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Clofarabine ± Fludarabine with Once Daily i.v. Busulfan as Pretransplant Conditioning Therapy for Advanced Myeloid Leukemia and MDS

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Although a combination of i.v. busulfan (Bu) and fludarabine (Flu) is a safe, reduced-toxicity conditioning program for acute myelogenous leukemia/myelodysplastic syndromes (AML/MDS), recurrent leukemia post-transplantation remains a problem. To enhance the conditioning regimen's antileukemic effect, we decided to supplant Flu with clofarabine (Clo), and assayed the interactions of these nucleoside analogs alone and in combination with Bu in Bu-resistant human cell lines in vitro. We found pronounced synergy between each nucleoside and the alkylator but even more enhanced cytotoxic synergy when the nucleoside analogs were combined prior to exposing the cells to Bu. We then designed a 4-arm clinical trial in patients with myeloid leukemia undergoing allogeneic stem cell transplantation (allo-SCT). Patients were adaptively randomized as follows: Arm I–Clo:Flu 10:30 mg/m^2, Arm II—20:20 mg/m^2, Arm III—30:10 mg/m^2, and Arm IV–single-agent Clo at 40 mg/m^2. The nucleoside analog(s) were/was infused over 1 hour once daily for 4 days, followed on each day by Bu, infused over 3 hours to a pharmacokinetically targeted daily area under the curve (AUC) of 6000 μMol-min ± 10%. Fifty-one patients have been enrolled with a minimum follow-up exceeding 100 days. There were 32 males and 19 females, with a median age of 45 years (range: 6-59). Nine patients had chronic myeloid leukemia (CML) (BC: 2, second AP: 3, and tyrosine-kinase inhibitor refractory first chronic phase [CP]: 4). Forty-two patients had AML: 14 were induction failures, 8 in first chemotherapy-refractory relapse, 7 in untreated relapse, 3 in second or subsequent relapse, 4 were in second complete remission (CR), and 3 in second CR without platelet recovery (CRp), 2 were in high-risk CR1. Finally, 1 patient was in first CRp. Graft-versus-host disease (GVHD) prophylaxis was tacrolimus and mini-methorexate (MTX), and those who had an unrelated or 1 antigen-mismatched donor received low-dose rabbit-ATG (Thymoglobulin™). All patients engrafted. Forty-one patients had active leukemia at the time of transplant, and 35 achieved CR (85%). Twenty of the 42 AML patients and 5 of 9 CML patients are alive with a projected median overall survival (OS) of 23 months. Marrow and blood (T cell) chimerism studies at day +100 revealed that both in the lower-dose Clo groups (groups 1+2) and the higher-dose Clo groups (groups 3+4), the patients had a median of 100% donor (T cell)-derived DNA. There has been no secondary graft failure. In the first 100 days, 1 patient died of pneumonia, and 1 of liver GVHD. We conclude that (1) Clo ± Flu with i.v. Bu as pretransplant conditioning is safe in high-risk myeloid leukemia patients; (2) clofarabine is sufficiently immunosuppressive to support allo-SCT in myeloid leukemia; and (3) the median OS of 23 months in this high-risk patient population is encouraging. Additional studies to evaluate the antileukemic efficacy of Clo ± Flu with i.v. Bu as pretransplant conditioning therapy are warranted.

