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Busulfan and Fludarabine Conditioning Regimen Given at Hematological Nadir of Cytoreduction Fludarabine, Cytarabine, and Idarubicin Chemotherapy in Patients With Refractory Acute Myeloid Leukemia Undergoing Allogeneic Stem Cell Transplantation

A Single Arm Pilot Consort Study

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Abstract: To improve the outcome of allogeneic stem cell transplantation in refractory acute myeloid leukemia (AML), we conducted a single-arm phase II clinical trial to evaluate the efficacy and feasibility of conditioning regimen following cytoreduction chemotherapy with 7-day interval.

Adult patients with refractory AML were enrolled in the study and received fludarabine, cytarabine, and idarubicin (FLAG-IDA) as cytoreductive chemotherapy followed by busulfan and fludarabine (Flu-BU) conditioning regimen and transfusion of mobilized peripheral stem cells from human leukocyte antigen-matched sibling or unrelated donor. The primary endpoint of the study was 2-year leukemia-free survival (LFS) and secondary endpoints included complete-remission rate, 2-year overall survival (OS), nonrelapse mortality (NRM), and relapse rate.

A total of 16 patients were enrolled with median age of 36 (16–60), which included 9 primary induction failure, 2 early relapse, and 5 with relapse/refractory disease. The median cycles of previous chemotherapy were 4 (3–10) with a median of 55% (1%–90%) blasts in bone marrow. Six patients received transplantation from matched sibling and 10 from matched unrelated donors. After transplantation, 15 patients achieved bone marrow remission (11 complete remissions [CRs] and 4 bone marrow remissions without platelet recovery) at day +28. A total of 8 patients remained alive in CR with median LFS of 29.5 months (9.5–40.5 months). Four patients relapsed and 3 of them died of disease and another 4 patients died because of transplantation-related toxicity. The 2-year NRM and relapse rates were 25.0% ± 10.8% and 33.4% ± 13.8%, respectively with 2-year OS at 53.5% ± 13.1% and LFS at 50.0% ± 12.5%. Based on the Simon 2-stage design, 5 out of first eligible 14 patients remained leukemia-free for more than 2 years after allogeneic hematopoietic stem cell transplantation; thus, the null hypothesis of the study will be rejected and the study protocol is accepted as being warranted for further study.

Based on the above data, our phase II study demonstrated that the sequential FLAG-IDA cytoreduction chemotherapy followed by Flu-BU conditioning regimen given at the hematological nadir was feasible and has sufficient activity to warrant further investigation prospectively with a larger patient sample (clinicaltrials.gov identifier: NCT01 496547).

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Abbreviations: aGVHD = acute GVHD, allo-HSCT = allogeneic hematopoietic stem cell transplantation, AML = acute myeloid leukemia, ATG = antithymocyte globulin, cGVHD = chronic GVHD, CIMBTR = Center for International Blood and Marrow Transplant Research, CR = complete remission, Crp = bone marrow remission without platelet recovery, CsA = cyclosporine, DLI = donor lymphocytes infusion, FLAG-IDA = fludarabine, cytarabine and idarubicin, Flu-Bu = busulfan and fludarabine, GI = gastric-intestine, GVHD = graft-versus-host disease, GVL = graft-versus leukaemia, LFS = leukemia-free survival, MDS = myelodysplasia syndrome, MMF = mycophenolate mofetil, MNC = mononuclear cells, MSD = HLA-matched sibling donor, MTX = methotraxate, MUD = HLA-matched unrelated donor, NRM = nonrelapse mortality, OS = overall survival, PFS = progression-free survival, PIF = primary induction failure, PTLD = post-transplant lymphoproliferative disorder, RIC = reduced-intensity conditioning, TPN = total parenteral nutrition.

INTRODUCTION

Refractory acute myeloid leukemia (AML) remains as the most difficult clinical scenario. Though different therapeutic regimens were tested in clinical trials, the overall outcome remained poor.[1–3] Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment for refractory AML.[4,5] However, allogeneic transplantation with active leukemia failed to improve significantly the long-term outcome. Standard myeloablative conditioning regimen in these patients was associated with significant regimen-related mortality, whereas reduced intensity conditioning had less treatment-related complications, but was associated with extremely high relapse rates.[6–8] The Center for International Blood and Marrow Transplant Research (CIMBTR) reported 3-year overall survival (OS) rates of 19% for AML patients with

