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ORIGINAL REPORT

Toward Optimization of Postremission Therapy for Residual Disease–Positive Patients With Acute Myeloid Leukemia

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ABSTRACT

Purpose
Despite the identification of several baseline prognostic indicators, the outcome of patients with acute myeloid leukemia (AML) is generally heterogeneous. The effects of autologous (AuSCT) or allogeneic stem-cell transplantation (SCT) are still under evaluation. Minimal residual disease (MRD) states may be essential for assigning patients to therapy-dependent risk categories.

Patients and Methods
By multiparametric flow cytometry, we assessed the levels of MRD in 142 patients with AML who achieved complete remission after intensive chemotherapy.

Results
A level of 3.5×10^{-4} residual leukemia cells (RLCs) after consolidation therapy was established to identify MRD-negative and MRD-positive cases, with 5-year relapse-free survival (RFS) rates of 60% and 16%, respectively ($P < .0001$) and overall survival (OS) rates of 62% and 23%, respectively ($P = .0001$). Of patients (n = 77) who underwent a transplantation procedure (56 AuSCT and 21 SCT procedures); 42 patients (55%) were MRD positive (28 patients who underwent AuSCT and 14 patients who underwent SCT) and 35 patients (45%) were MRD negative (28 patients who underwent AuSCT and seven who underwent SCT). MRD-negative patients had a favorable prognosis, with only eight (22%) of 35 patients experiencing relapse, whereas 29 (69%) of 42 MRD-positive patients experienced relapse ($P < .0001$). In this high-risk group of 42 patients, we observed that 23 (82%) of 28 of those who underwent AuSCT experienced relapse, whereas six (43%) of 14 who underwent SCT experienced relapse ($P = .014$). Patients who underwent SCT also had a higher likelihood of RFS (47% *v* 14%).

Conclusion
A threshold of 3.5×10^{-4} RLCs postconsolidation is critical for predicting disease outcome. MRD-negative patients have a good outcome regardless of the type of transplant they receive. In the MRD-positive group, AuSCT does not improve prognosis and SCT represents the primary option.

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INTRODUCTION

The use of modern intensive induction chemotherapies leads to complete remission (CR) in 60% to 80% of adult patients with acute myeloid leukemia (AML).[1-6] However, without additional chemotherapies, including intensive consolidation,[4,5,7] less intensive maintenance,[8,9] autologous (AuSCT), or allogeneic stem-cell transplantation (SCT),[1,2,4] nearly all cases of AML will eventually relapse. The goal of these additional treatments is to eradicate leukemia cells surviving the cytotoxic effects of the therapy that are undetectable by conventional light microscopy surveys. These cells, called minimal residual disease (MRD) units, are responsible for recurrence.

Although a number of baseline prognostic factors (including age, performance status, AML occurring as secondary disease, and WBC count) have been identified, the prognosis of patients within respective subgroups defined by these parameters is still quite heterogeneous. Acquired clonal chromosomal abnormalities are considered the most relevant prognosticators in patients with AML, who are then stratified by cytogenetics into good-, intermediate-, and high-risk AML.[10-12] Using

conventional cytogenetics analysis, approximately 40% to 50% of patients with AML bear a normal karyotype at diagnosis and are usually included in the intermediate-risk group, where broad variability in response to therapy and survival is observed. Thus the actual role of therapy intensification, including the use of AuSCT or SCT, is still under investigation in this group.[13,14]

On the basis of these premises, the implementation of therapy-dependent parameters into stratification systems has been approached. Indeed, previous studies have shown that MRD assessment impacts independently on the prognosis of AML and proves useful to modulate the intensity of postremission therapy.[15-17]

Currently, the most widely used techniques to assess MRD are based on detection of either molecular or immunophenotypical markers expressed by a leukemia clone. Despite its high sensitivity (one target cell per 10^3 to 10^6), the utility of polymerase chain reaction is confined to those AML cases (30% to 40%) characterized by the presence of fusion genes derived from chromosome translocations.[18,19] Multiparametric flow cytometry (MPFC) permits sensitivity to one leukemia cell per 10^4 to 10^5 normal bone marrow cells and can be successfully applied for nearly all patients with AML.[20,21]

MRD measured by MPFC provides prognostic information in AML expressing a leukemia-associated phenotype (LAP).[17,22] In particular, our results indicate that the postconsolidation time point is most informative for outcome prediction. By analyzing a larger group of de novo AML cases in the present study, we have confirmed the previously established threshold of 3.5×10^{-4} residual leukemia cells to discriminate risk categories and the significant prognostic role of MRD assessment at the end of consolidation therapy to predict disease outcome. As a major priority, we have focused on patients who remain MRD-positive after consolidation to evaluate the influence of transplantation procedures on outcome.

