The therapeutic response and clinical outcome of adults with *ALL1(MLL)/AF4* fusion positive acute lymphoblastic leukemia according to the GIMEMA experience

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ABSTRACT

The clinical outcome of 21 adults with *ALL1(MLL)/AF4* positive acute lymphoblastic leukemia enrolled in the GIMEMA LAL 2000 trial and of 25 patients entered into the previous 0496 study is reported. LAL 2000 included more intensive consolidation and transplants. Complete remission rates were 90% and 88% in the LAL 2000 and 0496 trials, respectively. Fifteen patients were transplanted (5 autologous, 10 allogeneic). At 36 months, overall and disease free survivals were 32.9%, 31.8%, 28% and 27.3%, in LAL 2000 and 0496 trials, respectively. Relapses remained the main reason of failure occurring in 10 and 16 of the 19 and 22 responding patients. In the LAL 2000 study, 4 relapses were observed before transplant. Thus, *ALL1(MLL)/AF4* abnormality characterized a subset of patients with adverse prognosis in which the overall strategy adopted in the LAL 2000 study, rather than transplants *per se*, failed to improve the patient clinical outcome.

Key words: acute lymphoblastic leukemia, *ALL1(MLL)/AF4*, transplant consolidation, adverse prognosis.

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Introduction

Acute lymphoblastic leukemia (ALL) accounts for less than 20% of adult acute leukemias. The t(4;11)(q21;q23) is a frequent abnormality in this disease.[1-3] This translocation fuses the *ALL1 (MLL, HRX, Hrtx1)*[4-8] gene to the *AF4* gene. This alteration identifies a subset of ALL with aggressive clinical features and poor outcome.[9,10] In addition, the *ALL1(MLL)/AF4* fusion is associated with a pro-B immunophenotype.[11] This fusion gene is detectable in the vast majority of pro-B ALL cases in infancy[12] and only in 30-40% of adults.[10] Due to this association with age, in infancy the pro-B immunophenotype and t(4;11) have often been considered as equivalent prognostic factors.

By contrast, in adults there is no such strong association and the lack, until 1990, of a centralized diagnostic procedure in large cooperative studies, such as the GIMEMA 0288 trial,[13] may have underscored the adverse prognosis conferred by these genetic alterations.

Recently, among a series of adults with pro-B ALL receiving the conventional chemotherapy regimen of the GIMEMA 0496 trial, we demonstrated that the *ALL1(MLL)/AF4* genotypic features was the only parameter conferring an adverse clinical outcome to this specific subset of ALL patients.[10] For these reasons, in the subsequent GIMEMA LAL 2000 study, the *ALL1(MLL)/AF4* ALL positive patients would be managed more intensively with one course of HD Ara-C/mitoxantrone and HSCT as consolidation treatments.

Therefore, herein, we report the clinical outcome of *ALL1(MLL)/AF4* positive ALL patients entered into the two consecutive GIMEMA trials looking for possible differences between the two adopted strategies.

Design and Methods

Patients

Twenty-one adult (18-60 years) patients with *ALL1(MLL)/AF4* positive ALL were enrolled into the GIMEMA LAL 2000 study between January 2000 and September 2004, while 25 patients entered into the GIMEMA 0496 study active between October 1996 and December 1999. The diagnosis of ALL was based on standard morphological and cytochemical evaluation[14] and on immunophenotypic criteria.

All patients gave informed consent for both treatment and diagnostic procedures. The two studies were approved by our institutional review board.

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Molecular analysis

Total RNA was extracted from cells cryopreserved in guanidium isothiocyanate according to the method of Chomczynsky and Sacchi.[15] The quality of RNA was assessed on an ethidium bromide-stained 1% agarose gel containing 2.2 M formaldehyde.