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INTRODUCTION

The combination of intravenous (i.v.) busulfan (i.v. Bu) with a nucleoside analog, fludarabine (Flu), rather than a second alkylating agent, was introduced as a myeloablative, reduced-toxicity pretransplant conditioning platform for patients with hematologic malignancies. This new regimen contributed to greatly increased safety of the allogeneic stem cell transplant (allo-SCT) procedure [1-10]. The low treatment-related mortality (TRM) demonstrated by these investigators is, at least in part, because of highly reproducible intra- and interpatient systemic Bu exposure [11-14], but also to the notable absence of a need for GSH in Flu metabolism and nonoverlapping end-organ toxicities between Flu and Bu. The available safety and efficacy data favor such a combination over that of double alkylating agent regimens such as Bu-cyclophosphamide (Cy) [8], or Bu-melphalan (Mel) [15-17], and also compare favorably with triple-alkylator regimens [18]. Some investigators expressed concerns about the antileukemic efficacy of Bu-Flu compared with BuCy2, particularly in patients with active leukemia at the time of transplantation [10,19,20], and although the remission (CR) rate of patients transplanted with active disease exceeds 80%, relapses start occurring within 2 months after transplant, yielding insufficient time for establishing a new immune system to exert its graft-vs-leukemia effect [10,19-22]. We decided to explore a later generation nucleoside analog, clofarabine (Clo) [23-28], in pretransplant conditioning therapy. When used in conventional acute leukemia therapy, Clo has demonstrated greatly improved antileukemic efficacy. Unfortunately, nothing was known about the cytotoxic interactions of Clo with Bu. In anticipation of supplanting Flu with Clo in pretransplant conditioning therapy, we investigated the in vitro antileukemic cytotoxic properties of Clo and Flu alone and combined with Bu in a human cell line model of Bu-resistant acute myelogenous leukemia (AML) [29]. In this model, as well as in additional human AML lines and primary explanted AML blasts from leukemic patients, we demonstrated that on a molar basis, Clo is about 50-fold more potent than Flu, and Clo synergizes to a higher degree than Flu with Bu. A combination of Clo and Flu exerted a much higher level of synergy than either nucleoside analog alone when combined with the alkylating agent. When Flu and Clo were followed by Bu, a major enhancement of the antileukemic efficacy was seen [30].

Further, although the immunosuppressive and engraftment-promoting capabilities of Flu are well documented [5,7,31], the extent to which Clo would support allogeneic progenitor cell engraftment is unknown. Hence, based on our in vitro data showing the greatest synergy when 2 nucleoside analogs were followed by Bu, and the lack of immunosuppressive information for Clo, we decided to investigate supplementing Flu with Clo in 4 different proportions, each modified from our standard 4-day Bu-Flu regimen [7], while continuously assessing donor-cell chimerism and disease control (being alive in CR) on day SCT +30. Our protocol utilized adaptive randomization based on a Bayesian continuous reassessment method [32-34], which takes into account the chimerism status, serious toxicity(-ies), and overall clinical result (alive and in CR) experienced in the first 30 days after allo–SCT. In this report, we present the translational in vitro background results and rationale for the clinical allo-SCT trial, together with early safety and efficacy data obtained with this second-generation myeloablative, reduced-toxicity [Clo ± Flu + i.v. Bu] regimen followed by allo–SCT. We will discuss clinical data obtained in 51 patients with advanced, largely chemotherapy-refractory, myeloid leukemia and myelodysplastic syndrome (MDS) that were treated on this program. Our analysis supports the incorporation of Clo together with Flu and i.v. Bu ± antithymocyte globulin (ATG) for patients with high-risk myeloid leukemia. Interestingly, both the in vitro data and the early clinical adaptive randomization results favor a combination of low-dose Flu together with a higher dose Clo-i.v. Bu to maximize benefit and without appreciable loss of the clinical safety, as opposed to simply replacing Flu with Clo in this second-generation reduced-toxicity conditioning regimen.

MATERIALS, METHODS, AND PATIENTS

Cell Line and Drugs for In Vitro Studies

KBM3/Bu250[6] is a Bu-resistant cell line derived from the human KBM3 AML cell line [29,35]. The cells were cultured in RPMI 1640 medium (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA) at 37°C in a humidified atmosphere of 5% CO_2 in air. Stock solutions of 3.3 mM Clo (Clolar®, Genzyme Corporation, Cambridge, MA) and 10 mM Flu (Sigma-Aldrich, St. Louis, MO) in dimethyl sulfoxide (DMSO) were stored at room temperature and −20°C, respectively, and diluted with cell culture medium immediately prior to use. Busulfan (Sigma-Aldrich) was freshly dissolved in DMSO immediately prior to cellular drug exposure(s).

