous infusion (preceded at the start of the infusion by a 100 mg bolus injection) on days 1 to 10; etoposide 100 mg/m^2/day i.v. on days 1 to 5. CR was defined as complete normalization of the morphological,[16] immunophenotypic and (when applicable) cytogenetic and molecular marrow picture, lasting for at least 1 month.

Single NOVIA consolidation therapy group (Figure 1a)

Seventy-three out of 91 consecutive AML patients achieved CR after ICE induction therapy and 70 proceeded to a high-dose NOVIA consolidation (Figure 1a). This group of patients has already been reported.[6] The consolidation regimen was as follows: cytarabine 500 mg/m^2/twice a day on days 1–6; mitoxantrone 12 mg/m^2/day on days 4–6. From January 1993 to May 1994, patients in CR then underwent a bone marrow harvest. From May 1994, all patients received G-CSF (10 μg/kg/day), to mobilize peripheral blood progenitor cells.[17,18] When mobilization was insufficient ($<$10 CD34$^+$ cells/μl), bone marrow was subsequently harvested in steady-state conditions.

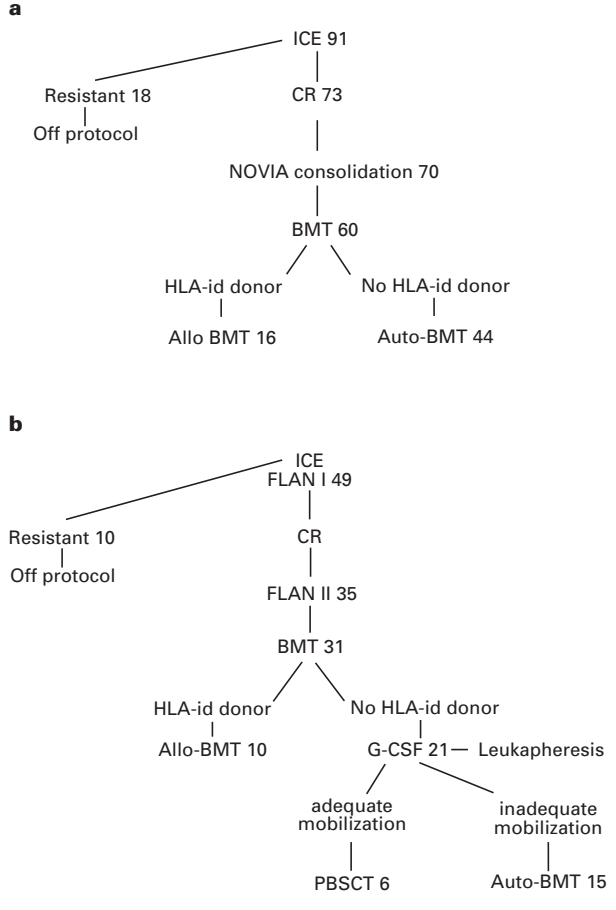

Figure 1 Flow charts of treatment strategy for AML patients submitted to (**b**) double-consolidation strategy with FLAN; (**a**) single consolidation with NOVIA.[6]

Double FLAN consolidation therapy group (Figure 1b)

All 49 patients regardless of their clinical outcome after ICE induction, received a first consolidation course of FLAN. This consisted of fludarabine 30 mg/m^2/day administered as a 30 min i.v. infusion, cytosine arabinoside 2 g/m^2/day for 5 days over 5 h i.v. infusion, 4 h after the end of fludarabine; mitoxantrone 6 mg/m^2/day as a 30 min infusion, 2 h after the end of cytosine arabinoside, days 1, 2 and 3. Patients who did not enter CR after this first course of FLAN were considered off-study. After hematopoietic recovery a second FLAN course was administered to patients in morphologically confirmed CR. Afterward, those patients who had an HLA-identical sibling were assigned to allogeneic bone marrow transplantation (allo-BMT). Patients without an HLA-identical sibling were assigned to auto-SCT, preferably PBSCT, otherwise ABMT. For PBSC mobilization, these patients received G-CSF (10 μg/kg/day), starting 13 days after FLAN and continuing until completion of leukaphereses. However, due to the severe impairment of PBSC mobilization observed after FLAN chemotherapy,[19] the vast majority of AML patients in first CR underwent bone marrow harvesting and subsequently received auto-BMT.

