

## ORIGINAL ARTICLE

# High antileukemic efficacy of an intermediate intensity conditioning regimen for allogeneic stem cell transplantation in patients with high-risk acute myeloid leukemia in first complete remission

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The goal of this analysis was to define the role of the moderate-intensity fludarabin Ara-C amsacrin (FLAMSA)-reduced intensity conditioning (RIC) regimen for patients with high-risk AML undergoing allogeneic SCT (alloSCT) in first CR1. High-risk was defined by (1) AML secondary to MDS or radio/chemotherapy, (2) unfavorable cytogenetics or (3) delayed response to induction chemotherapy. A total of 23 of 44 AML patients referred to the University of Munich for alloSCT in CR1 between 1999 and 2006 fulfilled these criteria and received FLAMSA chemotherapy, followed by RIC (4Gy TBI/cyclophosphamide/ATG) for alloSCT. Twenty-two patients engrafted, one died in aplasia. Two-year cumulative incidences for relapse and nonrelapse mortality (NRM) were 4.6 and 22.5%, respectively. Four-year overall and leukemia-free survival was 72.7% (median follow-up among survivors: 35 months). The results of this high-risk cohort were compared to the outcome of 21 consecutive standard-risk patients <55 years, who had received standard, myeloablative sibling SCT in CR1 AML within the same center and time period. Survival and cumulative incidences of relapse and NRM were identical in both groups. In conclusion, the FLAMSA-RIC regimen produces long-term remission in a high proportion of patients with high-risk AML transplanted in CR1. In this cohort, FLAMSA-RIC showed equivalent antileukemic activity as compared to the standard protocols.

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## Introduction

AML is a heterogeneous disease with an overall survival (OS) ranging from 70–90% in favorable to <20% in unfavorable subgroups.<sup>1</sup> Besides advanced stage and patient age, the leukemic karyotype is the most relevant prognostic factor.<sup>2–4</sup> Furthermore, delayed response to induction chemotherapy<sup>5,6</sup> and AML secondary to myelodysplastic syndrome (MDS) or previous chemotherapy<sup>7,8</sup> are associated with inferior outcome. The pejorative prognostic significance of these risk factors is also maintained after allogeneic SCT (alloSCT) following standard intensity conditioning (SIC).<sup>9–12</sup>

The introduction of reduced intensity conditioning (RIC) regimens has dramatically changed the landscape of alloSCT. Decrease in dosages of TBI and chemotherapy has reduced acute side effects of the conditioning and nonrelapse mortality (NRM). In RIC transplants, the antileukemic activity of alloSCT is shifted toward the GVL effect. However, in aggressive leukemia, RIC may not provide sufficient disease control to allow time for a GVL reaction. As a consequence, a variety of so-called moderate-intensity regimens were developed for alloSCT in myeloid leukemias.<sup>13–18</sup> In 1999, our group introduced the moderate-intensity FLAMSA-RIC protocol for high-risk patients with AML and MDS.<sup>19</sup> While promising results in refractory disease have been shown,<sup>20</sup> the role of the regimen in first CR1 has not yet been evaluated. Therefore, we retrospectively analyzed the outcome of all consecutive patients who had entered the protocol in CR1 at our center between 1999 and 2006. For comparison, a reference cohort of 21 consecutive standard-risk patients was used, who had

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received a SIC transplant from their HLA-identical sibling in CR1 within the same center and time period.

## Patients and methods

### Patients

Since 1999, all consecutive AML patients who were referred to the University of Munich for alloSCT and fulfilled the previously defined criteria for high-risk AML<sup>19</sup> had included into the FLAMSA-RIC protocol. In CR1, high-risk AML was defined by (1) unfavorable cytogenetics, (2) AML secondary to myelodysplastic syndrome or prior radio/chemotherapy or (3) delayed response to induction chemotherapy, that is, persistent leukemia after the first course. Per protocol, related and unrelated grafts were allowed in these patients. In contrast, patients in CR1 without any of these factors were regarded as standard risk. According to the respective protocols by the German AML study groups (summarized by Schlenk *et al.*<sup>21</sup>) these patients were offered a classical, standard intensity alloSCT in CR1, if they were  $\leq 55$  years of age, if an HLA-identical sibling donor was available and if they did not suffer from any comorbidity precluding standard conditioning.