Cytotoxicity Assay and Graphical Analysis

Cell suspensions were aliquoted (2×10^5 cells/well) into 96-well plates in the presence of drug(s) or

solvent alone (total volume of 100 μL/well), incubated as above at 37°C for 4 days, and analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [36]. Graphical analyses including calculations of IC_{50} values (the concentration of drug required for 50% growth inhibition) were done using Prism 5 (GraphPad Software, San Diego, CA). The combination effects of Bu and Clo were estimated based on the combination index (CI) values [37] calculated using the CalcuSyn software (Biosoft, Ferguson, MO). This program was developed based on the median-effect method: CI < 1.0 indicates synergy, CI = 1.0 is additive, and CI > 1.0 suggests antagonism.

Fluorescence-Activated Cell Sorter (FACS) Analysis

Cells (5×10^5 cells/mL) in RPMI medium were exposed to drugs at 37°C for 48 hours. Following drug or solvent exposure, the cells were centrifuged, resuspended in 70% ethanol in PBS, and fixed at −20°C overnight. Fixed cells were pelleted at 3000 × *g* at room temperature, washed with phosphate-buffered saline (PBS), and treated with 0.25 mL of 500 U/ml RNAse A in PBS containing 1.12% sodium citrate at 37°C for 30 minutes. After addition of 0.25 mL propidium iodide (PI, 50 μg/mL), the cells were kept in subdued light for at least 1 hour prior to FACS analysis. The DNA content of at least 10,000 cells was analyzed using BD FACSCalibur and CellQuest™ software (Becton Dickinson, Franklin Lakes, NJ).

Western Blot Analysis

Cells were collected by centrifugation, washed with PBS, and lysed with cell lysis buffer (Cell Signaling Technology, Danvers, MA) as recommended by the manufacturer. Total protein concentrations in cell lysates were determined using a BCA Protein Assay kit (Thermo Fisher Scientific, Rockford, IL). Western blot analysis was done by separating protein extracts on polyacrylamide-SDS gels and electrotransferring onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Immunoblot analysis by chemiluminescence was done using the SuperSignal West Pico/Dura Substrate (Thermo Fisher, Waltham, MA) or the Immobilon Western Chemiluminescent HRP Substrate (Millipore, Bedford, MA). Antibodies and their sources include phosphorylated histone 2AX (γ-H2AX; Millipore), PARP1 (Santa Cruz Biotech, Inc., Santa Cruz, CA), and β-actin (Sigma-Aldrich). The β-actin protein was used as an internal control.

Pretransplant Conditioning Program

The treatment program consisted of Flu (Fludara®, Genzyme Corporation, Cambridge, MA) given over 60 minutes daily for 4 days (days −6 to −3), each dose was followed by Clo (Clolar®, Genzyme Corp.), also given over 60 minutes daily for 4 days and then by i.v. Bu (IV Busulfex® [busulfan] Injection, Otsuka America Pharmaceuticals Inc., Princeton, NJ), over 3 hours once daily for 4 days at a dose calculated to target an average daily systemic exposure dose, represented by the area under the concentration versus time curve (AUC) of 6,000 μMol-min ±10%, or total course AUC of 24,000 μMol-min over 4 days. The Bu dose calculation was based on PK parameters derived from a Bu test dose of 32 mg/m^2 administered 48 hours before start of the therapeutic conditioning program (day −8). Our in vitro data suggested that [Clo + Flu] combination would greatly enhance the synergistic cytoreductive effects compared with individual nucleoside analog when combined with i.v. Bu, and the clinical study was designed as a randomized 4-arm trial:

Arm I : Flu 30 mg/m^2/day + Clo 10 mg/m^2/day

Arm II : Flu 20 mg/m^2/day + Clo 20 mg/m^2/day

Arm III : Flu 10 mg/m^2/day + Clo 30 mg/m^2/day

Arm IV : Clo alone at 40 mg/m^2/day

Patients who had a 1-antigen-mismatched related donor or a matched unrelated donor received antithymocyte globulin (rabbit, rATG) (Thymoglobulin®, Genzyme Corp.), 0.5 mg/kg on day −3, 1.5 mg/kg on day −2, and 2.0 mg/kg i.v. on day −1 for a total dose of 4 mg/kg. The progenitor cell preparation was infused on day 0. Graft-versus-host disease (GVHD) prophylaxis was based on tacrolimus and minidose methotrexate (MTX) [7,38].

