Results from a clofarabine-busulfan-containing, reduced-toxicity conditioning regimen prior to allogeneic stem cell transplantation: the phase 2 prospective CLORIC trial

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ABSTRACT

We prospectively evaluated the safety and efficacy of a clofarabine, intravenous busulfan and antithymocyte globulin-based reduced-toxicity conditioning (CloB2A2) regimen before allogeneic stem cell transplantation. Thirty high-risk patients (median age: 59 years; acute myeloid leukemia n=11, acute lymphoblastic leukemia n=13; myelodysplastic syndrome n=5, bi-phenotypic leukemia n=1) were included in this phase 2 study. At time of their transplant, 20 and seven patients were in first and second complete remission, respectively, while three patients with myelodysplastic syndrome were responding to chemotherapy or who had not been previously treated. The CloB2A2 regimen consisted of clofarabine 30 mg/m^2/day for 4 days, busulfan 3.2 mg/kg/day for 2 days and antithymocyte globulin 2.5 mg/kg/day for 2 days. The median follow-up was 23 months. Engraftment occurred in all patients. The 1-year overall survival, leukemia-free survival, relapse incidence and non-relapse mortality rates were 63±9%, 57±9%, 40±9%, and 3.3±3%, respectively. Comparing patients with acute myeloid leukemia/myelodysplastic syndrome *versus* those with acute lymphoblastic leukemia/bi-phenotypic leukemia, the 1-year overall and leukemia-free survival rates were 75±10% *versus* 50±13%, respectively (P=0.07) and 69±12% *versus* 43±13%, respectively (P=0.08), while the 1-year relapse incidence was 25±11% *versus* 57±14%, respectively (P=0.05). The CloB2A2 regimen prior to allogeneic stem cell transplantation is feasible, allowing for full engraftment and low toxicity. Disease control appears to be satisfactory, especially in patients with acute myeloid leukemia/myelodysplastic syndrome. The trial was registered at *www.clinicaltrials.gov no. NCT00863148.*

Introduction

Reduced-intensity conditioning (RIC) regimens emerged 15 years ago[1-3] with the aim of decreasing the toxicities and morbidities related to allogeneic stem cell transplantation (SCT). Progressively the concept of RIC switched to the concept of reduced-toxicity conditioning (RTC) regimens which are currently the majority of the conditioning regimens used worldwide.[4-5] Due to lower non-relapse mortality associated with RIC and RTC, such regimens allow older patients or patients with co-morbidities to be transplanted. The combination of fludarabine, an intermediate dose of intravenous busulfan and low-dose antithymocyte globulins is among the most popular RIC regimens used in Europe, particularly in France.[6-9] Nevertheless, relapse remains challenging after such regimens.[6-11] Therefore, attempts to intensify the RIC regimen without increasing non-relapse mortality might be an attractive option in order to obtain better disease control while waiting for the immune graft-*versus*-leukemia effect. Thus, incorporation of drugs with greater anti-tumor activity and acceptable toxicity merits further investigation as part of RTC approaches.

Clofarabine is a second-generation purine analog which requires intracellular phosphorylation to be active. Clofarabine's triphosphate impedes DNA synthesis and repair by inhibiting ribonucleotide reductase and DNA polymerase. It has been documented that clofarabine has significant anti-leukemic activity, particularly in relapsed acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), and the drug is approved by the United States Food and Drug Administration for the treatment of pediatric ALL patients after at least two prior regimens. Hand-foot syndrome and reversible liver function abnormalities are the two main complications of the drug.[12-14] Clofarabine combines the most favorable pharmacokinetic properties of the first-generation purine analogs, namely fludarabine and cladribine, with superior anti-leukemic activity, due to an increased resistance to deamination and phosphorolysis, conferring better drug stability.[12] Direct induction of apoptosis by activation of caspase 9 and a direct interaction with the mitochondrial membrane may also play a role in this better anti-leukemic effect.[14] Thus, one can also exploit the anti-leukemic, immunosuppressive effects and the favorable toxicity profile of clofarabine in the setting of a RTC regimen prior to allogeneic SCT. At present, experience with clofarabine-containing regimens before allogeneic SCT in acute leukemia