For the present analysis, all consecutive patients from both risk groups, who had been transplanted in CR1 at our center between October 1999 and March 2006, were evaluated. Characteristics and results of high-risk patients were analyzed, and were finally compared to the outcome of standard-risk patients.

### HLA typing

Standard serological typing was used for HLA-A, -B and -C. High-resolution molecular typing using PCR in sampled DNA with sequence-specific primers was performed for class II alleles (HLA DRB1 and DQB1).

### Treatment

Since conventional chemotherapy was regarded to be of minor efficacy in high-risk patients, they could be referred for SCT even after one course of induction therapy, once CR had been achieved. The FLAMSA-RIC protocol has recently been described in detail.<sup>19</sup> In brief, the sequential preparative regimen for SCT consisted of a 4-day course of chemotherapy (FLAMSA), followed by RIC (4 Gy TBI/cyclophosphamide/ATG) after a 3-day interval. PBSC were the preferred stem cell source, cyclosporin A (CYA) and mycophenolate mofetil were used for immunosuppression.

Standard conditioning for alloSCT in the control group consisted of either 12 Gy TBI given in three single fractions at days -7, -6 and -5 or 16 mg/kg oral busulfan ( $4 \times 1$  mg/kg per day) from day -8 to -5, followed in all patients by cyclophosphamide (CY) (60 mg/kg) on days -4 and -3 and ATG Fresenius (10 mg/kg) on days -4, -3 and -2. BM was used as stem cell source, and  $>2 \times 10^6$  CD34+ cells per kg and  $>2 \times 10^8$  mononuclear cells per kg were requested, respectively. Immunosuppression consisted of CYA from day -1, and methotrexate (15 mg/m<sup>2</sup> at day +1, 10 mg/m<sup>2</sup> at days +3 and +6).

### Evaluations and definitions

CR1, cytogenetic subgroups, comorbidities and GVHD were defined and graded as described.<sup>3,22-24</sup> Toxicities followed the National Cancer Institute Common Toxicity Criteria. Date of neutrophil engraftment was defined as the first of two consecutive days with a peripheral blood (PB) neutrophil count  $>500/\mu\text{l}$ . Date of platelet engraftment was defined as the first of 3 days with a platelet count  $>20$  g/l without transfusion. At day +30, disease response and chimerism were assessed in PB and BM. Since thrombocyte regeneration could be postponed by factors other than leukemia and cytotoxic therapy (that is, GVHD, drugs, virus), CR after transplantation was defined by  $<5\%$  blasts without evidence of dysplasia in BM, and  $>1500$  neutrophils per microliter in PB. Donor chimerism was studied using FISH<sup>25</sup> or short tandem repeat analysis.<sup>26</sup> Relapse was defined by reappearance of blasts in PB, by any leukemic infiltration outside the hematopoietic system, or by BM infiltration of  $>5\%$  blasts. NRM was defined as death from any cause without leukemic recurrence, including death from preexisting comorbidities. OS was calculated by time from transplantation to death or last follow-up, leukemia-free survival (LFS) by time from transplantation to relapse, death or last follow-up, whatever occurred first.

### Statistics

Results were analyzed as of 25 June 2007. Two-year OS was the primary end point. Secondary end points included LFS, cumulative incidence of relapse and NRM, and incidence and severity of acute and chronic GVHD. Numeric variables were analyzed as categories considering their value below or above the median of the entire cohort. For comparison of group characteristics (for example, sex, donor type, CMV status),  $\chi^2$ -test, Fisher's exact test and the Mann-Whitney test were used for univariate testing. Acute and chronic GVHD were analyzed as time-dependent variables. Additionally, landmark analyses were performed at several dates to evaluate the role of GVHD on survival. OS and LFS were estimated using the Kaplan-Meier method. Log-rank test was used for analysis of risk factors for time-to-event variables. Cumulative incidences of relapse and of NRM were simultaneously calculated, accounting for competing risks. R (a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>) and SPSS (SPSS Inc., Chicago, IL, USA) software were used for data analysis.