Bone marrow collection, leukapheresis procedures and PBSC processing

Characteristics of patients undergoing auto-SCT are shown in Table 1. Median time from CR to harvest was 3 months

Table 1 Characteristics of AML patients who completed auto-SCT after ICE induction and either single-NOVIA or double-FLAN consolidation

	ICE/NOVIA/ auto-SCT	*ICE/double-FLAN/ auto-SCT*
Patients	44	21
Sex (M/F)	22/22	12/9
Age (+/–s.d.)	43.1 (18–64)	42.7 (22–63)
FAB (%)		
M0	0	1 (5)
M1	9 (20)	5 (24)
M2	14 (33)	5 (24)
M4	12 (27)	8 (38)
M5	8 (18)	2 (9)
M6	1 (2)	0 (20)
Karyotype (%)		
Favorable	9 (20)	4 (19)
Intermediate	25 (57)	11 (52)
Unfavorable	10 (23)	6 (29)

(range, 2–6). Stem cells harvest, cryopreservation and thawing procedures have already been reported.[6,20] Progenitor cell apheresis was begun after an absolute count of 10 $CD34^+$ cells/μl was achieved during the recovery phase after consolidation therapy. As already reported,[17,18,21] circulating stem cells were collected by using either a Fenwal CS3000 continuous flow blood cell separator (Baxter, Rome, Italy) or a Cobe Spectra separator (Cobe BCT, Lakewood, CO, USA) to obtain a minimum yield of 2×10^6 $CD34^+$ cells/kg. Non-mobilizing patients underwent bone marrow harvest, as previously reported.[6–20] The final apheresis product was concentrated and cryopreserved as described. When applicable, cytogenetic and molecular analyses were performed in order to confirm CR.[22]

Conditioning regimen and PBSCT/auto-BMT

The preparative regimen consisted of busulfan 4 mg/kg/day for 4 days and cyclophosphamide (Cy) 60 mg/kg/day for 2 days given with uromitexan (Mesna; Asta Medica, Milan, Italy).[23] At the time of stem cell reinfusion (day 0), each bag was thawed and infused via a central line.

Supportive care and hematological recovery

All patients were nursed in reverse isolation single rooms until discharge and received antimicrobial prophylaxis that consisted of oral nystatin and ciprofloxacin. Broad spectrum intravenous antibiotics were promptly instituted in case of fever >38.5°C during neutropenia. Platelet support was given when the platelet count was $<10 \times 10^9$/l. Packed red cells were administered at a hemoglobin level <8 g/dl. Hemopoietic growth factors were not administered.

Statistical analysis

Age of patients and follow-up were expressed as median (range); number of cells infused and time to hematological recovery were expressed as median (range). The time of analysis was September 2000. Overall survival (OS), disease-free survival (DFS) and time to relapse were calculated according to the Kaplan–Meier estimate.[24] Comparison between the two groups was conducted by the log-rank test according to Peto *et al*.[25] Analysis was performed using the BMDP Statistical Software 1990 Edition. Two-sided *P* values were used throughout. *P* values were considered significant when <0.05.

Results

The two groups of patients were comparable with regards to clinical and biological characteristics and prognostic factors (age, karyotype, WBC) at diagnosis. Characteristics of patients receiving to auto-SCT are shown in Table 1.