PATIENTS AND METHODS

Patients

A total of 142 consecutive adult patients with de novo AML diagnosed at the Department of Hematology, University Tor Vergata/St Eugenio Hospital (Rome, Italy) were analyzed; this series includes 100 patients whose results have been already reported in previous publications.[17,22] All patients underwent intensive chemotherapy according to the European Organization for Research and Treatment of Cancer (EORTC)/Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) protocols AML-10, AML-12, and AML-13. Approval for this study was obtained from the institutional review board. Informed consent was provided according to the Declaration of Helsinki. The expression of an LAP at the time of diagnosis and the achievement of a morphologic CR after induction therapy were criteria for inclusion in the study.

Treatment Protocols

The EORTC/GIMEMA AML-10/12 trials included patients 18 to 60 years of age. Induction treatment combined cytarabine, etoposide, and anthracycline, as detailed elsewhere.[22,23] As consolidation, patients received cytarabine and anthracycline; thereafter, those with an HLA-compatible sibling were allografted. Patients without a donor were randomly assigned to peripheral or bone marrow AuSCT (AML-10) or underwent peripheral-blood AuSCT followed by no further therapy or subcutaneous interleukin-2 maintenance (AML-12). As induction, patients older than 60 years received mitoxantrone, cytarabine, and etoposide according to the EORTC/GIMEMA AML-13 randomized trial. Those in CR were randomly assigned to be given either an intravenous or an oral consolidation program (two cycles) consisting of idarubicin, cytarabine, and etoposide.[22,24]

Table 1. Clinical Characteristics of the Patients

Characteristic	AML10/AML12 Protocol (n = 92)	AML13 Protocol (n = 50)	Total (n = 142)
Age, years			
Median	45	68	52
Range	18-60	61-75	18-75
Sex, No. of patients			
Male	54	26	80
Female	38	24	62
FAB subtype, No. of patients			
M0	10	3	13
M1	23	11	34
M2	27	16	43
M4	11	8	19
M5	21	10	31
M6	0	2	2
WBC, No. of patients			
$< 50 \times 10^9$/L	67	45	112
50-100 $\times 10^9$/L	15	3	18
$> 100 \times 10^9$/L	10	2	12
Cytogenetic risk group, No. of patients*			
Favorable	16	2	18
Intermediate	67	35	102
Unfavorable	2	5	7
Induction, No. of patients	92	50	142
Consolidation, No. of patients	91	44	135
Consolidation II, No. of patients	—	30	
AuSCT/AlloSCT, No. of patients	51/21	5/—	77

Abbreviations: AML, acute myeloid leukemia; FAB, French-American-British; AuSCT, autologous stem-cell transplantation; AlloSCT, allogeneic stem-cell transplantation.

*Available in 127 of 142 patients. Patients were stratified, according to the Medical Research Council classification of cytogenetic risk, into favorable (cases with t(8;21), t(15;17) or inv(16), irrespective of the presence of additional cytogenetic abnormalities), adverse (cases lacking these favorable risk aberrations with complex cytogenetic changes [five or more unrelated abnormalities],−5, del(5q),−7, or 3q abnormalities), and intermediate (cases with normal karyotype, 11q23 abnormalities, trisomy 8 (+8), del(7q), del(9q), complex karyotypes [≥ 3 abnormalities but < five abnormalities] or all chromosomal changes of unknown prognostic significance) risk groups.

Immunophenotypic Studies and MRD Detection

At diagnosis, immunophenotypic, chromosomal, and genetic studies were performed as detailed elsewhere.[17,25,26] LAPs were detected by staining leukemia cells in triple/quadruple fluorescence assay. A given combination of markers was regarded as relevant if expressed in ≥ 50% of blasts. This step served to define a leukemia immunophenotypic fingerprint that, in turn, was used to track residual leukemia cells (RLCs) during follow-up at specific time points. At least two antibody combinations for each case were selected to minimize pitfalls owing to so-called phenotypic switches that have been described to be occasionally associated with relapses.[27,28] The CellQuest (Becton Dickinson, Mountain View, CA) software was used for flow cytometric data acquisition. Samples were then analyzed using the PAINT-A-GATE^{PRO} software program (Becton Dickinson), as previously described.[17,25,26] MRD studies during remission were performed on erythrocyte-lysed whole bone marrow samples using the same antibody combination defining the specific leukemia immunologic fingerprint. During data acquisition, a live gate that included the lymphomonocytic/granuloblastic region and excluded debris and platelet aggregates was used to acquire 10^6 total events in all samples. The acquired events were analyzed with PAINT-A-GATE software, applying the MouseTRAX Control option as described elsewhere.[17,25,26]