### Study conduct

The study was performed according to the modified Helsinki declaration. The protocol has been approved by the local ethical review board prior to its initiation. Every patient gave written informed consent.

## Results

### Patients

Forty-four AML patients consecutively referred in CR1 to the José-Carreras-Transplantation unit between 1999 and

2006 were analyzed (Table 1). Twenty-three patients with high-risk disease had been included into the FLAMSA-RIC trial. Eight of these patients had been previously reported<sup>19</sup> and were updated for the present analysis; data on 15 new patients were added. Median age was 47 (range 30–63) years. Inclusion criteria were delayed CR after induction therapy in 12, unfavorable cytogenetics (5q–,  $n=2$ ; t(6;9),  $n=1$ ; 7q–,  $n=4$ , translocations involving 11q23,  $n=3$  and complex karyotype,  $n=5$ ) in 15, and leukemia secondary to MDS ( $n=9$ ) or chemotherapy for solid tumor ( $n=3$ ) in 12 patients. A total of 10 patients had one, 10 had two and 3 had three risk factors. Donors were HLA-identical siblings in 12, a one antigen-mismatched family donor in 1 and matched unrelated donors in 10 patients. Due to a history of radiotherapy, three patients with sAML received oral busulfan (BU) ( $8 \times 1$  mg/kg) instead of TBI. Twenty-one patients with standard-risk disease with an HLA-identical sibling donor received classical conditioning, followed by BM transplantation.

#### High risk group

**Engraftment and chimerism.** PBSC grafts in the FLAMSA-RIC cohort contained a median of 9.1 (range: 4.0–18.6)  $\times 10^6$  CD34+ cells per kg. One patient died in aplasia, median time to neutrophil and thrombocyte engraftment among the remaining patients was 17 and 16 days, respectively. No graft failure occurred, and median donor chimerism in BM at day +30 was 100% (range: 96–100). Chimerism was not evaluated systematically in the peripheral T-cell compartment, but was 100% at day +30 in 12 informative cases. Complete hematological and cytogenetic remission was observed in all 22 patients achieving engraftment.

**Nonhematologic toxicities and nonrelapse mortality.** Twenty-six events of grade III/IV acute toxicity were observed, with liver ( $n=10$ ), lung (pneumonia;  $n=7$ ) and heart ( $n=3$ ) being most frequently affected (Table 2). One patient developed prostate cancer as a possible secondary neoplasm 5 years after transplantation. Five patients died from reasons not related to leukemia between days +34 and +1060. Cumulative incidence for NRM was 4.3% (95% confidence interval (CI) (0–8.7%)) at day +100 and 22.5% (95% CI (13.4–31.6%)) at 2 years. Acute GVHD (aGVHD) occurred in 17 patients between days +9 and +51 (median: +17), reaching grade I in 11, grade II in 3, grade III in 2 and grade IV in 1 case. Ten patients developed limited ( $n=4$ ) or extensive ( $n=6$ ) chronic GVHD.

**Relapse and outcome.** Two patients relapsed (days +215 and +1611). One died from refractory disease, the second one achieved complete blast clearance following low-dose Ara-C, but died from sepsis shortly before a planned donor lymphocyte transfusion. Two-year cumulative incidence of relapse was 4.6% (95% CI (0–9.2%)) Median follow-up of 16 surviving patients was 35.0 (range: 10.9–87.9) months. Estimated OS and LFS at 2 and 4 years from transplantation was 72.7%. Because of the small numbers, analysis of prognostic factors for survival was limited to univariate