ICE induction plus single-NOVIA consolidation

Three out of 91 patients died after induction therapy due to infective complications. Seventy-three out of the remaining 88 entered CR after ICE; 15 were resistant, and were considered off study. Three out of 73 CR patients were excluded because of toxicity. Seventy cases then underwent consolidation. Afterwards, 16 patients who had an HLA-matched identical sibling donor received allogeneic bone marrow transplants. Of the remaining 54 cases, three refused further therapy and seven experienced an early relapse. Forty-four patients proceeded to autologous transplantation. Twenty-nine patients received G-CSF to mobilize PBSC. Adequate numbers of PBSC were obtained in 23 out of 29 patients, with a mean of two collections. In six non-mobilizing cases, marrow was successfully harvested and the patients underwent ABMT. Bone marrow was then harvested from 21 patients from 2 to 6 months after CR; ABMT followed at a median of 6 months (range, 4 to 8 months) from CR; 25 (57%) relapses were recorded. Reinfusion parameters and hematological recovery are shown in Table 2.

ICE induction plus double-FLAN consolidation strategy

The treatment sequence of the 49 patients enrolled in the study is summarized in Figure 2. Thirty-four out of 49 (70%) patients entered morphological CR after ICE. Of the remaining 15 patients, 11 underwent the first cycle of FLAN despite being refractory to ICE, while four were considered off-study (one died, three had severe toxicities). Thus, 45 patients started consolidation. After the first cycle of FLAN, 34/49 (69%) patients reached morphological CR, (confirmed at the cytogenetic or molecular level where applicable). At this stage, 10 patients were moved to other treatments for a variety of reasons (two refusals, one hematopoietic toxicity, four early relapses, three resistant to ICE-FLAN I). Thus, a total of 35 out of 45 patients completed the double consolidation with a second course of FLAN. At this stage, four patients moved to other treatments for various reasons (two refusals, two early relapses). The double-consolidation strategy followed by transplantation was completed by 31/49 (63%) patients: 10 patients with HLA-identical sibling donors underwent allo-BMT, while the remaining 21 received auto-SCT. An adequate number of PBSC was obtained in 6/21 patients with a mean of two collections, who then underwent PBSCT. In the remaining 15/21 cases, bone marrow was successfully harvested (2–6 months after the second cycle of FLAN), and all these 15 patients underwent auto-BMT. Overall, auto-SCT was performed at a median of 5 months (range, 3–8) after the first CR; four (19%) relapses were recorded (two after PBSCT, two after ABMT). Reinfusion parameters and hematological recovery are shown in Table 3.

The median follow-up observation time for surviving patients was 38 months (range, 17–71) for the double-FLAN consolidation group and 70 months (range, 48–93) for the single-NOVIA consolidation group. With respect to the single-NOVIA consolidation strategy, the double-consolidation strategy with FLAN produced a superior DFS curve at 36 months (78.6% (95%CI: 59.4–97.8) in the FLAN/FLAN group *vs* 47.7% (95%CI: 33–62.4) in the NOVIA group; see Figure 3), a superior overall survival (Figure 4) and a lower cumulative relapse rate after auto-SCT ($P = 0.02$), albeit with a slightly lower overall feasibility in terms of percentage of patients able to complete

Table 2 Reinfusion parameters and hematological recovery of AML patients who completed auto-SCT after ICE induction and either single-NOVIA or double-FLAN consolidation

	ICE-NOVIA PBSCT (n = 23)	*ICE-NOVIA ABMT (n = 21)*	*ICE-FLAN-FLAN PBSCT (n = 6)*	*ICE-FLAN-FLAN ABMT (n = 15)*
Time (CR–SCT) (months) (range)	4.8 (2–8)	6.1 (5–8)	4.7 (2–8)	6.2 (4–8)
Nucleated cells reinfused ($\times 10^8$/kg) (range)	8.3 (2.4–23)	1.8 (1.1–5.2)	8.6 (2.4–21)	7.9 (2.1–18)
CFU-GM ($\times 10^4$/kg) (range)	33 (0.3–155)	1.6 (0.1–11.8)	17.4 (0.2–46)	4.0 (0.4–6.9)
$CD34^+$ cells ($\times 10^6$/kg) (range)	6.9 (2.5–14.6)	0.9 (0.3–43.6)	5.2 (3.2–12.4)	—
Days to				
ANC $>0.5 \times 10^9$/l (range)	17 (11–37)	36 (14–133)	21 (15–87)	25 (12–101)
Plt $<20 \times 10^9$/l (range)	20 (10–95)	150 (14–607)	125 (16–505)	97 (13–375)
Plt $>50 \times 10^9$/l (range)	37 (11–141)	279 (17–840)	198 (20–662)	156 (18–548)
Hospital discharge (range)	19 (11–38)	34 (17–42)	29 (18–41)	25 (15–39)