In vitro reverse transcription of 1 μg total RNA to cDNA was performed using the commercial kit Gene Amp RNA PCR kit (Applied Biosystems, Foster City, CA) according to the manufacture's instructions. RT-PCR amplification of the *ALL1(MLL)/AF4* fusion transcript and of the normal *ALL1* gene were performed according to the methods previously described.[16]

Treatment

The GIMEMA 0496 and LAL 2000 studies included an identical induction therapy with 4 drugs (prednisone [PDN], vincristine [VCR], daunorubicin [DNR], and asparaginase [ASP]) with high-dose DNR (270 mg/m^2).[10] A seven day prednisone pre-treatment was added in the GIMEMA LAL 2000 study. The two protocols differed in their post-induction treatments. The GIMEMA 0496 included two consolidation courses with high-dose Ara-C (2gr/m^2 every 12 hours as 3-hour infusion on days 1 and 2) and etoposide[11] followed by three years of maintenance treatment, whereas in the GIMEMA LAL 2000 patients received one course of high-dose Ara-C (two daily doses at 3 gr/m^2, days 1, 2, 3 and 4) and mitoxantrone (10 mg/m^2, days 3, 4 and 5) followed by an allogeneic or autologous HSCT according to the availability of an HLA-compatible donor of hematopoietic stem cells.

Response criteria

All patients starting induction therapy have been considered for statistical analysis. Therapeutic responses were evaluated at the end of induction treatment in all cases. Complete Remission (CR) was defined as the normalization of peripheral blood count and less than 5% blasts in the bone marrow (BM) with normal cellularity. Relapse was defined as the reappearance of leukemic cells in the bone marrow (> 5% blasts) and/or reappearance of clinical evidence of the disease.

Statistical analysis

Differences in the distribution of factors in subgroups were analyzed by χ^2 or Fisher's exact test, and by the Kruskal-Wallis test, as appropriate. Overall Survival (OS) was defined as the time from diagnosis to death or date of the last follow-up; Disease Free Survival (DFS) was calculated from the time of achieving CR to relapse, death or date of last follow-up. The probabilities of OS and DFS were estimated using the Kaplan-Meier method; differences between distribution were evaluated by means of the Log-Rank test; confidence intervals (C.I. 95%) were estimated using the Simon and Lee method. The hypothesis of proportionality was tested using Schoenfeld's partial residuals. All tests were two-sided; $P \leq 0.05$ was considered statistically significant. All analyses where performed using the SAS software (SAS Institute, Cary, NC).

Results and Discussion

In the present study, we describe the clinical course of 46 *ALL1(MLL)/AF4* positive adult ALL patients consecutively treated in two multicenter GIMEMA trials: this represents the largest cohort of patients showing this rare genetic alteration described in the literature. All patients had a pro-B ALL. As reported in Table 1, there was no difference in the main clinical and biological characteristics of the *ALL1(MLL)/AF4* positive ALL patients. The karyotype was available in 38 cases (83%) and a t(4;11)(q21;q23) cytogenetic alteration was detected in 31 cases. None of the cases showed a t(9;22) and/or expressed the *BCR/ABL* fusion.

Table 2 summarizes the therapeutic response and clinical outcome recorded in the two groups of patients. A hematologic CR was achieved in 19 (90%) and in 22 (88%) of the 21 and 25 ALL patients treated according to the LAL 2000 and 0496 trials, respectively (*P*= n.s.).

Among the 19 ALL cases in CR after the LAL 2000 induction, one patient received a conventional maintenance treatment without transplant because of a severe cardiomyopathy developed after induction-consolidation chemotherapy. The patient relapsed 24 months after CR. Thirteen patients received a transplant (5 autologous; 8 allogeneic) whereas 4 patients relapsed before transplant at a median time of 1.6 months (range 1.2-5.8) after CR; the remaining case was lost to follow-up. Since our analyses were based on the intention to treat, this latter patient was considered at risk for the time he persisted in follow-up, thereafter he was censored at the time he became lost to follow-up. Following a decision by the attending physician, 2 additional patients of the GIMEMA 0496 study were allotransplanted in first CR. Therefore, altogether 15 patients were transplanted. Eight cases received HSC from an HLA identical donor, one from an unrelated and one from an aploidentical donor, whereas the remaining 5 cases received autologous HSC.

All transplanted patients received standard intensive conditioning regimens that in the majority of cases consisted of total body irradiation (TBI) and/or cyclophosphamide (CY), and busulphan and CY.

The probabilities of OS and of DFS are reported in Figure 1. The clinical outcome of the two patient groups were similar; at 36 months, rates of OS and DFS were 32.9% (C.I. 95%: 26.6-40.7), 31.8% (C.I. 95%: 25.4-39.6), 28% (C.I. 95%: 23.5-33.4) and 27.3% (C.I. 95%: 22.6-32.9), respectively, for patients who received the LAL 2000 and the 0496 protocols.