 doi:10.3324/haematol.2014.108563.
This study was presented at an oral session during the 55th annual meeting of the American Society of Hematology (ASH) held in New Orleans, USA, in December 2013.
Manuscript received on April 3, 2014. Manuscript accepted on June 16, 2014.
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patients is still scarce and mostly includes series of patients with active disease at the time of transplantation.[15-18] We recently reported on a retrospective series of 88 AML/ALL patients (73% with active disease at the time of their transplant) receiving a clofarabine-containing RIC regimen: the 2-year overall survival rates of these AML and ALL patients were only 35% and 0%, respectively.[18]

Here we present the results of a prospective, multicenter phase 2 trial testing the use of clofarabine as part of the conditioning regimen in replacement of fludarabine, in combination with intravenous busulfan and antithymocyte globulin, in 30 patients with high-risk myelodysplastic syndromes (MDS) or acute leukemia in remission or not previously treated at the time of transplantation.

Methods

Study design

This prospective phase 2 study was conducted at six transplant centers in France (Bordeaux, Lyon, Nantes, Marseille, Strasbourg and Paris-Saint-Louis). The study was approved by each institutional review board of the participating centers, the Rennes' ethical committee and the cellular therapy committee of the *Agence Française de Sécurité Sanitaire des Produits de Santé* (AFSSAPS). Patients eligible for a RIC allogeneic SCT, aged between 18 and 65 years old, with high-risk MDS[19] not previously treated or in response after chemotherapy, or with high-risk AML or ALL[20-22] in first or second complete remission at transplant were considered for the purpose of this study. Patients were not eligible for a myeloablative regimen mainly because of age or because of their status beyond first complete remission. Other eligibility criteria included a Karnofsky performance status score ≥70% and a comorbidity score <3 according to Sorror *et al.*[23] (see *Online Supplementary Material*). All patients provided informed consent. Overall, 30 patients (male n=18, median age: 59 years) were included in this prospective study between October 2009 and August 2012. Only HLA-compatible sibling donors (n=14) or 10 out of 10 HLA-identical matched unrelated donors (n=16) were permitted. The characteristics of the patients and donors are shown in Table 1. Details of the conditioning regimen, graft source and graft-*versus*-host disease (GVHD) prophylaxis are given in Table 2. Definitions of hematopoietic reconstitution, engraftment, chimerism and immune reconstitution are provided in the *Online Supplementary Material*. Allogeneic or autologous reconstitution was evaluated by donor $CD3^+$ T-cell chimerism studies as previously described.[24]

Statistical analyses

The primary end-point of the trial was the assessment of leukemia-free survival at 1 year after allogeneic SCT. An A'Hern single-stage exact plan design was applied (see *Online Supplementary Material*). Secondary end-points were overall survival, relapse incidence, non-relapse mortality, engraftment, acute and chronic GVHD rates, percentage of donor chimerism and immune reconstitution post-allograft. Toxicity was evaluated according to the NCI criteria version 4. Overall survival was defined as the time from day 0 of allogeneic SCT to death or last follow-up for surviving patients. Leukemia-free survival was defined as the time from day 0 of allogeneic SCT to time without evidence of relapse or disease progression censored at the date of death or last follow-up. Probabilities of overall and leukemia-free survival were calculated using the Kaplan-Meier method and log-rank test. Relapse was defined as any event related to re-occurrence of the disease. Non-relapse mortality was defined as death from any cause without previous relapse or progression. Cumulative incidence curves were used for relapse incidence and non-relapse mortality in a competing risk setting.[25] Acute and chronic GVHD were diagnosed and graded according to standard criteria.[26,27] Death was considered as a competing event to GVHD. The log-rank test was used for univariate comparisons and the Gray test for cumulative incidence curves. For the purpose of this study, the single case of bi-phenotypic leukemia was considered as ALL for comparison of outcomes between AML/MDS and ALL patients. Statistical analyses were performed using SPSS 19 (SPSS Inc, Chicago, IL, USA), and R 3.0.1 (R Development Core Team, Vienna, Austria) software packages.