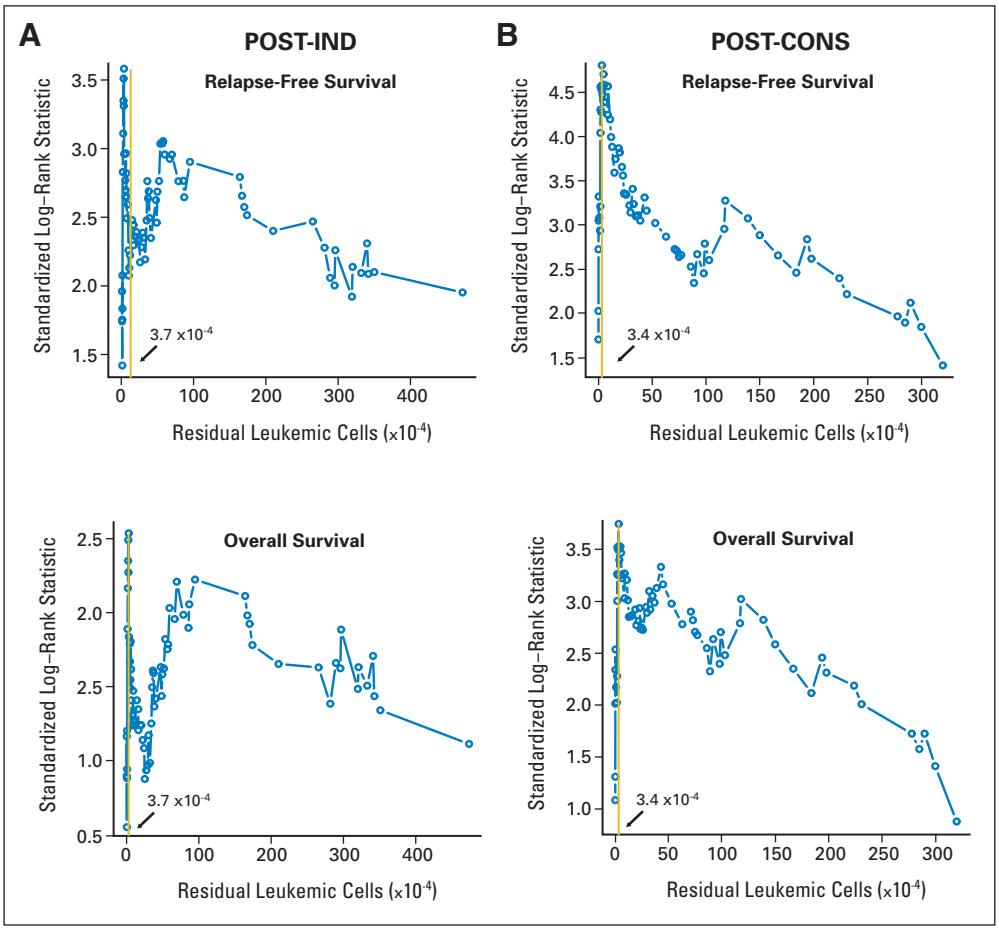

Fig 1. Maximally selected log-rank statistics were applied to residual leukemia values at (A) postinduction (POST-IND) and (B) postconsolidation (POST-CONS) check points to estimate the most appropriate cutoff for splitting patients into groups with different relapse-free survival (upper panels) or overall survival (lower panels) probabilities. X axis reports values of residual leukemia cells expressed as events $\times 10^{-4}$; the Y axis reports the corresponding standardized log-rank statistic values. For each plot, the dotted line and the arrow indicate the experimentally determined cutoff point.

Statistical Analyses

Overall survival (OS) was calculated from the date of entry onto the trial until death or last follow-up. Relapse-free survival (RFS) was measured from achievement of CR until relapse or death from any cause.[29] CR and relapse were defined by standardized criteria.[29] Values of MRD levels, evaluated after induction and consolidation course, were tested for possible cutoffs by means of maximally selected log-rank statistics.[30] Relationship of MRD to treatment response was estimated by two-sided χ^2 test. The Kaplan-Meier method[31] was used for estimation of OS and RFS. For comparison of survival and duration of RFS of two groups, the log-rank test was applied. To evaluate the independent effect of different variables on the duration of OS and RFS, multivariate analysis was performed, using a Cox proportional hazards model with predictive variables that were significant in univariate analysis. A P value of $\leq .05$ was considered significant in all cases.