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relapse disease or primary induction failure receiving myeloablative regimen.[9] To further improve the outcome of allo-HSCT in such high-risk and refractory patients, sequential schedule of cytoreduction therapy followed by nonmyeloablative conditioning has been developed.[10–14] Schmid et al had introduced a sequential conditioning regimen consisting of chemotherapy FLASMA followed by reduced-intensity conditioning (RIC) and prophylactic donor lymphocytes infusion (DLI) for patients with refractory AML. In their primary report, the 2-year OS and leukemia-free survival (LFS) were 40% and 37%, respectively and patients with only 2 cycles of previous chemotherapy might particularly benefit from the procedure.[10] In another study using 5-day clofarabine as cytoreduction followed by initiation of conditioning during the nadir 14 days later for relapsed/refractory AML, 1-year progression-free survival (PFS), and OS were 25% and 38% respectively. Bone marrow biopsy 12 days after clofarabine treatment with effective cytoreduction was correlated with improved PFS.[11]

To further improve the outcome of patients with refractory leukemia, we developed a transplantation strategy consisting of sequential cytoreductive chemotherapy with fludarabine, cytarabine, and idarubicin (FLAG-IDA) followed by a reduced toxicity conditioning with busulfan and fludarabine (Flu-Bu) at the hematological nadir of chemotherapy-induced aplasia in refractory AML. The rationale is based on the significant anti-leukemia efficacy of FLAG-IDA regimen, which has been demonstrated in relapse or refractory AML, whereas the Flu-Bu regimen have been reported to be effective and less toxic as standard conditioning regimen in AML and myelodysplasia syndrome (MDS).[15,16] In this study, we tested the hypothesis that maximizes the leukemia burden reduction by adding intensive chemotherapy regimen FLAG-IDA followed by the Flu-Bu conditioning given at the aplasia phase after cytoreduction chemotherapy may translate into better disease control after allo-HSCT.

METHODS

Study Design

Between June 15, 2011 and Jan 15, 2014, a total of 16 patients with refractory AML received allogeneic stem cell transplantation from HLA-matched donors in Blood & Marrow Transplantation Center, Department of Hematology, Rui Jin Hospital. All these patients were enrolled in a prospective, single-arm phase II clinical trial to study the efficacy and feasibility of new transplantation protocol consisting of intensive cytoreductive chemotherapy followed by sequential conditioning regimen given with a 7-day interval. The study was performed in accordance with the Declaration of Helsinki and the ICH Guidelines for Good Clinical Practice. The protocol was approved by the Ethics Committee of Rui Jin Hospital and was registered at www.clinicaltrials.gov (clinicaltrials.gov identifier: NCT01496547).

Eligibility Criteria

Patients were included if they fulfilled the following criteria defining refractory AML[10,17]: primary induction failure (PIF) after 2 cycles of chemotherapy, first early relapse after a remission duration of <6 months, relapse disease refractory to salvage chemotherapy containing high-dose Ara-C. Further inclusion criteria were age between 16 and 60 years with available donor (HLA-matched family or unrelated stem cell donor with 8/8 or 10/10 HLA matching) and written informed consent. The exclusion criteria were creatinine clearance <50 mL/min, bilirubin or transaminases >3 times the upper limit of normal, cardiac shortening fraction <30% and pregnancy.

Treatment Protocol

Before the treatment, bone marrow aspiration was performed to determine the disease status in all patients before a cytoreductive chemotherapy consisting of fludarabine (30 mg/m^2), AraC (2 g/m^2), and G-CSF 5 μg/kg from day $-20 \sim -16$ and idarubicin 12 mg/m^2 from day $-16 \sim$ day -14 (FLAG-IDA). A bone marrow aspiration was performed then on day -7 to access the marrow cellularity and residual blasts. A reduced toxicity conditioning regimen was consisted of fludarabine (30 mg/m^2) from day $-6 \sim$ day -2 and busulfan 3.2 mg/kg from day $-5 \sim$ day -3 (Flu-Bu). G-CSF-mobilized peripheral stem cells were infused on day 0 in all patients (Figure 1). The graft-versus-host disease (GVHD) prophylaxis regimen included cyclosporine (CsA) 3 mg/kg from day -1 to day+60 with short methotraxate (MTX; 15 mg/m^2 on day +1 and 10 mg/m^2 day +3 and +6) and mycophenolate mofetil (MMF; 1000 mg/day) from day 0 to day +30 for all patients. Rabbit antithymocyte globulin (ATG, Thymoglobulin®, Sanofi/France) was given with a total dose of 6 mg/kg for unrelated donor from day $-4 \sim -1$. The tapering of CsA started at Day +61 if no acute (GVHD) was documented. The prophylactic DLI was not eligible in the study. DLI was allowed only in case of documented loss of donor chimerism by polymerase chain reaction analysis of short-tandem repeat markers, increased minimal residual disease via flowcytometry or hematological relapse during follow-up.

Toxicity Assessment

The electronic medical record was reviewed to grade toxicities according to NCI CTC Toxicity scale Version 2.0. aGVHD and chronic GVHD (cGVHD) were diagnosed and graded accordingly.[18,19]

Sample Size and Statistical Consideration

The primary endpoint of the study was 2-year LFS, whereas LFS was defined as being alive without relapse of leukemia. A 2-year LFS of 15% with the study protocol will be of no interest clinically because it is not superior to the conventional or standard transplantation protocol according to our own historical control data and available literature, whereas a 2-year LFS reaching 45% will be of interest for further study.[9–11] All patients enrolled in the study must be followed-up to the occurrence of event (relapse of leukemia or death from any cause) or at least 2 years after allo-HSCT without any event defined above.