Disease relapses occurred in 10/19 (53%) patients in the LAL 2000 protocol and in 16/22 (72%) patients who received the GIMEMA 0496 treatment (*P*=n.s.). Source of HSCT did not affect the relapse rate after transplant. In fact, among patients treated with the LAL 2000 protocol, relapses occurred in 2 of the 5 patients autografted and in 3 of the 10 patients who received allogeneic HSCT. In addition, in the LAL 2000 protocol, the group of patients still in CR and those who had relapsed showed a similar median time to transplant, being 3.1 (1.8-6.1) and 2.8 (2-3.6) months, respectively.

In order to try to define the impact of HSCT on the clinical outcome of our patients, we first considered the occurrence of disease relapses and treatment failures (i.e. disease relapses and deaths in CR for transplant related comorbidities) with respect to transplant. Overall, we observed a relapse in 6/13 (excluding the 2 patients who died in CR) and 19/25 patients treated with HSCT or CHT alone, respectively (*P*=0.066), whereas 8 of 15 and 19 of 25 patients who received HSCT or CHT alone (*P*=0.175) failed the treatment. Secondly, we constructed a Cox model to evaluate the prognostic impact on DFS rates of the following variables: age and WBC count at diagnosis, type of treatment and transplant. This latter was considered as a time dependent covari-

ate (i.e. the effect of transplant on each patient was analyzed starting from the day of HSCT). Results of this analysis showed that DFS rate was not significantly affected by any of the variables considered. In particular, HSCT resulted in an HR of 0.676 (95% CI: 0.222-2.059; *P*=0.4904).

Although we observed a trend toward significance when considering the differences in relapse rates between transplanted and non-transplanted patients, the intensified strategy adopted in the LAL 2000 study, that included HSCT, did not favorably impact on the clinical outcome of *ALL1(MLL)/AF4* positive ALL patients.

The present findings are not powered enough to answer the question on the efficacy of HSCT for the treatment of this rare leukemic subtype because patients receiving HSCT were too few to have a significant impact on the DFS. However, it is worth noting that the lowest relapse rate was observed after allogeneic HSCT (3/10). Therefore, this latter should still be considered the treatment of choice for the treatment of this genetically characterized adult ALL subtype even if contrasting data have been reported. Results from the MRC/ECOG 2993 study[17] showed that, despite the use of HSCT in first CR, the t(4;11) alteration still identified patients with an adverse prognosis having low event free survival and OS rates, due in part to relapses but also to deaths in CR. By contrast, a superiority of allogeneic transplant was demonstrated by the results of the prospective multicenter LALA-94 study[18] which showed that allogeneic SCT was associated with a significantly improved DFS with a plateau at 18 months. The advantage of transplant procedures was also demonstrated by the German multicenter trials GMALL 0489[19] in which, however, t(4;11) positive cases were included in the larger pro-B immunophenotypic subgroup, a leukemic subtype with a heterogeneous prognosis, as previously reported.[10]

When considering the efficacy of HSCT in hematologic malignancies, it is necessary to consider several variables. One of the most important is the ability of pre-transplant treatments to eradicate the malignant clones to the greatest possible extent. This concept is based on several observations and has recently received further confirmation in *Ph*-positive ALL. The use of tyrosine kinase inhibitors before transplant, that results in an improved molecular remission rate, was associated with a significant improvement in disease outcome after transplantation.[20-22]

Due to the limited number of our patients, we cannot draw any definite conclusion. However, the failure of HSCT to improve results in our *ALL1(MLL)/AF4* positive ALL patients might, therefore, be due to the weakness of the pre-

Table 1. Clinico-biological characteristics of *ALL-1(MLL)/AF4* positive patients according to the treatment group.