Patient Eligibility

AML patients should have failed initial induction chemotherapy, or have high-risk disease in first complete remission (CR1), characterized by cytogenetics (CG) other than translocation (t) (8;21), inversion (inv) 16, or t(15;17), and/or by the need for more than 1 cycle of chemotherapy to achieve CR [39]. Patients who were refractory to induction chemotherapy or whose disease was beyond CR1 were also eligible, and were prioritized to include those with active leukemia at the time of transplantation. Subjects with MDS were eligible if they had an International Prognostic Score System (IPSS) score of ≥2 [40], or if they progressed after previous chemotherapy. Patients with advanced chronic myeloid leukemia (CML) must have completed tyrosine-kinase inhibitor-based (TKI) therapy at least 10 days prior to the start of

conditioning treatment to avoid a serious hepatic interaction with Bu metabolism (B.S. Andersson, unpublished data).

The eligibility criteria included acceptable renal (creatinine ≤1.5 mg%) and hepatic function with normal bilirubin, SGPT ≤3 times the upper normal limit, a ZUBROD performance status ≤2, negative serology for hepatitis B and C, and HIV, LVEF ≥45%, FEV1, FVC, and DLCO ≥50% of predicted, absence of uncontrolled infection, and no chemotherapy within 30 days of study entry. A human leukocyte antigen-(HLA) compatible related (fully matched or 1 antigen mismatched), or a matched unrelated donor (MUD), assessed with high-resolution DNA typing, was required. All patients signed informed consent according to institutional guidelines. Clinical serious adverse events were assessed according to the NIH Common Terminology Criteria v3.0 [41], and engraftment was documented with DNA chimerism from blood and marrow using polymerase chain reaction (PCR)-based technology [8].

Treatment Groups and Adaptive Randomization

The adaptive randomization schedule began with an initial phase where the first 20 patients were fairly randomized to each of the 4 treatment arms. After this phase, the outcome parameters (1) degree of chimerism (>90% donor), and (2) being alive in CR on day SCT +30 determined the adaptive randomization probabilities. If a treatment arm were to be closed early, the remaining arms(s) would stay open until the targeted total number of patients were accrued.

RESULTS

Sensitivity of KBM3/Bu250^6 Cells to Nucleoside Analogs and Synergistic Effects of Clofarabine, Fludarabine, and Busulfan

The cytotoxicity of Clo and Flu to leukemic cells has been reported [30,42]. We sought to determine how these drugs would affect the survival of KBM3/Bu250^6 AML cells. Drug exposure for 4 days and MTT assay indicated IC_{50} values of 0.06 μM Clo and 3.0 μM Flu (data not shown), suggesting an approximately 50-fold difference in their cytotoxic activity on a molar basis. The higher activity of Clo prompted us to examine its possible synergistic effects in combination with Flu and Bu on the survival of Bu–resistant KBM3/Bu250^6 cells. Exposure of cells to individual drug at concentrations corresponding to their IC_{20} values for 48 hours, to combinations of 20 μg/mL Bu + 0.015 μM Clo, or to 20 μg/mL Bu + 0.6 μM Flu resulted in marginal proportion of cells with sub-G1 DNA content (Figure 1A) or cells positive to TUNEL assay (Figure 1B). The synergistic cytotoxicity of 0.015 μM Clo and 0.6 μM Flu is shown by an increase to 22% and 42% cells with sub-G1 DNA content and TUNEL-positive, respectively (Figure 1A and B). These effects of the [Clo + Flu] combination were further enhanced when 20 μg/mL Bu was added into the mixture and resulted in 40% cells in sub-G1 and 65% cells positive to TUNEL assay (Figure 1A and B), suggesting an increase in the proportion of cells undergoing apoptosis. These results are confirmed by immunoblot analysis, which shows increased phosphorylation