Results

Engraftment, hematopoietic recovery and chimerism

Engraftment was observed in all patients (100%). The median time for neutrophil recovery (>500/μL) was 18 (range, 14-26) days, while that for platelet recovery (>20,000/μL) was 9 (range, 0-18) days. The median time for the platelet count to be >50,000/μL was 12 (range, 0-23) days. No secondary graft failure occurred. Full donor chimerism was documented in 48% (n=11/23), 59% (n=13/22) and 90% (n=9/10) of evaluable patients at day +30, +90 and 1 year post-transplant, respectively.

Table 1. Characteristics of the patients and donors.

Patients	N=30
Gender: male	18 (60%)
Median age at transplant: years (range)	59 (20.6-64.5)
Karnofsky score at transplant:	
100%	19 (63%)
90%	6 (20%)
80%	4 (13%)
Unknown	1 (4%)
Type of diseases:	
Myelodysplastic syndrome	5 (17%)
Acute myeloid leukemia	11 (37%)
Acute lymphoblastic leukemia	13 (43%)*
Bi-phenotypic leukemia	1 (3%)
Status at transplant	
First complete remission**	20 (67%)
Second complete remission***	7 (23%)
MDS in response	2 (7%)
Non-treated MDS	1
Median interval between diagnosis and graft: months (range)	6 (3.8-124)
Cytomegalovirus serology status: positive	11 (37%)
Donors	
Sibling	14 (47%)
Matched unrelated	16 (53%)
Gender: male	20 (67%)
Median age: years (range)	44.7 (20-72.8)
Cytomegalovirus serology status: positive	11 (37%)
Graft (all peripheral blood stem cells)	
Median nucleated cell dose ($x10^8$/Kg): median (range)	8.13 (0.59-18.29)
Median $CD34^+$ cell dose ($x10^6$/Kg): median (range)	6.68 (1.51-16.99)

**including two patients with Philadelphia-positive ALL. **including ten AML/MDS cases and ten ALL/bi-phenotypic cases. ***including three AML and four ALL cases.*

Acute and chronic graft-versus-host disease

Grades 2, 3 and 4 acute GVHD occurred in ten, one and one patients respectively. The cumulative incidence of grades 2-4 acute GVHD at day +100 was 38±9%. The cumulative incidences of chronic GVHD at 1 and 2 years were 40±9% and 51±9%, respectively. Overall 15 patients presented with chronic GVHD (limited n=7; extensive n=8). The incidence of chronic GVHD was similar between patients with related or unrelated donors (42% *versus* 54%; *P*=0.54).

Toxicity

No unexpected events occurred after the transplants. The 1-year and 2-year non-relapse mortality incidences were 3.3±3% and 3.3±3%, respectively, as only one patient died due to the graft procedure of acute GVHD while not in relapse. Two reversible hand-foot syndromes occurred during the conditioning phase despite systematic administration of steroids. With regards to infectious events, reactivation of Epstein-Barr virus and cytomegalovirus occurred in six and five patients, respectively, while only one case of invasive aspergillosis infection was observed. Epstein-Barr virus-related lymphoma occurred in one patient after reactivation of the virus; the patient was treated effectively with rituximab. Bacterial sepsis was documented in eight patients. Finally, only two reversible grade 3 hepatic events were reported.