The sample size of 14 patients was determined based on Simon 2-stage minimax design with type one error rate α at 0.05 and power at 0.8.[20] If no patient among the first 5 enrolled in the study maintained leukemia-free at 2 years after allo-HSCT, the trial will be stopped early for futility. At the end, if only ≤4 patients out of 14 enrolled patients in the study maintained leukemia-free at the landmark of 2 years after allo-HSCT, no further investigation of the study protocol is warranted. Otherwise, the FLAG-IDA/Flu-Bu protocol will be considered as being warranted for larger-scale clinical trial.

The secondary endpoints of study included complete remission rate (CR) on day 28 after allo-HSCT, 2-year nonrelapse mortality (NRM), 2-year relapse rate, and 2-year OS (the time from enrollment to death from any cause). The distribution of time-to-event endpoints such as LFS and OS

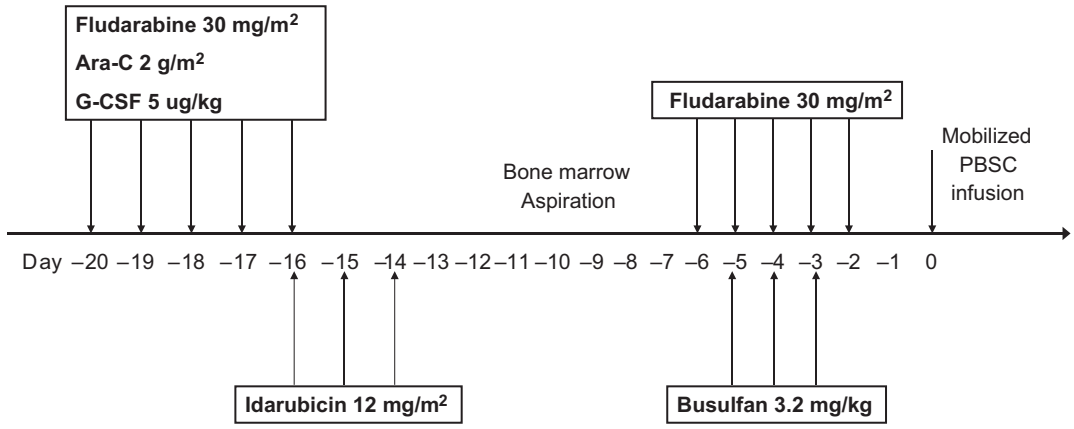

FIGURE 1. This figure illustrates the treatment scheme of the study protocol.

was estimated using the Kaplan–Meier method. Probability of NRM and relapse rate was calculated using reciprocal cumulative incidence estimates. Additionally, we compared the overall outcome of the study with our historical control series in terms of OS, LFS, NRM, and relapse rate. Log-rank test was used for the analysis with P values reported as 2-sided and <0.05 as statistical significance.[21] SPSS (SPSS Inc, Chicago, IL) software packages were used for data analysis.

RESULTS

Patients' Characteristics

Between June 15, 2011 and Jan 15, 2014, a total of consecutive 20 patients with refractory AML were referred to Blood & Marrow Transplantation Center and received allo-HSCT as salvage therapy. Among them, 4 patients without HLA-matched donor sibling (MSD) and unrelated donor (MUD, not available in 3 and refusal from donor in 1) received allo-HSCT from related haploidentical donors were excluded from the analysis. The remaining 16 patients with MSD ($n = 6$) or MUD ($n = 10$) were enrolled in the study. For those patients enrolled for MSD transplantation, no salvage chemotherapy was given, whereas for those patients for MUD transplantation, salvage chemotherapy was allowed during the work-up phase to MUD transplantation based on the decision of their primary hematologists.

Initially, 14 patients enrolled received cytoreductive chemotherapy and conditioning regimen on schedule except for 2 patients (UPN4 and 8) who experienced severe neutropenic fever with sepsis after FLAG-IDA chemotherapy, which lead to a 3-day delay of Flu-Bu conditioning delivery after successful antibiotics treatment and the day-7 bone marrow evaluation was not performed. Thus, 2 more patients were enrolled who received the FLAG-IDA chemotherapy, day-7 bone marrow assessment, and Flu-Bu conditioning on schedule. All these 16 patients completed the cytoreduction chemotherapy, conditioning regimen, and mobilized peripheral stem cells infusion sequentially. The data of first 14 patients enrolled were eligible for determination of either rejection or acceptance of the study null hypothesis in the statistical consideration, whereas data of all 16 patients were included for the analysis of overall transplantation outcome, toxicity, and comparison study to the historical control.