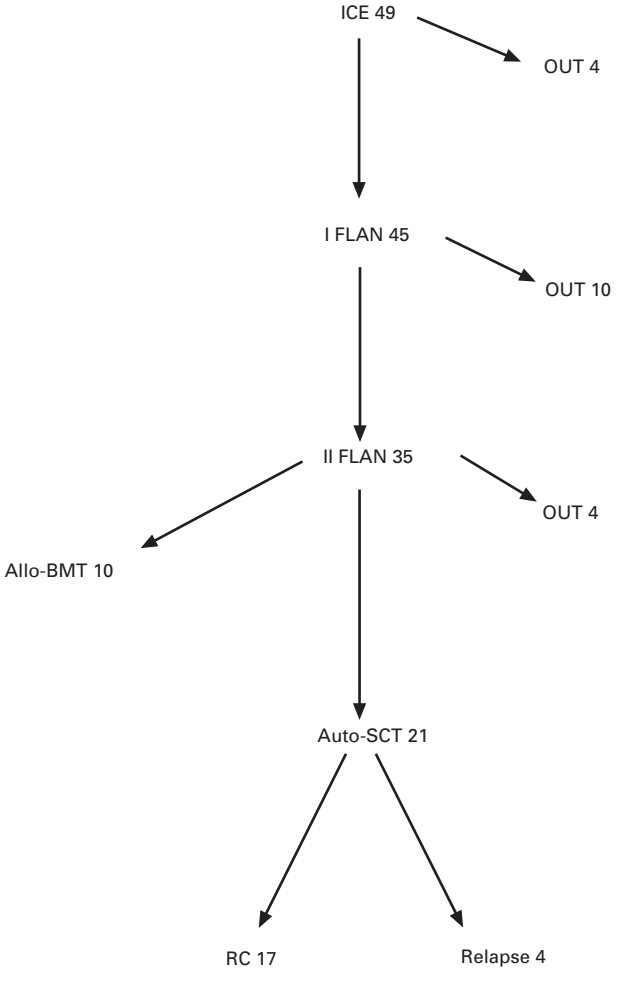

Figure 2 Flow chart showing the outcomes of 49 AML patients submitted to a double-consolidation strategy after induction with ICE, involving two cycles of FLAN, prior to auto-SCT (or allo-BMT).

the full program (31/49 (63%) *vs* 60/88 (68%)). When intention to treat was considered, the double-consolidation strategy again produced a superior DFS curve ($P = 0.04$; Figure 5). Time to relapse is shown in Figure 6.

Table 3 Transplant-related toxicity

	ICE-NOVIA-ASCT (n = 44)	*ICE-FLAN-FLAN-ASCT (n = 21)*
FUO	59%	57%
Documented infections	27%	33%
Mucositis grade I	63%	66%
Mucositis grade II	27%	28%
Mucositis grade III	—	—
Late complications	11%	9%