Outcomes (Figure 1)

With a median follow-up of 23 (range, 14-48) months after allogeneic SCT, the 1-year overall survival, leukemia-free survival and relapse incidence rates were 63±9%, 57±9% and 40±9%, respectively. The corresponding figures at 2 years were 58±10%, 53±9% and 43±9%, respectively. At 1 year, 12 patients had relapsed (40%) within a median time of 3.5 (range, 2.3-11.6) months after transplantation. One additional relapse occurred at 13.1 months. Eleven patients died within the first year. Overall, 14 patients died, including 13 patients already in relapse. The causes of death were mainly relapse in 11, then GVHD in two (including one after donor lymphocyte infusion for relapse) and sepsis in one.

Comparison of outcomes between patients with different malignant conditions (Figure 2)

Compared to patients with ALL/bi-phenotypic leukemia patients, those with MDS/AML had a higher 1-year overall survival rate (75±10% *versus* 50±13%; *P*=0.16) and a higher 1-year leukemia-free survival rate (69±12% *versus* 43±13%; *P*=0.15). The 1-year relapse incidence was lower (25±11% *versus* 57±14%; *P*=0.09) among the MDS/AML group, whereas the 1-year non-relapse mortality was not significantly different between the two groups (MDS/AML: 6±6% *versus* 0%, *P*=0.36). The 2-year overall and leukemia-free survival rates remained higher for MDS/AML patients than those for patients with ALL/bi-phenotypic leukemia (75±10% *versus* 38±14%, *P*=0.07; and 69±12% *versus* 36±13%, *P*=0.08) while the 2-year relapse incidence was still lower (25±11% *versus* 64±14%; *P*=0.05). Finally, the 2-year non-relapse mortality was similar in the two groups (MDS/AML: 6±6% *versus* 0%; *P*=0.36), as was the incidence of chronic GVHD (MDS/AML: 38% *versus* 64%, *P*=0.14).

Immune reconstitution

A normal median count of lymphocytes was not reached at 1 year post-transplant (median: 1110/μL range: 680-4080) while the median monocyte count was always within the normal range before and within the first year post-transplant. All median counts increased between month +3 and month +12: $CD3^+$ T cells: 466/μL *versus* 639/μL; $CD4^+$ T cells: 84/μL *versus* 214/μL; $CD8^+$ T cells: 386/μL *versus* 419/μL; $CD19^+$ B cells: 23/μL *versus* 267/μL; natural killer cells: 210/μL *versus* 333/μL. Normal values of natural killer cells and $CD8^+$ T cells were achieved as soon as day +100. The median counts of $CD3^+$, $CD4^+$ T cells and $CD19^+$ B cells were below the normal range during the first year post-transplant. A normal median immunoglobulin count was not achieved at 1 year post-transplant (median: 3.6 g/L, range: 1.7-12.2).

Discussion

This prospective phase 2 trial is the first to report the feasibility, safety and efficacy of a RIC/RTC regimen, based on clofarabine, intravenous busulfan and antithymocyte globulins (CloB2A2), prior to allogeneic SCT. Thirty patients with high-risk MDS or acute leukemias who were ineligible for standard conventional myeloablative transplantation, were enrolled in the study. The main differences between previous studies using clofarabine and busulfan and ours are the fact that patients were considered prospectively and did not have active disease at the time of transplantation. Full engraftment was observed in all cases suggesting a sufficient immunosuppressive activity of clofarabine in combination with intermediate doses of busulfan and anti-thymocyte globulin. Because the study targeted a high-risk population, the choice was made

Table 2. The "CloB2A2" RIC/RTC regimen.