RESULTS

Clinical characteristics for the 142 patients included in this study are listed in Table 1. Previously, it was shown that a postconsolidation MRD level $\geq 3.5 \times 10^{-4}$ had an independent impact on RFS and OS.[17,22] In the present study, we used a statistical outcome-oriented method to identify the cutoff with the most significant relation to prognosis. We evaluated the trend of standardized log-rank statistics using RFS (Fig 1, upper panels) and OS (Fig 1, lower panels) as dependent variables and the values of RLCs, determined at postinduction and postconsolidation time points, as independent variables (Fig 1A and 1B, respectively). Experimental cutoff points, empirically identified as the absolute peak in standardized log-rank statistics plots (vertical dotted lines in Fig 1), were 3.7×10^{-4} and 3.4×10^{-4} RLCs for postinduction and postconsolidation values, respectively. Accordingly, we decided to maintain the previously established cutoff of 3.5×10^{-4} RLCs to distinguish MRD-negative ($<3.5 \times 10^{-4}$) from MRD-positive ($\geq 3.5 \times 10^{-4}$) cases.

Determination of MRD After Induction

After induction, the median level of RLCs was 2.3×10^{-3} (range, 0 to 2.2×10^{-1}). At this stage, 28% of the patients (40 of 142 patients) were MRD negative and 72% (102 of 142 patients) were MRD positive. Among MRD-negative patients, 16 patients (40%) experienced relapse at a median of 11 months (range, 3 to 39 months), whereas of MRD-positive patients, 69 patients (67%) experienced relapse at a median of 8 months (range, 2 to 55 months; $P = .004$). The 5-year actuarial probabilities of RFS and OS (Fig 2A) were 50% and 49%, respectively for patients in the MRD-negative group or 22% and 29%, respectively, for those in the MRD-positive group ($P = .0009$ and $P = .014$, respectively).

Determination of MRD After Consolidation

Seven patients experienced an early relapse after induction (all were MRD positive) and 135 patients received consolidation; of these, one patient died early of septic shock. Therefore, 134 patients were