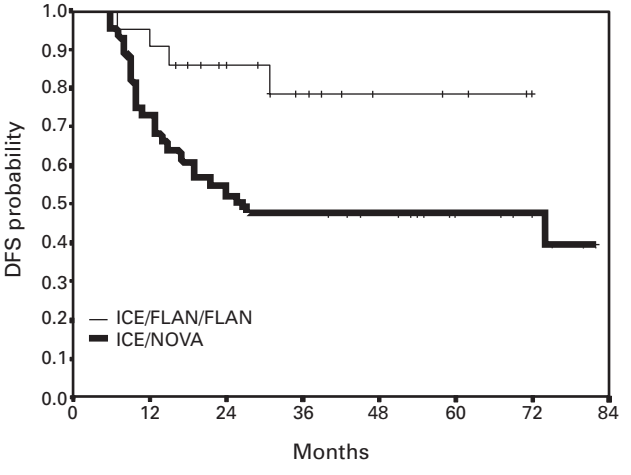

Figure 3 Comparison of DFS curves for either double consolidation (ICE + FLAN/FLAN) or single consolidation (ICE + NOVIA) plus autologous SCT ($P = 0.02$, log rank test).

Transplant toxicity and complications (Table 3)

Mild or moderate mucositis was the most frequent toxicity both after the double-FLAN consolidation strategy and the single-NOVIA consolidation strategy. There was no significant difference in other parameters (days of fever, i.v. antibiotic therapy, FUO, documented infections, non-hematological toxicity, late complications) between the two groups.

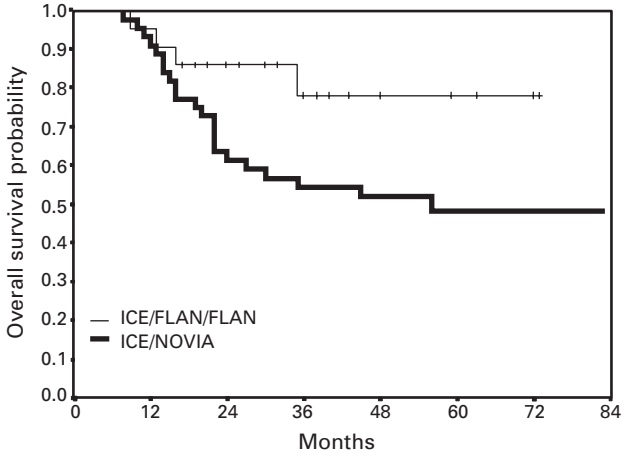

Figure 4 Comparison of OS curves for either double consolidation (ICE + FLAN/FLAN) or single consolidation (ICE + NOVIA) plus autologous SCT.

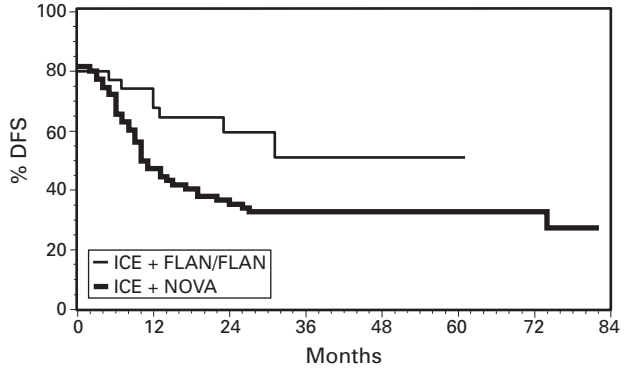

Figure 5 Comparison of intention-to-treat DFS curves for either double consolidation (ICE + FLAN/FLAN) or single consolidation (ICE + NOVIA) ($P = 0.04$, log rank test).

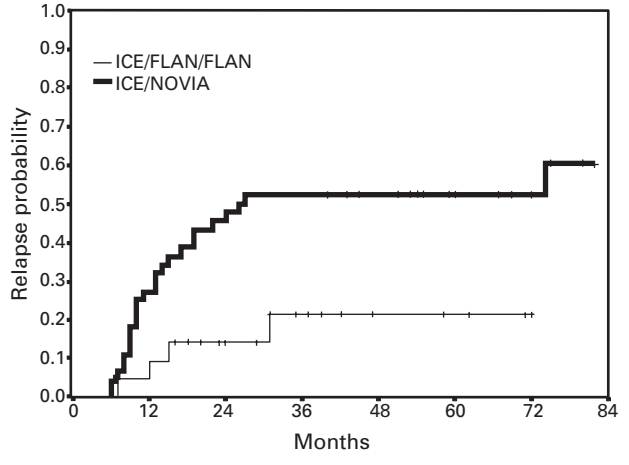

Figure 6 Comparison of time to relapse curves for either double consolidation (ICE + FLAN/FLAN) or single consolidation (ICE + NOVIA) plus autologous SCT.