**Table 1** Patients characteristics

	FLAMSA-RIC	Standard conditioning	P
Number of patients	23	21	
Patient age (years)	48 (30–63)	43 (21–55)	1.0
<i>Patient's sex</i>			
Female	13	12	1.0
Male	10	9	
<i>Donor's sex</i>			
Female	7	7	1.0
Male	16	14	
<i>AML subtype</i>			<0.0001
De novo	11	21	
Secondary to MDS	5	—	
Treatment related	7	—	
<i>Cytogenetic subgroups<sup>a</sup></i>			<0.0001
Favorable	—	2	
Intermediate	8	19	
Unfavorable	15	—	
<i>Treatment before transplantation (no. of courses)</i>			0.002
One	5	—	
Two	14	7	
> Two	4	14	
Delayed response to induction therapy	12 (52%)	—	<0.0001
Interval from diagnosis to transplantation (days)	144 (58–527)	161 (106–246)	0.23
<i>CMV status (patient/donor)</i>			0.89
Negative/negative	7	5	
Negative/positive	2	3	
Positive/negative	8	7	
Positive/positive	6	6	
<i>Donor</i>			<0.0001
HLA-identical sibling	12	21	
Mismatched family	1	—	
Matched unrelated	10	—	
<i>Stem cell source</i>			<0.0001
Bone marrow	3	21	
Mobilized PBSC	20	—	
Median year of transplantation	2004	2002	0.07
<i>HCT-CI score<sup>b</sup></i>			0.008
0	13	13	
1	1	7	
2	1	0	
3	4	1 <sup>c</sup>	
4 or more	4	0	
History of pneumonia (bacteria/aspergillus)	4/8	2/4	0.14

Abbreviations: FLAMSA-RIC; HCT-CI = hematopoietic cell transplantation comorbidity index; MDS = myelodysplastic syndrome.

<sup>a</sup>Cytogenetic subgroups were classified according to the Southwest Oncology Group/Eastern Cooperative Oncology criteria.

<sup>b</sup>HCT-CI scores were assessed as described by Sorror *et al.*<sup>23</sup> Accordingly, the following comorbidities were included: arrhythmia, cardiac, inflammatory bowel disease, diabetes, cerebrovascular disease, psychiatric disturbances, hepatic (mild, moderate/severe), obesity, active infection, rheumatologic, peptic ulcer, renal (moderate/severe), pulmonary (moderate/severe), prior solid tumor, heart valve disease.

<sup>c</sup>Combination of mild elevated transaminases and moderate dyspnea, not classified as contraindication against standard conditioning for SCT.

**Table 2** Nonhematological toxicities<sup>a</sup> and GVHD

	Number of events	
	FLAMSA-RIC	Standard conditioning
Number of patients	23	21
Oral mucositis	1	14
Hepatic	10	9
Cardiac	3	3
Bleeding	1	1
Renal	1	2
Pneumonia	7	4
Multiorgan failure	1	1
Septic shock	1	0
Pulmonary leakage	1	0
<i>aGVHD</i>		
Total II–IV (%)	25	29
II	<i>n</i> = 3	<i>n</i> = 5
III	<i>n</i> = 2	—
IV	<i>n</i> = 1	<i>n</i> = 1
<i>cGVHD</i> <sup>b</sup>		
Total (%)	45%	55%
Limited	<i>n</i> = 4	<i>n</i> = 7
Extensive	<i>n</i> = 6	<i>n</i> = 4

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; CTC = common toxicity criteria; FLAMSA-RIC.

<sup>a</sup>Limited to toxicities grade III/IV according to CTC.

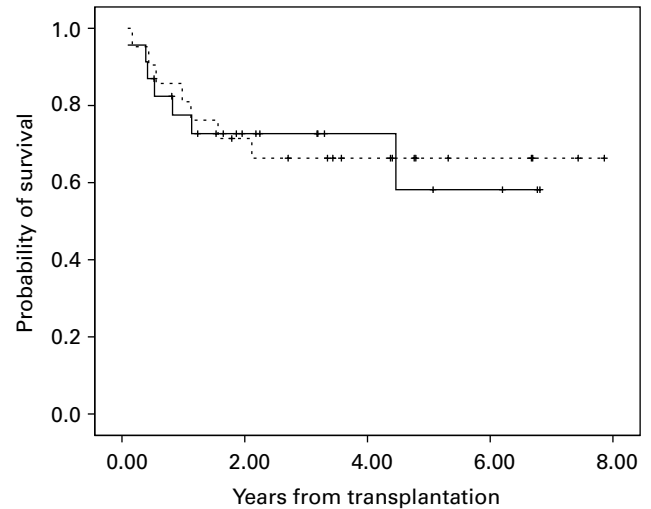
<sup>b</sup>Analysis limited to patients alive at day +100.

