Fludarabine and busulfan as a reduced-toxicity myeloablative conditioning regimen in allogeneic hematopoietic stem cell transplantation for acute leukemia patients

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Abstract. The optimal conditioning regimen for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in acute leukemia remains undefined. We evaluated the outcomes in 30 patients with acute leukemia who underwent allo-HSCT from human leukocyte antigen-matched donors after conditioning with busulfan and fludarabine (BuFlu). The regimen comprised injection of busulfan 3.2 mg/kg daily on 4 consecutive days and fludarabine 30 mg/m² daily for 4 doses. All 30 patients achieved hematopoiesis reconstitution with full donor chimerism confirmed by short tandem repeat DNA analysis. The most common regimen-related toxicity was mucositis (86.7%), followed by cytomegalovirus infection (80%). Serious regimen-related toxicities were rare. Acute graft vs. host disease (aGVHD) was detected in 46.7% of the patients; 33.4% had grade I-II aGVHD and 13.3% had grade III-IV aGVHD. Chronic GVHD (cGVHD) was noted in 20% of the patients. The overall survival and disease-free survival rates were 66.7 and 53%, respectively, with a median follow-up of 25 months for surviving patients. Therefore, BuFlu was an effective conditioning regimen with a low rate of transplant-related adverse effects and increased antileukemic effects in patients with acute leukemia undergoing allo-HSCT.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered to be a potential effective treatment strategy for patients with hematological malignancies, particularly acute leukemia. Allo-HSCT has been shown to cure leukemia via a graft vs. leukemia (GVL) effect mediated by immunological cells (1). Currently, high-dose busulfan plus cyclophosphamide (BuCy) has been widely used as a myeloablative conditioning regimen for HSCT. Although BuCy is generally well-tolerated, high exposure to cyclophosphamide metabolites after HSCT may cause serious adverse events and an increase in non-relapse mortality, particularly in elderly patients. By reducing the toxicity, reduced-intensity conditioning (RIC) allows the extension of allo-HSCT to a significantly wider patient population; however, RIC is associated with an increased risk of relapse following HSCT (2,3).

Fludarabine may inhibit lymphocyte proliferation and promote lymphocyte apoptosis by affecting DNA replication and repair, and is considered as the conventional therapy for chronic lymphocytic leukemia (4). As an immunosuppressive purine analogue, fludarabine is used in chemotherapy regimens for acute leukemia, replacing cyclophosphamide in myeloablative and non-myeloablative conditioning regimens (5-8). In addition, busulfan plus fludarabine (BuFlu) exert a synergistic effect, impairing alkylator-induced DNA damage repair. Previous studies demonstrated that fludarabine, when used in a RIC regimen, allows adequate engraftment of allogeneic hematopoietic cells to bring about immunosuppression (9,10). Several clinical trials have also demonstrated that, as a myeloablative conditioning regimen, BuFlu is associated with fewer regimen-related toxicities (RRTs), a lower incidence of non-relapse mortality, and higher disease-free survival (DFS) rates compared with BuCy for allo-HSCT (5,11,12). Fludarabine appears to be well-tolerated by patients undergoing allo-HSCT and is a feasible conditioning regimen alternative to cyclophosphamide.

Our study retrospectively analyzed the efficacy of fludarabine 30 mg/m² [intravenous (i.v.) injection daily, 4 doses] and busulfan 3.2 mg/kg (i.v. daily, 4 consecutive days) as a myeloablative conditioning regimen, considering RRT, engraftment, hematological relapse/disease progression, acute and chronic graft vs. host disease (GVHD), overall survival (OS) and DFS, in 30 patients undergoing allo-HSCT for acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).

Patients and methods

Patient population. Between January, 2008 and January, 2013, a total of 30 patients with AML or ALL were enrolled in this...
follow-up study at the Department of Hematology and Hema-
topoietic Stem-cell Transplantation Center of the Second
Affiliated Hospital of Xi’an Jiaotong University (Xi’an, China).
The patient characteristics, status at HSCT and donor source
are outlined in Table 1. All the patients had a Karnofsky
performance score of ≥70; normal cardiac, hepatic, and renal
function; and no uncontrolled bleeding or severe infection.
High-risk disease status was defined as patients who were
beyond the first remission, had sustained non-remission, had
multiple relapses, or were chemoresistant. All the study partic-
ipants provided written informed consent for the analysis of
transplant outcome data.