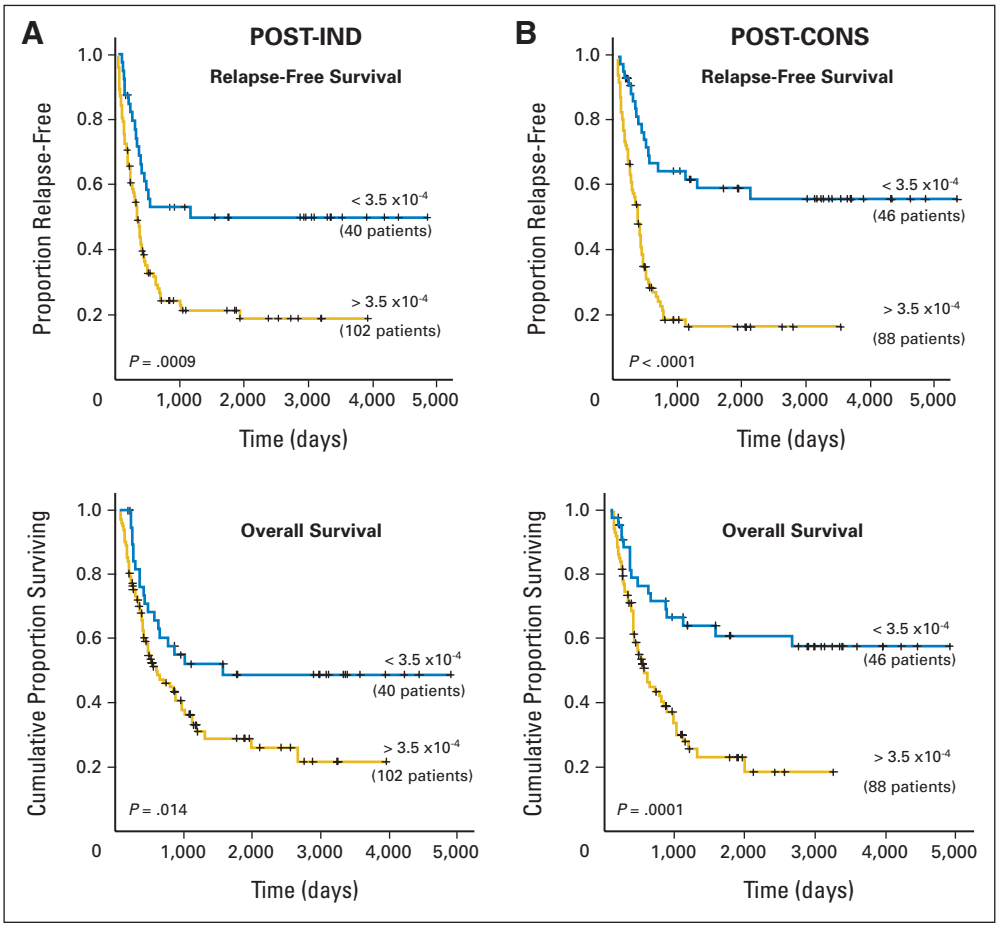

Fig 2. Relapse-free survival and overall survival were evaluated at (A) postinduction (POST-IND) and (B) postconsolidation (POST-CONS) check points by grouping the patients according to minimal residual disease values less than or $\geq 3.5 \times 10^{-4}$ residual leukemia cells.

evaluated for successive MRD assessments. At postconsolidation analysis, the median level of RLCs in the whole series was 1.6×10^{-3} (range, 0 to 1.9×10^{-1}). Forty-six patients (34%) were MRD negative and 88 patients (66%) were MRD positive. In the MRD-negative group, 12 patients (26%) experienced relapse at a median of 15 months (range, 5 to 65 months), whereas in MRD-positive group, 66 patients (75%) experienced relapse at a median of 9 months (range, 2 to 33 months; $P < .0001$). The 5-year actuarial probabilities of RFS and OS (Fig 2B) were 60% and 62%, respectively, for the MRD-negative patients, compared with 16% and 23%, respectively, for MRD-positive patients ($P < .0001$ and $P = .0001$, respectively).

On the basis of the levels of MRD after induction and consolidation check points, the 134 patients completing both treatment phases were divided into four groups: (1) induction negative/consolidation negative (31 patients), for patients who were MRD negative at both time points; (2) induction positive/consolidation negative (15 patients), for patients who were MRD positive after induction but reverted to MRD negative after consolidation; (3) induction positive/consolidation positive (79 patients), for patients who were MRD positive after induction and consolidation; (4) induction negative/consolidation positive (nine patients), for patients who were MRD negative after induction but who became MRD positive at the end of consolidation. Analysis of RFS and OS rates showed that MRD-negative status at the end of consolidation was the most important predictor of prognosis. Indeed (Fig 3), the 5-year actuarial probabilities of RFS and OS were 65% and 64% or 48% and 55% for patients with an induction negative/consolidation negative and induction positive/consolidation negative status, respectively, suggesting a good treatment outcome even in those with a slow response in terms of MRD clearance.

Prognostic Determinants

All the relevant prognostic variables with a statistical significance in univariate analysis were challenged into a multivariate model. In this analysis, postconsolidation MRD-positive status was found to be an independent variable significantly associated with a shorter duration of RFS and OS ($P = .001$ and .004, respectively; Table 2).

Outcome of Patients Who Did Not Undergo Transplantation

Fifty-seven patients received chemotherapy alone; 11 patients (19%) were MRD negative and four patients (36%) experienced relapse at a median of 28 months (range, 14 to 65 months). Forty-six patients (81%) were MRD positive, and 37 patients (80%) experienced relapse at a median of 5 months (range, 2 to 34 months; $P = .007$). The 5-year actuarial probabilities of RFS and OS were 60% and 72%, respectively, for the MRD-negative patients, compared with 11% and 11%, respectively, for MRD-positive patients ($P < .0001$ and $P = .00007$, respectively).