testing. *De novo* leukemia as compared to secondary AML was identified as the most important favorable factor (4-year OS: 91 vs 56% ( $P=0.03$ )). In contrast, no influence on outcome was detected for age, cytogenetic subgroups, donor type, hematopoietic cell transplantation (HCT)-comorbidity index (CI), intensity of pretreatment and chronic GVHD (cGVHD). Patients with aGVHD limited to the skin had a slightly better OS than patients without aGVHD, whereas outcome after visceral GVHD was inferior ( $P=0.04$  and  $0.05$ , respectively).

#### Comparison to standard-risk patients

The two groups were comparable with respect to age, sex and CMV status of patient and donor, history of prior pneumonia including pulmonary aspergillosis and time from diagnosis to transplantation. In contrast, as defined by the respective protocols, the use of unrelated donors and PBSC grafts and the presence of leukemia-associated risk factors were limited to the FLAMSA-RIC cohort. Furthermore, the HCT-CI revealed significantly higher comorbidity scores for the FLAMSA-RIC patients (Table 1).

As expected due to the higher number of PBSC recipients in this group, neutrophil engraftment was faster after FLAMSA-RIC conditioning (17 vs 22 days). With respect to acute toxicity, grade III/IV oral mucositis occurred in 14 of 21 SIC recipients, but was seen in one FLAMSA-RIC patient only ( $P<0.001$ ). All other nonhematological toxicities, as well as aGVHD and cGVHD, were equally distributed. (Table 2) Neither 2-year cumulative incidence of NRM (22.5% (95% CI, 13.4–31.6%) vs 14.3% (95% CI, 6.6–22.1%)), nor cumulative incidence of relapse (4.6%



**Figure 1** Comparable overall survival (OS) for high-risk patients following FLAMSA-RIC (solid line) and standard-risk patients following standard conditioning (dashed line) for allogeneic SCT.

(95% CI, 0–9.2%) vs 14.3% (95% CI, 8.1–20.5%)) was increased in the high-risk as compared to the standard risk group. With a median follow-up of 50 months among survivors, 4-year OS of the standard-risk cohort was 66.3%, thereby nearly superimposing the survival curve of the FLAMSA-RIC cohort (Figure 1).

#### Discussion

The sequence of intensive chemotherapy and reduced conditioning was aimed to improve the antileukemic efficacy of alloSCT by establishing a state of minimal residual disease as a platform for an early GVL reaction. As described,<sup>19</sup> only leukemia-specific risk factors, but not increased risk for NRM after alloSCT, were considered for inclusion into this moderate-intensity protocol. Hence, patients fulfilling high-risk criteria were included, whether or not they had contraindications against conventional conditioning. The purpose of the present analysis was to evaluate the role of this strategy in CR1 AML. Unfavorable cytogenetics, secondary AML after MDS or radio/chemotherapy or delayed response to induction therapy defined high-risk disease in CR1. For comparison, a homogenous group of standard-risk patients  $\leq 55$  years were used, who had received classical SIC BMT from an HLA-identical sibling donor. Time and center effects were excluded by transplantation in the same center and time period. Survival analysis showed that in spite of the inherent risk for relapse, long-term remission could be achieved in  $>70\%$  of patients using the FLAMSA-RIC regimen in CR1. Early engraftment and full donor chimerism were observed. The results compared well to the results obtained in the standard risk, i.e. standard conditioning, cohort. This is in contrast to the results from earlier studies evaluating the outcome of alloSCT in different prognostic subgroups.<sup>9,27</sup> Furthermore, OS and LFS were superior to the results of a similarly defined cohort of poor-risk patients published recently.<sup>28</sup> These

findings underscore the antileukemic activity of the FLAMSA-RIC protocol, although the inclusion of unrelated donors might have contributed to the positive results by applying a more profound GVL efficacy as compared to related grafts. Acute toxicity and NRM were comparable between the two cohorts, except oral mucositis, which occurred significantly more frequently after SIC. Given the higher risk for NRM within the FLAMSA-RIC cohort, as indicated by the higher HCT-CI scores and the inclusion of unrelated donors, the regimen was relatively well tolerated.