Human leukocyte antigen (HLA) typing and engraftment.
HLA genotyping was performed in the same manner as in all
centers: The presence of class I antigens was tested using stan-
dard serological techniques; and class II alleles were resolved
with low-resolution molecular typing using polymerase
chain reaction (PCR) amplification with sequence-specific
oligonucleotide primers for hybridization of amplified DNA,
followed by high-resolution typing in all patients and donors.
Donor-recipient pairs were considered fully matched in cases
of HLA-A, HLA-B and HLA-DRB1 compatibility.

Engraftment was defined as an absolute neutrophil count
of >0.5 x10^9/l on the first 3 consecutive days and a platelet
count of >20 x10^9/l after at least 3 days without the need for
transfusion. Chimeric status was evaluated in nuclear cells of
peripheral blood T cells and polymorphonuclear neutrophils
on days +20, +30 and +60 by using the short tandem repeat
PCR (STR-PCR) method.

Conditioning regimens, GVHD prophylaxis and supportive
care. The myeloablative conditioning regimen consisted of
fludarabine 30 mg/m² infused over 30 min daily for 4 doses
days -5 to -2), followed by intravenous busulfan 3.2 mg/kg of
actual or adjusted ideal body weight over 4 h daily on 4 consecu-
tive days (days -5 to -2). Acute GVHD (aGVHD) and chronic
GVHD (cGVHD) were diagnosed and classified according to
previously described clinical criteria (13-15). Depending on
the donor type, transplant recipients received traditional cyclo-
sporin A (CSA) and short-course methotrexate (MTX) (sibling
donor) or CSA, MTX and mycophenolate mofetil (unrelated
donor) for GVHD prophylaxis. The serum CSA concentration
was maintained between 100 and 300 ng/ml, and the dose
was tapered off by 5% every week from day +60 to day +90.
Supportive care comprised prophylactic transfusion of plate-
lets if platelet counts decreased to <20 x10^9/l, or prophylactic
transfusion of red blood cells if hemoglobin levels decreased
to <80 g/l.

Antimicrobial therapy and other medications per-protocol.
The patients underwent HSCT treatment in rooms with a
positive-pressure filtered flow. The patients were monitored for
cytomegalovirus (CMV) DNA with quantitative PCR once a
week from the first day of conditioning (day -6) until day +100,
and then twice a month until the discontinuation of GVHD
prophylaxis. Patients positive for CMV DNA were treated
with ganciclovir and/or foscarnet until two consecutive nega-
tive test results were obtained. Neutropenic fever was managed
according to the Infectious Diseases Society of America Fever
and Neutropenia guidelines (16). Co-trimoxazole was initiated
after engraftment to prevent Pneumocystis carinii pneumonia.
Prostaglandin E1 and Danshen injections were used for
veno-occlusive disease (VOD) prophylaxis, beginning with
the initiation of conditioning.

Statistical analysis. The day of stem cell infusion was
defined as day 0. Descriptive statistics were used to describe
the baseline characteristics of disease status at conditioning.
Categorical variables are summarized as frequency counts
and percentages, and continuous variables are summarized
as median and range. DFS was defined as the time between
transplantation and the earliest occurrence of relapse or death
due to any cause. Cumulative incidence or survival was plotted
according to the Kaplan-Meier method and the log-rank test
was used to analyze differences between groups. Basic statis-
tical data were obtained using the SPSS software package,
version 17.0 (SPSS Inc., Chicago, IL, USA). A cut-off value of
0.05 indicating statistically significant differences was
adopted for all statistical analyses.