	Day-8	Day-7	Day-6	Day-5	Day-4	Day-3	Day-2	Day-1	Day 0
IV CLOFARABINE* 30 mg/m²/day	x	x	x	x					
IV BUSULFAN 3.2 mg/kg/day					x	x			
ANTITHYMOCYTE GLOBULIN (Thymoglobuline) 2.5 mg/kg/day							x	x	
GVHD prophylaxis= cyclosporine alone						x	x	x	x
Graft (PBSC only)**									x

*RIC/RTC: redused-intensity/toxicity conditioning; IV: intravenous; GVHD: graft-versus-host disease; PBSC: peripheral blood stems cells. *Corticosteroids at a dose of 1 mg/Kg/day were used as prophylaxis during clofarabine administration in order to prevent hand-foot syndrome. Supportive care was at the discretion of the investigators of each participating center.*

to use cyclosporine alone for GVHD prophylaxis. Our goal was to obtain some GVHD in these high-risk patients as a guarantee of the graft-*versus*-leukemia effect and lower relapse rate. Our objective of at least 16 patients not in relapse at 1 year post-transplant was achieved, allowing us to envisage a phase 3 trial in which fludarabine will be compared to clofarabine as part of the RTC regimen. Moreover, we were able to study some aspects of immune reconstitution after the CloB2A2 regimen. We show here that monocytes, T, B, and NK cells as well as gammaglobulin reconstitution were similar to those that can be observed after fludarabine-based RIC regimens.[9] Furthermore, the toxicity was very low compared to other clofarabine-based conditioning regimens which included cytarabine[15] or melphalan,[16,17] with a non-relapse mortality rate below 5% at 1 year, and only two cases of reversible grade 3 hepatic events and no renal toxicity. This could be explained not only by the fact that none of the patients had active disease at the time transplantation and had not been heavily previously treated (most cases were if first complete remission), but also by the particular schedule we used here, with the drugs being administered successively and not concomitantly.

Results were particularly interesting in MDS/AML cases with 1-year overall and leukemia-free survival rates of 75% and 69%, respectively. No relapses occurred after 1 year post-transplant and the same survival rates were observed at 2 years. This latter fact suggests effective disease control in such high-risk patients. Some of the largest RIC allogeneic SCT studies for MDS/AML (including various RIC regimens) had showed 2-year overall survival rates of less than