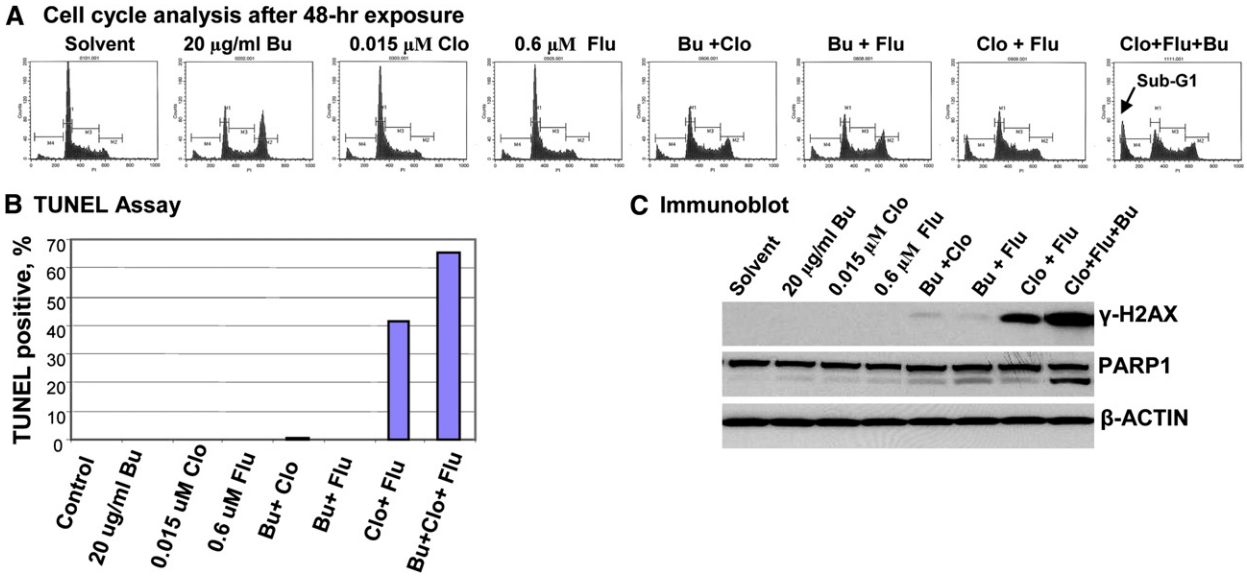

Figure 1. The synergistic cytotoxicity of 0.015 μM Clo and 0.6 μM Flu is shown by an increase to 22% and 42% cells with sub-G1 DNA content and TUNEL-positive, respectively (Figure 1A and B). These effects of the [Clo + Flu] combination were further enhanced when 20 μg/mL Bu was added into the mixture and resulted in 40% cells in sub-G1 and 65% cells positive to TUNEL assay (Figure 1A and B), suggesting an increase in the proportion of cells undergoing apoptosis.

of histone 2AX and cleavage of PARP1 protein (Figure 1C) when cells were exposed to the triple [Clo + Flu + Bu] combination for 48 hours, suggesting DNA-damage response and induction of apoptosis. The possibility of a synergistic cytotoxicity of combined [Clo + Flu + Bu] was further indicated by analysis using the median-effect method [37]. Analysis of cell survival data shows combination indices (CI) much lower than 1.0, signifying strong synergism between the agents in these concentration intervals (data not shown).

Clinical Treatment Results

Patients and disease characteristics

There were 32 males and 19 females, with a median age of 45 years (range: 6-59). Nine patients had CML (BC: 2, second AP: 3, and TKI-refractory first CP: 4), and 42 patients had AML: 14 were induction failures, 8 in first chemotherapy-refractory relapse, 7 in untreated relapse, 3 in second or subsequent relapse, 4 were in CR2, and 3 in second CRp. Two subjects were in high-risk CR1 (high-risk CG, and 1 of these had achieved CR after salvage treatment after failing primary induction chemotherapy), and 1 patient was in CR1p. Twenty-one of the 42 AML patients had an unfavorable CG risk pattern, 20 had intermediate CG, and data were unavailable for 1 patient.

Engraftment and chimerism

All 51 patients engrafted at a median time of 12 days (range: 10-22) for neutrophils and a median of 15 days (range: 8-53) for a platelet count of 20×10^9 per Liter. Marrow and peripheral blood myeloid- and T cell chimerism studies around days SCT +30 and +100 revealed that both Arms I + II (lower doses of Clo, n = 17) and Arms III + IV (higher doses of Clo, n = 34) groups had a median of 100% donor-derived DNA. There has been no late (secondary) graft failure.