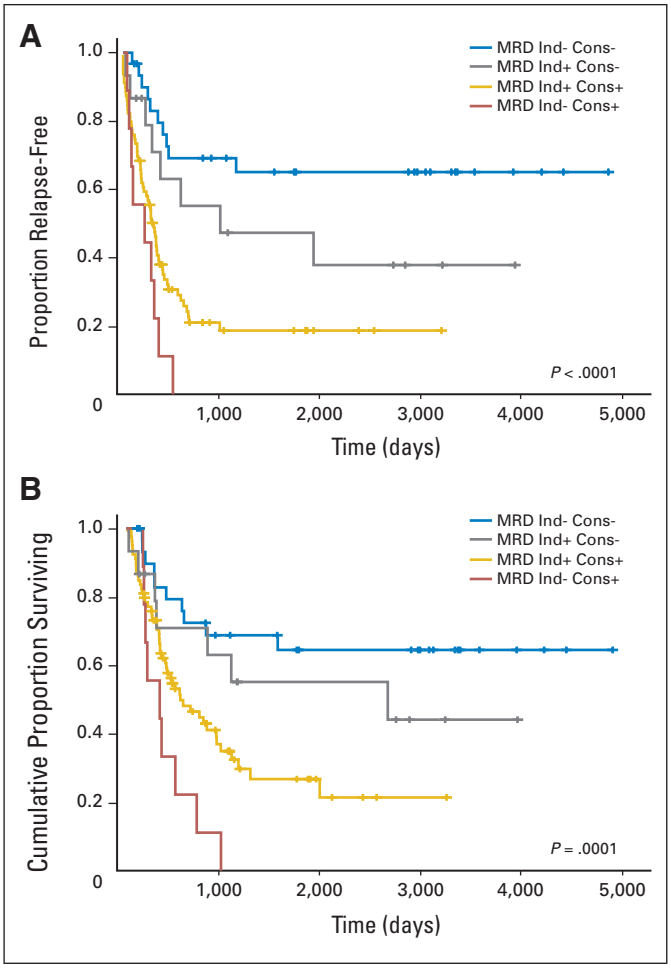

Fig 3. (A) Relapse-free survival (RFS) and (B) overall survival (OS) of 134 patients grouped according to the levels of minimal residual disease (MRD) after induction (Ind) and consolidation (Cons). Four different categories were identified: (1) Ind−/Cons− (31 patients), MRD negative at both time points; (2) Ind+/Cons− (15 patients), MRD positive after induction, reverted to MRD negative after consolidation; (3) Ind+/Cons+ (79 patients), MRD positive both after induction and consolidation; (4) Ind−/Cons+ (nine patients), MRD negative after induction, positive at the postconsolidation assessment.

Table 2. Impact of Prognostic Factors on Relapse-Free and Overall Survival by Multivariate Analysis

Clinical Variable	Hazard Ratio	95% CI	*P*
Relapse-free survival			
Postconsolidation MRD status, positive *v* negative	3.02	1.56 to 5.85	.001
MRC cytogenetic risk class, poor *v* intermediate/good	6.97	2.69 to 18.05	.0001
MDR-1 phenotype, positive *v* negative	1.90	1.07 to 3.37	.03
Postinduction MRD status, positive *v* negative			NS
Overall survival			
Postconsolidation MRD status, positive *v* negative	3.56	1.50 to 8.43	.004
MRC cytogenetic risk class, positive *v* negative			NS
MDR-1 phenotype, positive *v* negative			NS
Postinduction MRD status, positive *v* negative			NS

Abbreviations: MRD, minimal residual disease; MRC, Medical Research Council; MDR-1, multidrug resistance-1; NS, not significant.

Impact of Transplantation on MRD-Positive Patients

Of the 92 patients recruited under AML10/AML12 protocols, 72 underwent stem-cell transplantation. Twenty patients did not undergo transplantation because of early death (n = 1), medical decision (n = 4), and relapse (n = 15). Five additional patients who were older than 60 years were grafted with AuSCT according to the AML13 protocol for fit patients. Overall, 77 patients underwent stem-cell transplantation: 56 patients underwent AuSCT and 21 patients underwent SCT (Table 1). Before AuSCT, 28 (50%) of 56 were classified as MRD negative and 28 (50%) of 56 were classified as MRD positive at the postconsolidation check point. Before SCT, 14 (67%) of 21 patients were classified as MRD positive, whereas seven (33%) of 21 patients were MRD negative at the postconsolidation check point. Because the median time from completion of consolidation to AuSCT or SCT was 2.5 months, MRD was investigated again in all cases within 7 to 10 days before AuSCT/SCT to confirm the individual MRD status observed at hematologic recovery after consolidation.

When the analysis was broken down according to MRD status after consolidation, the 5-year actuarial probabilities of RFS and OS were 55% and 58%, respectively, for the MRD-negative patients or 22% and 33%, respectively, for MRD-positive patients ($P = .004$ and $P = .077$, respectively). These results suggest that postconsolidation MRD-negative patients have a favorable prognosis regardless of the type of transplantation they undergo. In fact, only eight (22%) of 35 patients in this group experienced treatment failure because of relapse or transplant-related mortality (TRM), whereas 29 (69%) of 42 MRD-positive patients did ($P < .0001$). Therefore, in the latter group of high-risk patients, we decided to examine the issue of transplantation procedure, between AuSCT and SCT, which might affect prognosis. We observed that 23 (82%) of 28 patients who underwent AuSCT experienced relapse, whereas six (43%) of 14 patients who underwent SCT experienced treatment failure for relapse or TRM ($P = .014$). The 5-year actuarial probabilities of RFS and OS were 47% and 44%, respectively, for patients who underwent SCT or 14% and 28%, respectively, for those who underwent AuSCT (Fig 4).