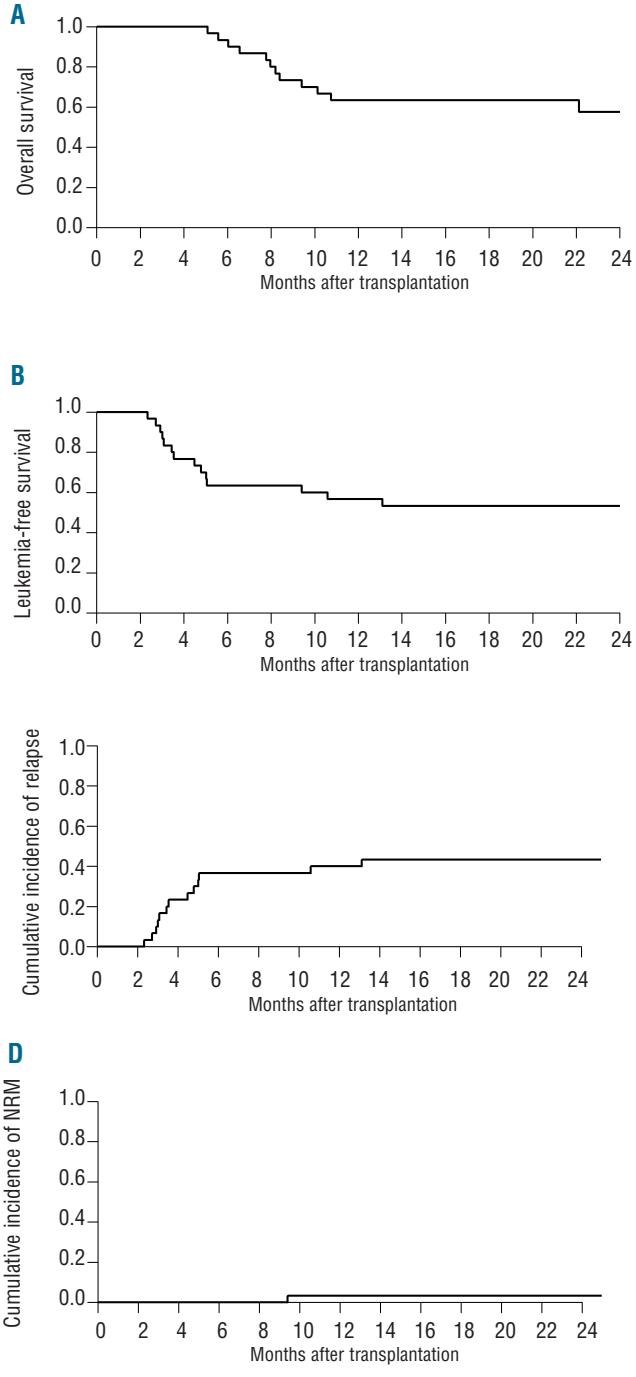

Figure 1. Overall survival (A), leukemia-free survival (B), relapse incidence (C) and non-relapse mortality (NRM) (D) for the whole cohort (n=30).

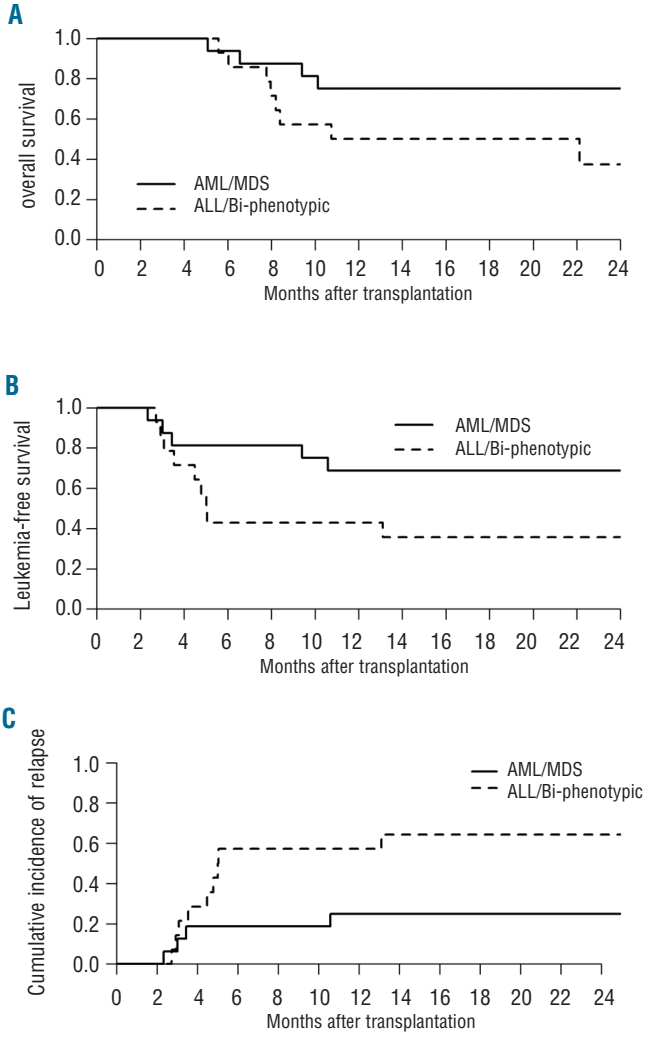

Figure 2. Comparison of outcomes between MDS/AML patients (n=16) and ALL/bi-phenotypic patients (n=14): Overall survival (A), leukemia-free survival (B) and relapse incidence (C).