The patients' characteristics of all 16 patients are summarized in Table 1. The median age of patients was 36 (16–60). Among 16 patients enrolled, 9 had PIF, 2 early relapse and 5 had relapse/refractory disease. All these patients received at least 3 cycles of chemotherapy (median 4, range 3–10). Fifteen patients had active disease and only 1 patient (UPN 8) who failed to obtained CR after first 2 cycles of induction chemotherapy (idarabicin + cytarabine) with a 9/10 matched MUD donor identified was enrolled in the study. During the work-up period for MUD transplantation with delay for almost 2 months, the very patient obtained CR with 2 additional cycles of salvage chemotherapy (Fludarabine + cytarabine + G-CSF, FLAG). Since the patient insisted to undergo the study protocol, the allo-HSCT was performed accordingly. Overall, the median percentage of blasts in bone marrow before the FLAG-IDA chemotherapy was 55% (1%–90%). The graft contained a median of 7.15×10^8/kg mononuclear cells (MNC, range 3.8–12.7) and 6.45×10^6 CD34+/kg (range 2.26–22.0).

Transplantation Outcome

As to the cytoreduction, the bone marrow analysis on day -7 was evaluable in a total of 14 patients (except for UPN 4 and 8) and all demonstrated an extremely hypocellularity without any blasts ($n = 12$) or only few blasts (<5 in per bone marrow smear, $n = 2$).

As to the engraftment, 1 patient (UPN 4) died before engraftment, whereas the remaining 15 patients achieved neutrophil engraftment ($>0.5 \times 10^9$ cells/L) with median of 15 days (range 9–24). The engraftment of platelet ($>20 \times 10^9$ cells /L) occurred in 12 patients with a median of 19.5 days (range 10–30), whereas remaining 3 patients had poor platelet recovery dependent on platelet transfusions for at least 3 months after transplantation (Figure 2).

Among the 15 patients with evaluable chimerism data at day 28, all had complete donor chimersim (>99%) in unfractionated bone marrow nucleated cell compartments in which 11 patients achieved CR and 4 with bone marrow remission without platelet recovery (CRp, Table 2).

Up to the last follow-up at Nov 30, 2014, with a median follow-up of 10.5 months (0.5–40.5) for all patients, 4 patients died because of transplantation-related toxicity including infection before engraftment, post-transplant lymphoproliferative disorder (PTLD), sudden death, and pulmonary fungal infection respectively (as shown in Table 2). Accumulated 100-day NRM

TABLE 1. Patients' Characteristics

UPN	Sex	Age	Diagnosis	Donor	Disease Status	Cytogenetics/ Molecular	Blasts in BM Pre-Tx	Cycles of Prior Chemo	Cycles of HD-Ara-C/ FLAG
1	F	16	AML	MUD	PIF	46 XX	90%	4	1/0
2	F	38	AML	MUD	ERel	46 XX	18%	4	1/0
3	F	34	AML	MUD	PIF	46, XX	29%	4	0/0
4	F	25	AML	MUD	Ref/Rel	46, XX; HOX11 (+)	85%	3	1/1
5	F	39	AML	MUD	PIF	46, XX	25%	5	0/0
6	F	19	AML	Sib	Ref/Rel	NA	90%	6	1/0
7	F	37	AML	MUD	Ref/Rel	45, XX, −22	80%	4	1/2
8*	M	25	AML	MUD	PIF	47, XY, +der(6)	1%	4	0/2
9	M	22	AML	MUD	Ref/Rel	46 XY	18%	10	1/0
10	M	19	AML	MUD	Ref/Rel	NA	75%	5	1/0
11	F	22	AML	MUD	PIF	46, XX, inv(3) (q21q26), −7	80%	3	2/0
12	M	42	AML	Sib	PIF	46XY, ins(1;3) (p22;q26q21)	60%	6	1/0
13†	F	48	AML	Sib	PIF	46, XX, 6P−	35%	3	1/0
14	M	60	AML	Sib	ERel	46, XY	45%	3	0/0
15	M	44	AML	Sib	PIF	−5q, −7q, MLL+	85%	3	1/0
16	F	45	AML	Sib	PIF	45−46, XX, t(7;11)	8.5%	3	1/0

Blasts in BM Pre-Tx = bone marrow blast percentage before transplantation, Chemo = chemotherapy, E-rel = early relapse, F = female, FLAG = Fludarabine, Ara-C and G-CSF, HD-Ara-C = high-dose cytarabine, M = male, MUD = matched unrelated donor, MUD = matched unrelated donor, NA = not available, PIF = primary induction refractory, Ref/Rel = refractory relapsed patients, Sib = matched sibling donor.

* Patients failed to obtained remission after first 2 cycles of chemotherapy (IA) and obtained remission after additional 2 cycles of FLAG during the work-up for unrelated donor.