	GIMEMA LAL 0496 N=25	GIMEMA LAL 2000 N=21	*P*
Sex			
males	14	8	0.2259
Females	11	13	
Age			
median	38.8	38.6	0.8694
range	(14.9-59.4)	(19.6-56.1)	
WBC at diagnosis			
median	60.0	60.1	0.8522
range	(2.2-663.0)	(2.4-872.3)	
HB at diagnosis			
median	9.0	8.9	0.8782
range	(4.9-15.1)	(3.0-12.7)	
PLTs at diagnosis			
median	33.5	33.0	0.5520
range	(1.0-153.0)	(14.0-136.0)	
BLAST % BM at diagnosis			
median	95	93.0	0.2737
range	(70.0-100.0)	(75.0-99.0)	
BLAST % PB at diagnosis			
median	91.5	87.0	0.1029
range	(18.0-100.0)	(32.0-99.0)	

Table 2. Therapeutic response and clinical outcome of ALL-1(MLL)/AF4 positive of all patients and according to the two different protocols.

	All patients (n=46)	GIMEMA 0496 (n=25)	GIMEMA LAL 2000 (n=21)
Response to induction therapy			
CR	41	22	19
Resistant	5	3	2
Post-remission treatment			
Allo-HSCT	10	2	8
Auto-HSCT	5	-	5
Maintenance	21	20	1
Clinical course			
Pts. in CCR	13	6	7
Relapses	26	16	10 (4)*
Died in CR	2	0	2

**patients relapsed before HSCT; CCR: continuous complete remission; Allo-HSCT: allogeneic HSCT; auto-HSCT: autologous HSCT.*

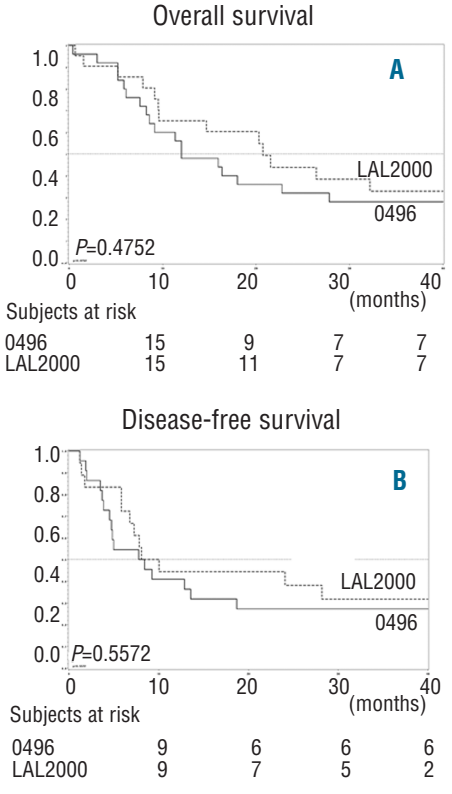

Figure 1. Actuarial probability of OS (A) and DSF (B) according to treatments [LAL 2000 (————) or LAL 0496 (– – – – – –)].

transplant strategy rather than to the effect of HSCT *per se*. In fact: 1) the occurrence of several relapses before HSCT (4 of 10); 2) the fact that the more aggressive pre-transplant regimen used in the LAL2000 protocol did not significantly increase the molecular responses in comparison with the GIMEMA-0496 protocol. In fact, at the end of induction/consolidation treatments, the observed molecular CR rates (i.e. absence of the specific *ALL1(MLL)/AF4* amplification band in the presence of RNA integrity) were 66% and 50% in the LAL 2000 and 0496 studies, respectively; 3) the use of HSCT after even more aggressive regimens, including cyclophosphamide, high-dose methotrexate and a second course of high-dose AraC-mitoxantrone, resulted in a better clinical outcome.[17,18]

It, therefore, appears that HSCT, to be effective, should be preceded by a more effective pre-transplant treatment.

The risk of an early relapse in *ALL1(MLL)/AF4* positive ALL patients should prompt the search for a potential donor as soon as a diagnosis has been made.

In conclusion, *ALL1(MLL)/AF4* positive ALL remains an attractive leukemic subtype in which to evaluate novel strategies in order to improve chemotherapy activity and/or reduce treatment toxicity.

Authorship and Disclosures

FM, GC, GM and RF designed the study and revised the manuscript. GC interpreted clinical data and prepared the manuscript. LE provided molecular diagnosis and helped in writing. MV and FP managed clinical date and performed statistical analyses. NC, ML and SS provided clinical data on patients and helped in writing.

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