Disease Response, Overall and Event-Free/ Progression-Free Survival (OS, EFS, PFS)

Forty-one patients had active leukemia at the time of transplant, and 35 achieved CR (85%). Thirty-two of 51 patients are alive with a projected median OS of 23 months (range: 4-39). Seventeen of the 32 patients transplanted with active AML are in continuous CR at a median follow-up of 14 months (range: 4-39). The adaptive randomization based on 30-day chimerism and 30-day survival in CR favored the higher doses of Clo, with Arm III (Flu 10 mg/m^2 and Clo 30 mg/m^2), being allocated 22 patients, whereas Arm IV (Clo 40 mg/m^2 only) received 12 patients. Arms I and II, which had lower doses of Clo, were allocated 10 and 7 patients, respectively. The 2-year OS and PFS are 48% and 41%, respectively, with a median OS of 23 months (Figure 2).

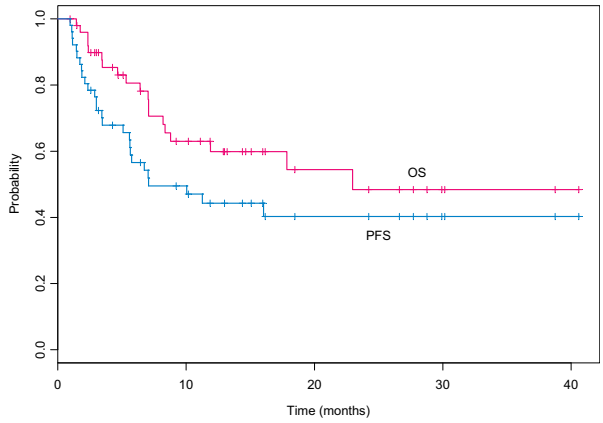

Figure 2. Kaplan-Meier estimates for the overall (red line) and progression-free (blue line) survival of all 51 patients treated on the Clo ± Flu with i.v. Bu protocol.

The multivariate analysis suggests an early trend for improved OS and EFS for AML patients treated in arms with higher Clo doses (Arms III, and IV) (Figures 3 and 4). There also appeared to be an improved OS of patients receiving a MUD graft. To assess whether the improved OS of patients treated in Arms III and IV was accidentally confounded by a higher relative distribution of MUD donors to these arms, we reanalyzed the outcome for patients receiving MUD donor grafts only, and the improved OS trend with the higher doses of Clo remained in effect (not shown). There were 2 treatment-related deaths (4%) in the first 100 days: 1 was because of infectious complications (pneumonia, day SCT +77), whereas the other was because of liver GVHD (day SCT +45). Between day SCT +100 and SCT +1 year, there were 5 more deaths in CR (cumulative

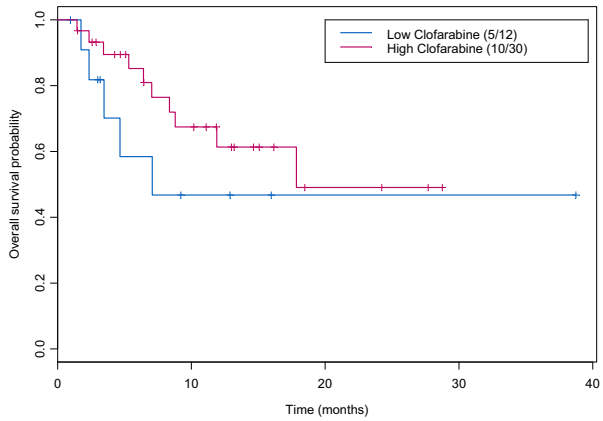

Figure 3. Kaplan-Meier estimates for overall survival of the 42 AML/ MDS patients. The low clofarabine group is Arms I and II (the lower doses of 10 or 20 mg/m^2/day clofarabine together with fludarabine at 30 and 20 mg/m^2/day, respectively, blue line), and the high clofarabine group refers to Arms III and IV (30 or 40 mg/m^2/day clofarabine with fludarabine at 10 mg/m^2/day and 0 mg/m^2/day, respectively, red line). The numbers within the brackets refer to the number of events in the respective groups (5/12 in the low Clo group and 10/30 in the high Clo group).