† Patients failed to obtained remission after 3 cycles of chemotherapy with active leukemia cutis when enrolled in the study.

and 2-year NRM were 18.7% ± 9.8% and 25.0% ± 10.8%, respectively.

A total of 4 patients relapsed 3.5, 4.5, 5.5, and 7 months, respectively after transplantation with 2-year accumulated relapse rate of 33.4% ± 13.8%. One patient (UPN3) declined any further treatment and 2 patients (UPN 7 and 9) failed to respond to rescue chemotherapy and all died of disease. Only 1 patient (UPN 14) achieved remission with salvage therapy with decitabine and low-dose chemotherapy followed by DLI and remained in remission up to last follow-up (as shown in Table 2).

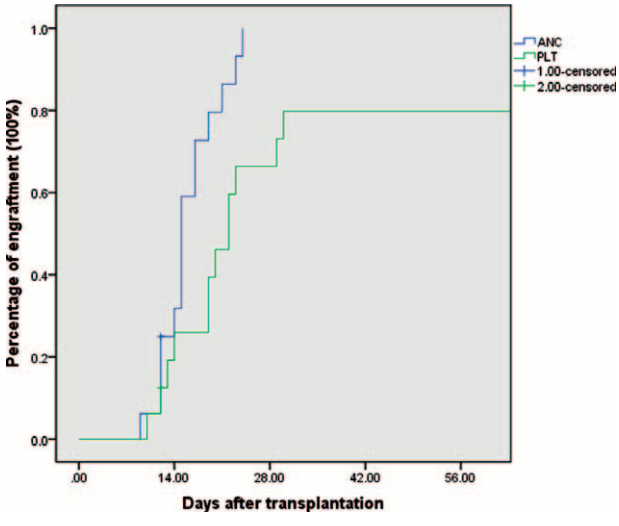

FIGURE 2. The probability of neutrophils and platelet engraftment after transplantation in 16 patients.

Overall, 8 patients remained alive without relapse for a median of 29.5(9.5−40.5) months and 6 of them already maintained leukemia-free for >2 years after allo-HSCT. Based on a median follow-up of 26.5 months for all alive patients, the estimated 2-year OS and LFS were 53.5% ± 13.1% and 50.0% ± 12.5%, respectively.

Transplantation Toxicity

The toxicities of this transplantation protocol were mainly hematological toxicities and toxicity of gastric intestinal tract as shown in Tables 3 and 4. All patients developed grade IV neutropenia and thrombocytopenia. The median duration of neutropenia was 24 days (18−36). All these patients experienced at least 1 episode of neutropenic fever (14 grade III and 2 grade IV) and septic shock (grade IV) was documented in 2 patients after FLAG-IDA chemotherapy causing a 3-day delay of conditioning regimen given. The gastric intestine (GI) toxicity such as anorexia, mucositis, and diarrhea occurred almost in all patients and total parenteral nutrition (TPN) support had to be given in 12 of 16 patients. Grade III GI bleeding was documented in 1 patient after conditioning regimen and recovered well after intensive supportive care. The liver toxicity was mild to moderate and only 1 veno-occlusion disease was documented (as shown in Tables 3 and 4).

As to the aGVHD, in 15 evaluable patients, 8 developed aGVHD in which grade II in 6 and no grade III-IV aGVHD occurred. For 12 evaluable patients for cGVHD, 3 patients had limited cGVHD and another 3 had experienced extensive cGVHD (as shown in Table 2).

Comparison with Historical Control

In the next analysis, we compared the transplantation outcome of patients enrolled in the clinical trial with our

TABLE 2. Clinical Outcome

UPN	Engraftment ANC	Engraftment Plt	BM D+28	Current Status	GVHD	DFS, Months	OS, Months	Event
1	15	30	CRp	Alive in CR	I aGVHD/limited cGVHD	39.5+	40.5+	/
2	12	14	CR	Alive in CR	II aGVHD/no cGVHD	37.0+	38.0+	/
3	23	/	CRp	Death—relapse	No GVHD	6.0	10.5	Relapse 7 months after Tx
4	/	/	NE	Death	/	0.0	0.5	Sepsis
5	15	29	CR	Alive in CR	No GVHD	34.5+	35.5+	/
6	9	13	CR	Alive in CR	No aGVHD/Ext cGVHD	31.5+	32.5+	/
7	17	22	CR	Death—relapse	II aGVHD/limited cGVHD	4.5	8.0	Relapse 5.5 months after Tx
8	15	23	CR	Alive in CR	II aGVHD/limited cGVHD	25.5+	26.5+	/
9	12	12	CR	Death—relapse	No aGVHD/no cGVHD	2.5	11.0	Relapse 3.5 months after Tx
10	14	20	CR	Alive in CR	II aGVHD/ext cGVHD	23.0+	24.0+	/
11	12	10	CR	Death—remission	II aGVHD/–	2.5	3.5	PTLD
12	15	19	CR	Death—remission	No aGVHD/–	1.5	2.5	Sudden death
13	17	22	CR	Alive in CR	Ext cGVHD	11.0+	12.0+	/
14*	19	19	CR	Alive in remission after relapse	no GVHD	3.5	10.0+	Relapse 4.5 months after Tx
15	24	/	CRp	Death—remission	II aGVHD/no cGVHD	4.5	5.5	Pulmonary fungal infection
16	21	/	CRp	Alive in CR	I aGVHD/no cGVHD	8.5+	9.5+	/