50%.[10,28] In the current study, the relatively good results in MDS/AML are not explained by a higher incidence of chronic GVHD occurrence after transplant which is generally associated with a higher allogeneic GVL effect and higher survival rate in patients.[29] One may hypothesize that it is rather related to the stronger anti-leukemic activity of clofarabine in AML itself, as suggested by the EBMT survey[18] and the study by Mageneau *et al.*,[30] in which patients with AML with active disease at transplantation were more likely to achieve complete remission after allogeneic SCT than were patients with other diseases, especially ALL. Thus, it would be interesting in the future to confirm these results in a larger cohort and to compare the CloB2A2 regimen prospectively to the results of other widely used fludarabine-based RIC regimens (e.g. fludarabine, intravenous busulfan, antithymocyte globulin *versus* clofarabine, intravenous busulfan, antithymocyte globulin) in the particular setting of patients with MDS/AML in order to demonstrate its superiority.

Despite a very low non-relapse mortality rate, results for ALL/bi-phenotypic leukemia cases were within the range previously published in literature for older patients, with overall and leukemia-free survival rates of 38% and 36% at 2 years.[11,31-33] In the retrospective registry-based study by Mohty *et al.*, which included the largest number of ALL cases for RIC allogeneic SCT (n=127, median age 56 years),[11] the 2-year overall and leukemia-free survival rates were 48% and 32%, respectively (51% and 35% for patients in first complete remission and 33% and 20% for those in second complete remission). Although better results may be obtained in younger patients,[34,35] considering the prospective aspect of the trial and the high-risk and older population here, our results appear somewhat encouraging. Lymphoblasts may not be as sensitive as the malignant cells in AML/MDS to clofarabine. One of the reasons may be that, while the anti-apoptotic Bcl2 protein seems to play a crucial role in the survival of lymphoid cells,[36] Bcl2 over-expression itself confers resistance to clofarabine in the particular setting of ALL.[37] Thus, anti-Bcl2 therapy, such as ABT-737 may overcome the resistance to clofarabine in ALL patients,[37] and should be tested as part of the conditioning regimen.

One possible way of improving the results of the current CloB2A2 regimen for both myeloid and lymphoid acute leukemia could be to increase the dose of either clofarabine (given for 5 days rather than 4 days, for example), as is already the case when considering induction or salvage chemotherapy for patients with *de novo* or relapsed disease,[12-14] or busulfan, as has been tested recently by our group (the FB3A2 regimen),[38] or of both drugs (CloB3 or CloB4 regimens).[39,40] Another way would be to administer clofarabine and busulfan concomitantly since it has been demonstrated that there is a synergistic effect between the two drugs.[41] Another interesting perspective is to investigate the combination of clofarabine, fludarabine and busulfan in patients with acute leukemia, using pharmacokinetically-guided doses of busulfan in order to guarantee the safety of the procedure.[39,40]

In conclusion, this phase 2, prospective, multicenter trial showed that a CloB2A2 RIC/RTC regimen prior to allogeneic SCT in patients with high-risk MDS/leukemia is feasible, enabling full engraftment and very low toxicity. Disease control appeared to be satisfactory, especially in AML/MDS, warranting a prospective comparison with other widely used fludarabine-based RIC regimens.

Acknowledgments

We want to thank all participating SFGM-TC centers, local investigators and data managers and the DRC of Nantes. Our research activities are supported by educational grants from the "Association for Training, Education and Research in Hematology, Immunology and Transplantation" (ATERHIT). We also thank the "Région Pays de Loire", the "Association pour la Recherche sur le Cancer (ARC; grant #3175 to MM)", the "Fondation de France", the "Fondation contre la Leucémie", the "Agence de Biomédecine", the "Association Cent pour Sang la Vie", the "Association Laurette Fuguain", the IRGHET and the "Ligue contre le Cancer Grand-Ouest" for their generous and continuous support of our clinical and basic research work. Our group is supported by several grants from the French National Cancer Institute (PHRC, INCa to MM and PC).The authors would like to acknowledge the continuous support of the cell banking facility ("tumurotheque") of the CHU de Nantes.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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