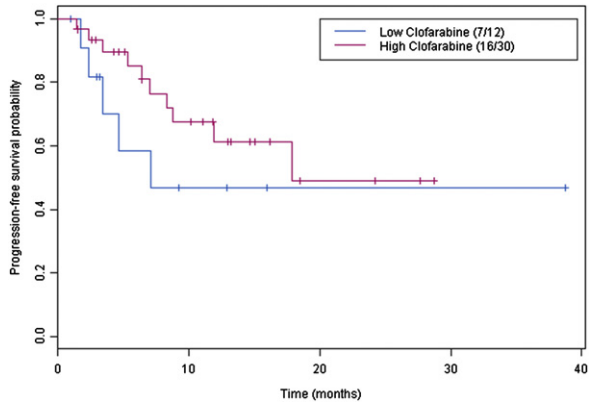

Figure 4. Kaplan-Meier estimates for progression-free survival in the 42 AML/MDS patients. The low clofarabine group is Arms I and II (the lower doses of 10 or 20 mg/m^2/day clofarabine together with fludarabine at 30 and 20 mg/m^2/day, respectively, blue line), and the high clofarabine group refers to Arms III and IV (30 or 40 mg/m^2/day clofarabine with fludarabine at 10 mg/m^2/day and 0 mg/m^2/day, respectively, red line). The numbers within the brackets refer to the number of events in the respective groups.

1-year TRM estimated at 15%), related to infections (n = 3) and GVHD (n = 2). The most common toxicity was, not unexpectedly, grade 2-3 mucositis, which occurred in about 80% of the patients. There was 1 reversible case of veno-occlusive disease (VOD), but no serious neurologic or renal toxicity was encountered (Table 1).

Acute GVHD (aGVHD)

With this second-generation reduced-toxicity regimen, we encountered a similar incidence of GVHD as in our previous experience with i.v. Bu-Flu, the incidence of grades II-IV aGVHD was 31% (17/51), and the incidence of grades III-IV aGVHD was 8% (4/51) (Table 2).

DISCUSSION

Replacing a second alkylating agent in the pretransplant conditioning program with a nucleoside analog (Flu) with i.v. Bu in a myeloablative, reduced-toxicity pretransplant conditioning program [5,7], contributed to significantly increased early safety of the allo-SCT procedure [5-10], with 100-day and 1-year TRM estimates of about 3% to 5% and 8% to 10%, respectively [6,8]. The low TRM demonstrated in these reports is critical when considering the importance of dose intensity for long-term disease control in AML [19,20,22]. Further, all available safety and efficacy data currently favor a nucleoside analog-Bu combination over that of double (or triple) alkylating agent regimens, or over Mel-based conditioning programs [1-10,15-18,43,44]. However, there are lingering concerns about the antitumor efficacy of the variant Bu-Flu regimens compared with

Table 1. Treatment-Related Toxicity within First 30 Days Posttransplant*

	Clinical Grade and Incidence, Number (%)			
Attributed to the Preparative Regimen	Grade	2	3	4
Fever not associated with neutropenia or infection		1(2)		
Mucositis		37 (73)	5 (10)	1 (2)†
Nausea		17 (33)	1 (2)	
Diarrhea		4 (8)	1(2)	
Hepatic–transaminitis		4 (8)	1(2)	
Hepatic–total bilirubin elevation		3 (6)		
VOD			1(2)	
Skin rash		1(2)	1(2)	

VOD indicates veno-occulusion disease.
*Toxicity scored with the NCI criteria.
†This individual had to be transiently intubated for airway protection.