ANC = Absolute neutrophil counts, CR = complete remission, CRp = bone marrow remission without platelet recovery, NE = not evaluable, Plt = platelet, PTLD = post-transplant lymphoproliferative disorder.

* Remission after chemotherapy (decitabine + low-dose Ara-C + Aclamycin) followed by DLI.

TABLE 3. Major Toxicities

		GI Toxicity					Liver Toxicity				Neutropenia	
UPN	Fatigue	Anorexia	Mucositis	GI Bleeding	Diarrhea	TPN Requirement	Bil	SGPT	SGOT	GGT	Days of Neutropenia	Neutropenic Infection
1	4	4	3	3	1	+	2	0	0	1	24	3
2	2	2	1	0	1	–	0	0	0	0	21	3
3	3	2	2	0	1	+	1	1	0	1	35	3
4	4	4	2	0	3	+	2	0	1	2	22	4
5	3	3	2	1	3	+	0	0	0	1	26	3
6	2	2	3	0	1	+	1	0	0	0	24	3
7	2	3	1	0	2	–	0	1	0	2	23	3
8	4	3	1	1	2	+	1	0	1	1	26	4
9	3	1	2	0	2	+	1	1	0	1	18	3
10	2	1	1	0	1	–	2	2	1	2	19	3
11	3	3	2	0	2	+	0	0	0	1	22	3
12	2	2	2	0	1	+	3	4	4	2	36	3
13	3	3	2	0	1	+	1	1	1	1	29	3
14	3	1	1	0	0	–	0	1	1	1	27	3
15	3	2	2	0	1	+	1	2	1	1	22	3
16	4	3	2	0	1	+	0	1	1	1	35	3

Bil = bilirubin, GGT = glutamyl transpeptidase, GI = gastric-intestine, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic-pyruvic transaminase, TPN = total parenteral nutrition.

TABLE 4. Overall Toxicity Grading

	Any, n (%)	Grade III, n (%)	Grade IV, n (%)
Fatigue	16 (100)	7 (43.8)	4 (25)
GI toxicity			
Anorexia	16 (100)	6 (37.5)	2 (12.5)
Mucositis	16 (100)	2 (12.5)	0 (0)
GI bleeding	3 (18.8)	1 (6.3)	0 (0)
Diarrhea	15 (93.8)	2 (12.5)	0 (0)
Liver toxicity			
Bilirubin	10 (62.5)	1 (6.3)	0 (0)
SGPT	9 (56.3)	0 (0)	1 (6.3)
SGOT	8 (50)	0 (0)	1 (6.3)
GGT	14 (87.5)	0 (0)	0 (0)
Hematological toxicity			
Neutrophils	16 (100)	0 (0)	16 (100)
Platelets	16 (100)	0 (0)	16 (100)
Neutropenic infection	16 (100)	14 (87.5)	2 (12.5)

GGT = glutamyl transpeptidase, GI = gastric-intestine, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic-pyruvic transaminase.

historical control series of 26 patients with refractory AML received allogeneic transplantation from Jan 1st, 2000 to May 30, 2011 in our Blood and Marrow Transplantation Center (patient's characteristics as show in Table 5). When compared to the study group, the historical control group had significant lower bone marrow blasts before transplantation and all other clinical features were not significantly different between 2 groups. As to the transplantation outcome, most patients in the historical control group relapsed or died of transplantation toxicity within 6 months with only 3 patients remained disease-free 6 months after transplantation and 2 remained alive in continuous remission for >3 years up to the last follow-up. When compared with the historical control group, the outcome of patients enrolled in the study group was significantly improved in relapse rate (vs $81.2\% \pm 9.1\%$, $P = 0.002$), LFS (vs $11.1\% \pm 6.0\%$, median 3.7 months, $P = 0.01$), and OS (vs. $11.19\% \pm 6.0\%$, median 4.5 months, $P = 0.002$), whereas the NRM was not significantly different (vs $41.9\% \pm 14.8\%$, $P = 0.5$) as shown in Figure 3.