Results

Engraftment. As shown in Fig. 1, patients achieved absolu-
tute neutrophil count (ANC) recovery and received platelet
engraftment at 11 days (range, 7-17 days) and 13 days
(range, 7-25 days), respectively. The median time to ANC
recovery or platelet engraftment was not statistically different
between the sibling and unrelated donor groups. Complete
donor chimerism was achieved in all patients, with neutrophil
count recovery being confirmed by STR-DNA detection on
days +20, +30 and +60.

Regimen-related toxicity. Of the 30 patients, 1 experienced
hemorrhagic cystitis (HC), which was resolved by the discon-
tinuation of drugs that may cause or aggravate HC, and the
initiation of diuretic, hemostatic and anti-infection treatment.
CMV viremia was noted in 24 patients, of whom 2 developed
CMV-associated interstitial pneumonia. All 24 patients
received antiviral treatment with ganciclovir and foscarnet.
A total of 26 patients developed mucositis, which resolved
with symptomatic therapy without any serious or permanent
sequelae. There was one case of cardiac toxicity with tachy-
cardia and no reported cases of VOD or regimen-related death.
Grade II, III and IV toxicities were observed in 14 (46.7%),
8 (26.7%) and 1 (3.3%) patient, respectively.

GVHD. As shown in Table 1 and Fig. 2, 14 patients (46.7%)
 experienced aGVHD. Of those, 9 (13.3%) had grade II-IV
aGVHD, 4 of whom succumbed due to severe rejection.
cGVHD was observed in 6 patients (20%), including 2 (6.7%)
with extensive cGVHD. The incidence of aGVHD did not
differ significantly between the AML and ALL groups or
between the sibling and unrelated donor groups.

Survival data. With a median follow-up period of 25 months
(range, 2-78 months) for surviving patients, 20 of the
30 patients remained alive. A total of 4 patients succumbed
to disease relapse, whereas 6 deaths were due to non-relapse
causes: aGVHD (n=4), uninduced epileptic seizures (n=1),
and multifactorial respiratory failure following severe pulmonary infection (n=1) (Table II). The OS and DFS rates were 66.7 and 53%, respectively, at the end of follow-up (Fig. 3). Three (10%) and one (3.3%) relapses occurred in ALL and AML patients, respectively. The median time-to-relapse was 79 days (range, 65-155 days) after transplantation. The 3 patients with ALL who relapsed were chemoresistant, and the patient with AML who relapsed 155 days after transplantation was in non-remission. After receiving a full chimerism sibling donor lymphocyte infusion, the patient achieved a transient complete remission but relapsed twice and succumbed 1 year later. The probability of OS at 2 years of AML vs. ALL patients was 73.3 vs. 60%, respectively, which was not statistically significant (P=0.70). Furthermore, the cumulative incidence rate of 2-year OS did not differ significantly between high-risk and standard-risk patients (53.8 and 76.5%, respectively; P=0.76).

Table I. Patient characteristics (n=30).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years [median (range)]</td>
<td>30 (13-59)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (47.0)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (53.0)</td>
</tr>
<tr>
<td>Acute myeloid leukemia (n=15)</td>
<td></td>
</tr>
<tr>
<td>Complete remission 1</td>
<td>8 (54.0)</td>
</tr>
<tr>
<td>Complete remission 2</td>
<td>5 (33.0)</td>
</tr>
<tr>
<td>Non-remission</td>
<td>2 (13.0)</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia (n=15)</td>
<td></td>
</tr>
<tr>
<td>Complete remission 1</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Complete remission 2</td>
<td>5 (33.0)</td>
</tr>
<tr>
<td>Non-remission</td>
<td>1 (7.0)</td>
</tr>
<tr>
<td>Donor gender</td>
<td></td>
</tr>
<tr>
<td>Matched</td>
<td>19 (63.0)</td>
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<tr>
<td>Mismatch</td>
<td>11 (37.0)</td>
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<tr>
<td>Disease risk</td>
<td></td>
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<tr>
<td>Standarda</td>
<td>17 (57.0)</td>
</tr>
<tr>
<td>Highb</td>
<td>13 (43.0)</td>
</tr>
<tr>
<td>Donor type</td>
<td></td>
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<tr>
<td>Sibling</td>
<td>18 (60.0)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>12 (40.0)</td>
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<tr>
<td>Stem cell source</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>4 (13.0)</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>26 (87.0)</td>
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<tr>
<td>Mononuclear cells, x10^9/kg [median (range)]</td>
<td>8.71 (0.87-15.97)</td>
</tr>
<tr>
<td>CD34+ cells, x10^6/kg [median (range)]</td>
<td>3.62 (0.66-12.9)</td>
</tr>
<tr>
<td>GVHD prophylaxis</td>
<td></td>
</tr>
<tr>
<td>CSA+MTX</td>
<td>18 (60.0)</td>
</tr>
<tr>
<td>CSA+MTX+MMF</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>I</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>II</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>III-IV</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td></td>
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<tr>
<td>Limited</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Extensive</td>
<td>2 (6.7)</td>
</tr>
</tbody>
</table>