A broad variety of RIC regimens, ranging from truly nonmyeloablative to moderate-intensity protocols, has been evaluated in AML. However, comparison of results in CR1 is difficult for two reasons: first, many studies have included patients at various disease stages, and do not allow to assess cytogenetics and other risk factors of CR1 patients. Second, in studies reporting on patients with 'increased risk', the definition of high-risk disease often combines criteria for aggressive leukemia as used in our study, and risk factors for NRM, as age and comorbidities. Therefore, any comparison must be interpreted with caution.

Among nonmyeloablative regimens, the Seattle/Leipzig consortium has recently shown extended data from their minimal intensity protocol (2 Gy TBI +/- fludarabin).<sup>29</sup> Two-year OS rates between 50 and 67% were reported, with unrelated transplants achieving better results. These studies included CR1 patients from all risk groups, but the results are impressive, in particular, because of the high-median patient age up to 62 years. Relapse was the most frequent cause of death.

Various moderate-intensity protocols have been studied. The combination of fludarabin, melphalan and alemtuzumab was used by groups from Birmingham<sup>30</sup> and Chicago.<sup>31</sup> In the British study, patients in CR1 were included based on similar risk factors as chosen by us. Disease-free survival for CR1 patients at 3 years was 39%, 3-year OS for all patients transplanted in remission was 48%. The Chicago group reported 1-year OS and PFS of 66 and 61% in patients transplanted in remission. The MD Anderson group used i.v. BU/fludarabin, and reported a 1-year OS of >80% for patients transplanted in remission.<sup>17</sup> Similar results were observed by a German study, using 8 Gy TBI/fludarabin.<sup>18</sup> However, in both studies, the risk profiles of patients transplanted in CR1 were not presented in detail. Recently, a French group retrospectively reported on 33 AML patients in CR1, 21 of them with high-risk leukemic features as used in our study.<sup>32</sup> They also used a sequence of intensive chemotherapy followed or not by autologous SCT, and RIC for alloSCT. Two-year OS and LFS were 79 and 76%, respectively, with low NRM and relapse rates.

In spite of the limitations of these nonrandomized or retrospective studies, promising results of different RIC regimen prompted the question whether patients in CR1, who are eligible for SIC, could receive less intensive regimen without increased incidence of relapse. This is of particular interest, since the importance of dose intensity has been shown in SIC<sup>33</sup> and RIC<sup>34</sup> transplantation. Several retrospective analyses have compared myelo-

ablative regimen with RIC. The Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation analyzed patients >50 years receiving RIC or SIC for sibling SCT.<sup>35</sup> In both cohorts, >50% of the patients were in CR1. Following RIC, lower NRM and higher relapse incidence occurred, resulting in identical outcome (53% 2-year OS for CR1 patients) as found after SIC. Whereas no data on leukemia-associated risk profiles were available in this analysis, two recent studies reported analogous results in patients with increased leukemia-associated risk: The Seattle Group analyzed patients >40 years with MDS or tAML after myeloablative vs nonmyeloablative conditioning. OS and LFS were identical among patients transplanted with <5% BM blasts.<sup>36</sup> An Israeli study included high-risk patients in CR1, using the same definition as in our study, and in more advanced stages. Whereas the RIC regimen showed clearly inferior results in active disease, the outcome was identical in patients transplanted in remission.<sup>37</sup> These data suggest to consider RIC instead of standard regimen for alloSCT in CR1 of AML, even in high-risk disease, which is further supported by our analysis. However, final evidence for equality of RIC in this situation will come from prospective, randomized trials, as currently performed in Germany and by the Seattle consortium. Furthermore, prospective, randomized multicenter trials will have to evaluate optimal RIC regimens for defined subgroups, based on disease- and patient-specific characteristics.

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