Discussion

Post-remission therapy is necessary to prevent relapse in patients with AML. However, the optimal form of treatment is uncertain. Auto-BMT, as well as PBSCT, has been shown to be feasible and effective in AML, provided that adequate induction/consolidation treatment has previously accomplished effective *in vivo* purging.[4,5,16,25–28] Nevertheless, the severity of intensive induction/consolidation regimens can result in a substantial reduction in the number of patients who reach transplantation. Moreover, the two modalities of performing auto-SCT are potentially afflicted by problems. After auto-BMT, delayed hematological recovery occurs in a substantial proportion of patients, causing significant morbility and mortality.[29–33] PBSCT results in a faster engraftment than does conventional BMT. However, the much larger number of stem cells infused, and perhaps residual leukemia cells, may create a higher relapse risk. However, the experiences of other authors do not suggest that reinfusion of PBSC translates into a higher relapse rate.[27,28,34–37] Either way, a therapeutic strategy based on feasible, reinforced induction/consolidation therapy exerting an adequate antileukemic effect combined with tolerable post-consolidation treatment may help overcome these problems. Reinforced chemotherapy mainly based on a double high-dose consolidation regimen may be a new way to enhance *in vivo* purging prior to auto-SCT.

With this aim in mind, we planned the present study addressing the question of dose effect of consolidation treatment by comparing two different strategies of early intensification of induction therapy. Objectives of the study were to determine the overall feasibility, expressed in terms of the percentage of patients able to complete double-consolidation strategy with FLAN and efficacy of auto-SCT performed in first CR, with regard to hematopoietic recovery and antileukemic activity.

Concerning the feasibility of double-FLAN consolidation after ICE, 31 of the 49 (63%) evaluable patients were able to complete the full program, proceeding either to auto-SCT or allo-BMT, compared with 60/91(66%) after ICE-NOVIA. This suggests that a considerable proportion of AML patients undergoing this intensive double-consolidation strategy should eventually reach transplantation.

Considering the patients who received auto-SCT after double-FLAN consolidation (the main end-point), their data compare favorably with the single-NOVIA consolidation group. In particular, the FLAN/FLAN group showed a significantly lower relapse rate ($P = 0.02$) and a significantly better DFS (Figure 3) and OS (Figure 4), despite the limited number of cases. However, it should be emphasized that the present study was not randomized. Thus, our suggestions should be taken with caution.

Our data suggest that double FLAN consolidation may exert a superior antileukemic effect. However, we have no way of knowing whether this superiority is due to the higher overall dosage, to the use of fludarabine, or to a combination of these two factors. Despite these encouraging data concerning antileukemia activity, it has to be remembered that fludarabine severely impairs stem cell potential and PBSC mobilization. This makes auto-BMT an obligatory choice in the majority of patients, with conse-

quent delays in hematological recovery and increased morbidity and mortality. Further studies are needed to investigate how fludarabine impairs stem cell potential: priming with G-CSF prior to bone marrow harvest may be a new approach in auto-SCT after fludarabine consolidation.

In conclusion, the present study suggests that clinical benefits may result from a further intensification of consolidation based on a double FLAN schedule. These benefits may translate into an increased antileukemia effect for patients who undergo auto-SCT. Despite the impairment of stem cell mobilization induced by FLAN, this double consolidation program following ICE induction appears to provide the basis for a safe and feasible approach for intensifing AML treatment.

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