Landmark Analysis

Accordingly to the statistic consideration, patient (UPN 8) was excluded from analysis for statistical hypothesis rejection or acceptance because a bone marrow remission was documented before the FLAG-IDA chemotherapy. Among first 14 patients (UPN 1–15, UPN 8 excluded) enrolled in the study, 8 patients relapsed or died from either relapse or transplantation-related toxicity within 2 years after allo-HSCT. One patient (UPN 13) remained leukemia-free up to the last follow-up but did not complete the 2-year landmark. Importantly, the remaining 5 patients completed the designed follow-up for at least 2 years after allo-HSCT without relapse or other lethal event, thus based on the statistical analysis, the null hypothesis of the trial is rejected and the FLAG-IDA/Flu-Bu transplantation protocol is considered being possible to result a potential 2-year LFS around 45%, which is of clinical interest and required confirmation in further large-scale clinical trial.

TABLE 5. Patients' Characteristics for Patients in Historical Control Group Compared With Study Group

	Historical Control	Study Group	*P*
Age, median (range)	35 (18–51)	36 (16–60)	0.78
Sex			0.20
Male	15	6	
Female	11	10	
Disease status:			0.43
Primary-refractory	10	9	
Early-relapse	7	2	
Relapse-refractory	9	5	
Cycles of chemotherapy before transplantation	4 (2–12)	4 (3–10)	0.90
Bone marrow blasts (%): median (range)	19 (1–90)	55 (1–90)	0.02
Blasts <5%	6	1	0.15
Blasts ≥5%	20	15	
Donor			0.30
HLA-matched sibling	14	6	
HLA-matched unrelated	12	10	
Conditioning-regimen			<0.001
Bu-Cy ± VP-16	19	0	
Cy-TBI ± VP16	3	0	
Fludarabine-Bu ± Ara-C	4	0	
FLAG-IDA/Flu-Bu	0	16	
Graft			0.43
Bone marrow	1	0	
Mobilized peripheral blood stem cells	25	16	

FLAG-IDA = fludarabine, cytarabine, and idarubicin, Flu-Bu = busulfan and fludarabine, HLA = human leukocyte antigen.

DISCUSSION

Treatment options for adult patients with refractory AML are extremely limited.[1,2,22] Although HSCT is generally the only curative option, active disease before HSCT leads to limited survival.[4–8] The reported long-term survival in young patients undergoing fully myeloablative regimens approaches 25% at 3 years and <10% with RIC regimens at 5 years.[23,24] One strategy to improve the outcomes is to give preconditioning cytoreduction therapy with active chemotherapy regimen and the feasibility of this strategy has been shown prospectively in refractory AML patients with active disease.[10–13]

Several lines of evidence demonstrated that lower leukemia burden before transplantation was prognostic factor for achievement of CR and improvement of long-term outcomes after allo-HSCT. In an EBMT Registry, analysis of 168 primary refractory AML underwent unrelated donor transplantation, a lower percentage of bone marrow blasts at transplant was associated with improved survival.[25] In a retrospective study, 17 patients with active refractory AML and extensive previous therapy received clofarabine as cytoreduction chemotherapy followed by 14–21 days conditioning regimen with fludarabine, alemtuzumab, and melphalan (Flu/Mel/Alem) or busulfan (Flu/BU/Alem). The effective cytoreduction after clofarabine at the hematological nadir in terms of blast clearance (blasts <10% in bone marrow) was important prognostic factor. The PFS was 6.4 months for patients who achieved cytoreduction after initial induction, compared with 3.8 months ($P = .035$) for those who did not. OS was