DISCUSSION

To determine the optimal postremission therapy, a modern prognostic stratification of patients with AML should be based on parameters available at diagnosis and parameters derived from response to chemotherapy. Therefore, monitoring of MRD has become essential for the management of patients with AML. Although polymerase chain reaction–based methods are available for less than half of all AML cases (20% to 40%),[19,32] MPFC is applicable to virtually all patients.[22,33]

Identification of the most useful check point for outcome prediction is an open issue in MRD monitoring of AML. Previous studies have shown that early determination during aplasia after chemotherapy or at the end of induction therapy provides significant prognostic information.[34-36] On the contrary, there are data suggesting that MRD levels after induction are less strongly associated with prognosis compared with those determined after consolidation.[17,22,32,37,38]

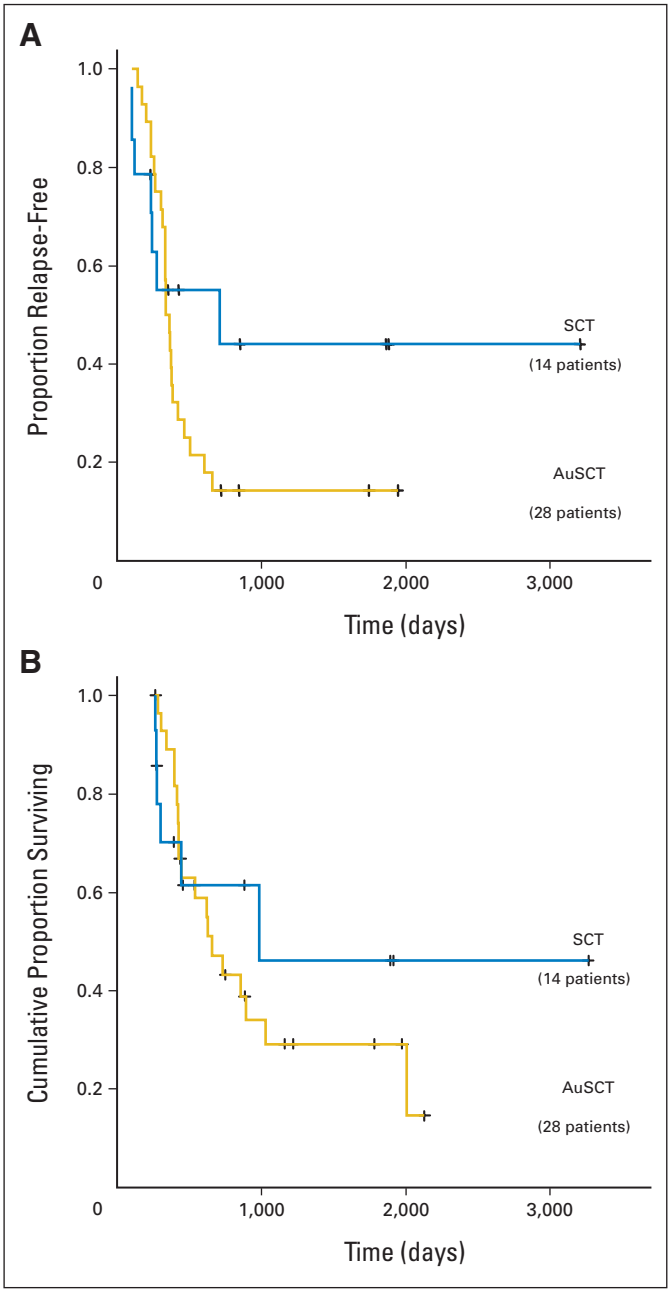

Fig 4. The 5-year actuarial probability of (A) relapse-free survival and (B) overall survival of 42 high-risk patients (postconsolidation minimal residual disease positive) was 47% and 44%, respectively, for patients who underwent allogeneic stem-cell transplantation (SCT), compared with 14% and 28%, respectively, for those who underwent autologous stem-cell transplantation (AuSCT).

In the present study, we confirmed previous observations that postconsolidation evaluation is highly predictive of patient outcome.[17,22,26,37] Levels of MRD $\geq 3.5 \times 10^{-4}$ leukemia cells after consolidation were indeed associated with unfavorable prognosis. By applying the maximally selected log-rank statistics, a test was specifically developed to find out the optimal cutoff for a given biologic variable.[22] This threshold was the most significant for prognostic purposes.