Bu-Cy, most importantly in patients with active disease at the time of transplant [10,19,20]. A randomized comparison of Bu-Flu with BuCy2 would address this question. However, regardless of whether Bu-Flu or BuCy-based therapy were considered the preferable regimen, patients transplanted with active leukemia have a high relapse rate within the first year [21]. Ultimately, neither Flu nor Cy, which as single agents have, at best, limited antileukemic efficacy, may be the ideal partner(s) to i.v. Bu in a pretransplant conditioning regimen for patients who are at high risk for rapidly recurrent disease. We therefore decided to explore the second-generation nucleoside analog Clo, which has a greatly enhanced antileukemic activity [23-28], in combination with Bu in conditioning therapy. Prior to embarking on a clinical study, we examined the in vitro antileukemic cytotoxic properties of Clo and Flu alone and in various combinations with Bu in the KBM3 cell line model of Bu-resistant AML [29,30,35]. In this mechanistic in vitro model we concluded that (1) on a molar basis Clo is at least 50-fold more potent than Flu, (2) Clo synergizes to a higher degree than Flu with Bu. Interestingly, we also found that (3) a combination of Clo and Flu exerted a greatly increased level of synergistic cytotoxicity compared with either

Table 2. Acute GVHD, Clinical Stage

		Organ Involvement			
No. Patients (%)	Grade	Skin	Liver	GI	UGI
26 (51)	0	0	0	0	0
5 (10)	I	5	0	0	0
16 (31)	II	10	1	5	6
2 (4)	III	1	0	2	1
2 (4)	IV	1	1	0	0
Total: 51					

GI indicates gastrointestinal GVHD, UGI, upper gastrointestinal GVHD in the absence of lower GI findings; GVHD, graft-versus-host disease.

nucleoside analog alone or if individually combined with Bu. This augmented cytotoxicity was significantly increased when the 2 nucleoside analogs were followed by Bu. In designing a clinical trial, we then had to consider that although the immunosuppressive and engraftment-promoting capabilities of Flu were well documented, there was no information regarding the immunosuppressive effect of Clo in support of allogeneic progenitor cell engraftment. Hence, based on our in vitro data and the lack of immunosuppressive information for Clo, we decided to supplement and gradually replace Flu with Clo in a 4-arm schedule, modified from our standard 4-day reduced-toxicity i.v. Bu-Flu regimen [7]. The use of adaptive randomization, based on a Bayesian continuous reassessment method [32,33], took into account the chimerism status, disease control/remission status, and whether any serious toxicity(–ies) were experienced in the first 30 days posttransplantation. The adaptive randomization paralleled the in vitro cell line data and preferentially allocated patients to Arm III (Flu 10 mg/m^2 and Clo 30 mg/m^2) and Arm IV (only Clo at 40 mg/m^2) with 34 of the 51 patients distributed to these treatment arms.

In consideration of the high-risk nature of our patient population, we are impressed by the overall safety and antileukemic activity of the redesigned regimen; the 100-day TRM of 4% and 1-year TRM of 15% are not significantly different from our experience with the Bu-Flu regimen [7,8], and the median OS of 23 months in this high-risk patient population is very encouraging. The acute GVHD rate did not appear to increase with the addition of a second nucleoside analog. Longer follow-up and a larger number of patients are likely to continue favoring gradual replacement of Flu with Clo together with Bu in pretransplant conditioning therapy for high-risk patient populations, but the optimal design of a Clo ± Flu-i.v. Bu conditioning regimen is still uncertain. It is intriguing that both the mechanistic in vitro studies and the early adaptive randomization favored a combination of Flu and Clo over that of Clo alone.

Overall, our clinical findings suggest that (1) Clofarabine has sufficient immunosuppressive capability to consistently support allogeneic progenitor cell engraftment. (2) Further, Clo with i.v. Bu ± ATG appears to be a highly active conditioning regimen in patients with advanced, largely chemotherapy-refractory myeloid leukemia; and finally, (3) using both Flu and Clo together with i.v. Bu will likely be as safe as using 1 nucleoside analog combined with the alkylating agent in the studied dose range. The combined in vitro and clinical data indicate that in pretransplant conditioning treatment, analogous to the experience with conventional AML therapy, a combination of 2 nucleoside analogs may synergistically increase antileukemic efficacy/patient benefit without a concomitant increase in clinical toxicity.

Although the Bayesian continuous reassessment approach as presently used is no substitute for a randomized trial that would compare Flu with Clo in combination with i.v. Bu in the described setting, it can be used to obtain a reasonable estimate of the value of Clo either used by itself with i.v. Bu, or when added to Flu together with IV Bu. Therefore, our study may be used to support therapeutic decision making in patients who are at high risk for recurrent leukemia after allo-SCT.

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