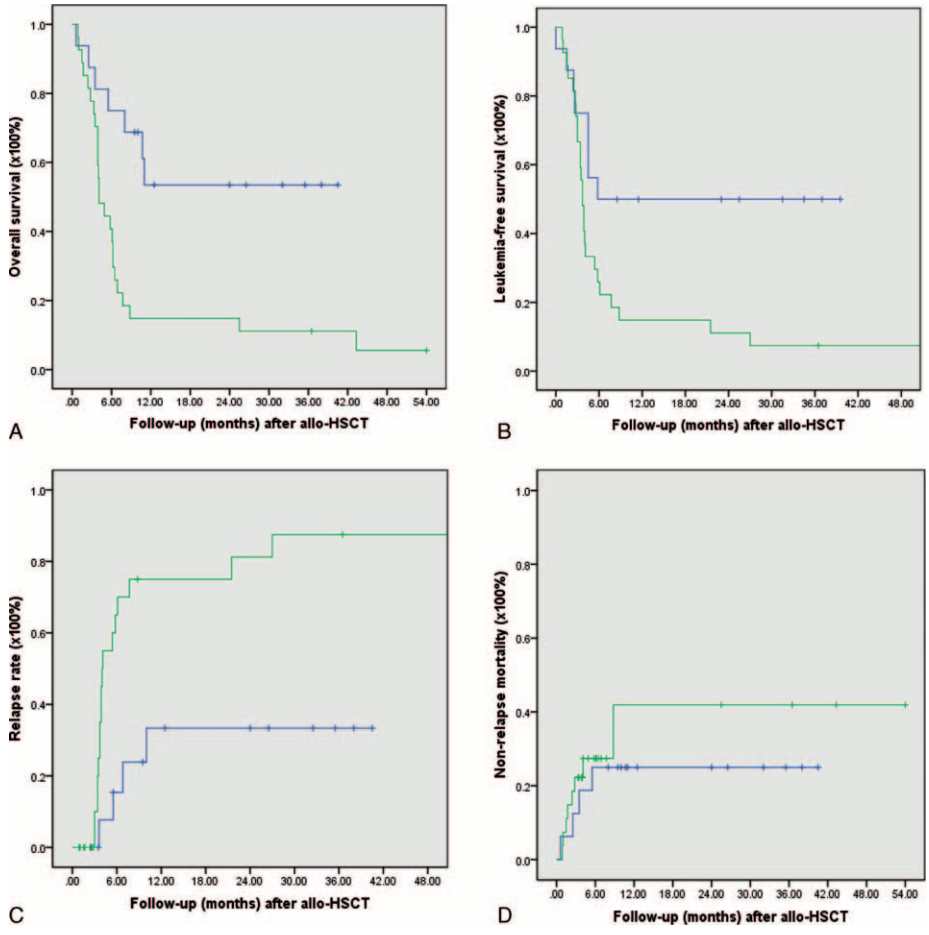

FIGURE 3. (A) The 2-year overall survival of patients in study group (53.5% ± 13.1%) compared with historical control (11.1% ± 6.0%, $P = 0.002$). (B) The 2-year leukemia-free survival of patients in study group (50.0% ± 12.5%) compared with historical control (11.1% ± 6.0%, $P = 0.01$). (C) The 2-year relapse rate of patients in study group (33.4% ± 13.8%) compared with historical control (81.2% ± 9.1%, $P = 0.002$). (D) The 2-year nonrelapse mortality in study group (25.0% ± 10.8%) compared with historical control (40.9% ± 14.8%, $P = 0.50$).

16.6 months for patients who achieved cytoreduction compared with 5.1 months ($P = 0.053$) for patients who did not.[11]

In our historical control series with conventional conditioning regimen such as Bu-Cy ± Vp16 or Cy-TBI ± Vp16, although remission can be achieved after allo-HSCT in these patients with refractory AML with median LFS of 3.7 months and OS of 4.5 months which was comparable to the group without success cytoreduction by clofarabine in the study reported by Locke et al.[11] Most patients relapsed rapidly within 6 months (70.0% ± 10.2% as shown in Figure 3C) after transplantation, which was before the development of potential allogeneic graft-versus leukemia (GVL) effect, which is more likely associated with cGVHD and believed to have greater impact on the long-term disease control.[10,26–28]

In this prospective study, the goal was to evaluate whether the new strategy to give conditioning regimen at the nadir of cytoreductive chemotherapy could result in significantly better disease control after transplantation in refractory AML. The enrolled patients with primary or relapsed/refractory AML were heavily pre-treated with extreme high risk because of a median marrow blast of 55%. Of note, 7 days after FLAG-IDA chemotherapy, almost all evaluable patients achieved an extreme hypocellular bone marrow with significant clearance of blasts. Moreover, 8 of 16 patients remained in CR at last follow-up with a median follow-up of 29.5 months (9.5–40.5) without prophylactic DLI. Actually 5 of them remained leukemia-free for >2 years (including 2 for >3 years). Interestingly, 7 of 8 patients who remained alive in remission had either aGVHD or cGVHD. Based on these observations, we speculated that for refractory AML the sequential administration of Flu-Bu conditioning at the aplasia phase of intensive FLAG-IDA cytoreductive chemotherapy, which significantly depleted the leukemia burden, may lead to improved early disease control (7 of 15 evaluable patients remained in CR at the first 6 months after allo-HSCT). This benefit can be possibly enhanced or maintained by the subsequent GVL effect associated with aGVHD or cGVHD and eventually translates into better long-term disease control.[10]

The major challenge of this protocol was the toxicity such as prolonged myelo-suppression and GI toxicity associated with intensive chemotherapy and conditioning regimen given with a short interval. Indeed, we observed significant myelosuppression, which leads to prolonged neutropenia and increased risk of infection in which all patients had at least 1 episode of neutropenic fever, including 2 patients with septic shock. Aggressive supportive care such as transfusion blood products and

TPN were required in most patients. Fortunately, the overall accumulated NRM of patients following HCT was comparable to the historical control.

Overall, we present the results of phase II trial of conditioning regimen Flu-Bu sequentially given at the hematological nadir of previous cytoreductive chemotherapy. FLAG-IDA was feasible and can be considered as effective salvage treatment for patients with refractory AML. The small number of patients studied in the trial precludes any definitive conclusions, but this strategy has sufficient activity to warrant further investigation prospectively with a larger patient sample.

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