On the basis of MRD levels after induction and consolidation check points, we were able to identify four groups of patients. From a prognostic point of view, patients who obtained an early MRD negativity, soon after induction, and maintained it after consolidation treatment (induction negative/consolidation negative) had the best outcome. However, slow responders, namely those entering MRD negativity only after consolidation (induction positive/consolidation negative), showed an RFS and OS not significantly different from those achieving early negativity (induction negative/consolidation negative; Fig 3). In the present study, 67% (31 of 46) of patients who were MRD negative after consolidation were negative after induction. Therefore, an early response to the therapy might represent a predisposing factor for favorable prognosis, probably reflecting a higher chemotherapy sensitivity of leukemia cells. However, the final outcome is likely due to the net debulking effect achieved with comprehensive (induction-consolidation) upfront therapy. This assumption is supported in two ways. First, it is supported by the statistical observation that MRD status after induction was significantly associated with RFS and OS in univariate analysis alone, whereas MRD status after consolidation retained statistical significance in univariate and multivariate analyses (Table 2). Second, it is supported by the clinical observation that the achievement of MRD-negative status after induction does not guarantee persistence of negativity after consolidation and, therefore, a good outcome; we observed nine patients who were MRD negative after induction who converted to MRD-positive status after consolidation. These patients had a very poor prognosis, which might reflect the selection and emergence of cells resistant to chemotherapeutic drugs. In fact, the blasts of seven of nine patients showed robust expression of multidrug resistance-1 protein. Overall, the evaluation of the MRD status at the end of consolidation therapy facilitated the identification of two patient risk categories.

The first category of MRD-negative patients had a favorable prognosis, with a 5-year RFS of approximately 60%, and were likely to be cured. When the analysis was restricted to patients who underwent AuSCT and SCT, we found that MRD-negative patients had a significantly better outcome regardless of the type of transplantation they underwent. In fact, only 22% of these patients experienced treatment failure (relapse or TRM), contrasting with 69% of MRD-positive patients. Therefore, a standard intensification by means of AuSCT is probably effective and sufficient to these chemotherapy-sensitive patients, thereby avoiding the risk of SCT-related complications. On the basis of this assumption, our preliminary analysis of 35 MRD-negative patients submitted to transplantation procedure showed that 2-year actuarial probability of RFS was 67% for 28 patients who underwent AuSCT, compared with 57% for seven who underwent SCT (P = non significant, data not shown). In the latter group, TRM was the main reason for treatment failure. Despite the relatively small number of patients and the short follow-up, our results suggest that, for MRD-negative patients submitted to SCT, it should be carefully considered that the reasons for treatment failure comprise relapse and therapy-related issues. Our findings are in agreement with those observed in core binding factor leukemias, a well recognized subgroup with good prognosis, where patients treated with SCT were not found to have a better outcome than those who underwent chemotherapy or AuSCT.[6,39,40] However, as the relapse rate in the postconsolidation MRD-negative group was approximately 25%, these patients certainly need to be monitored to anticipate overt relapse on the basis of MRD expansion.

The second group consisted of patients who were MRD positive at postconsolidation and who were considered high risk, with a 16%

5-year RFS. On the basis of previous work[25] and the present experience, an additional intensification by AuSCT has no impact on the dismal outcome of disease. In the present study, 28 of 56 patients who underwent AuSCT were MRD positive, and 82% experienced relapse. High levels of MRD are indicative of a status of incomplete remission that is probably the consequence of different mechanisms of chemotherapy resistance. In agreement with this, we found that the number of RLCs detected before and after AuSCT did not significantly differ from those quantified after induction and consolidation therapy. This indicates that high-risk patients may be identified prematurely and that they will probably not benefit from this type of postremission therapy; alternative or additional therapies are needed to improve their prognosis. Therefore, we evaluated the influence of transplantation procedure. Unlike what we observed in the MRD-negative group, we found that in MRD-positive patients, SCT is able to offer an advantage in terms of the prolongation of RFS and OS (Fig 4). In fact, treatment failure of SCT as a result of TRM is counterbalanced by the extremely high number of relapses in the AuSCT group. We hypothesize that the lack of statistical significance was due to the low number of cases in both groups; therefore, further studies in larger series are warranted to confirm these preliminary results.

In conclusion, our data support the utility of incorporating sequential MRD assessment in the current protocols for the treatment of AML to deliver tailored therapies on a risk-based assessment. According to this point of view, improving the prognosis of AML must require that patients are assigned to an allogeneic transplantation procedure (either related or unrelated-donor transplants or full-haplotype mismatched transplants), not on the basis of donor availability, but on the basis of biologic, genetic, and therapy-dependent risk categories.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Final approval of manuscript: Sergio Amadori, Adriano Venditti

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