aDisease in first complete remission.
bMore advanced status than standard-risk disease. CSA, cyclosporin A; MTX, methotrexate; MMF, mycophenolate mofetil; GVHD, graft vs. host disease.
Table II. Number of deaths according to primary disease.

<table>
<thead>
<tr>
<th>Causes of death</th>
<th>ALL, n (%)</th>
<th>AML, n (%)</th>
<th>HR, n (%)</th>
<th>SR, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse-related mortality</td>
<td>3 (30.0)</td>
<td>1 (10.0)</td>
<td>4 (40.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Non-relapse-related mortality</td>
<td>3 (30.0)</td>
<td>3 (30.0)</td>
<td>2 (20.0)</td>
<td>4 (40.0)</td>
</tr>
<tr>
<td>GVHD</td>
<td>2 (20.0)</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
<td>3 (30.0)</td>
</tr>
<tr>
<td>Infection</td>
<td>1 (10.0)</td>
<td>0 (0.0)</td>
<td>1 (10.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other a</td>
<td>0 (0.0)</td>
<td>1 (10.0)</td>
<td>0 (0.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (60.0)</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td>4 (40.0)</td>
</tr>
</tbody>
</table>

*Percentage of total deaths. aSuccumbed to uninduced epileptic seizures. HR, high-risk; SR, standard-risk; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; GVHD, graft vs. host disease.

**Discussion**

BuCy is a traditionally used myeloablative conditioning regimen for HLA-matched allo-HSCT. However, the high RRT due to the additive effect of the two alkylators is a major concern; in particular, cyclophosphamide metabolism is associated with sinusoidal obstruction syndrome, hemorrhagic cystitis and bilirubin level elevation, in addition to increased non-relapse mortality (17). Although a non-myeloablative transplant is associated with lower conditioning regimen-related mortality, it has a higher rate of leukemia relapse compared with a classical myeloablative transplant regimen.

The BuFlu regimen has been confirmed to be safe and effective for allo-HSCT in patients with hematological malignancies. Slavin et al (18) first reported the efficacy of fludarabine (180 mg/m²) in an RIC regimen, with an OS rate of 85% and a DFS rate of 81% after a median follow-up of 8 months for HLA-matched peripheral blood stem cell transplant. The replacement of cyclophosphamide with fludarabine appeared to decrease toxicity, while exhibiting efficacy comparable to that of BuCy. However, the graft failure rate was higher in patients treated with the RIC regimen (18,19). Other studies have demonstrated that intense conditioning may decrease the incidence of graft failure (20,21). Or et al (22) reported a 54% rate of aGVHD with negligible toxicity after a median follow-up of 42 months with the same conditioning regimen as that used by Slavin et al (18). Bornhauser et al (23) reported that an ablative dose of BuFlu resulted in 100% engraftment and 7% RRT in 42 patients with high-risk chronic myeloid leukemia or myelodysplastic syndrome, with estimated OS and DFS rates of 42.4 and 34.9%, respectively, at a median follow-up of 18 months. The MD Anderson Cancer Center reported that a regimen of fludarabine 40 mg/m² and i.v. busulfan 130 mg/m² once daily for 4 days was well-tolerated and effective (11). In that study, patients who received a transplant in first complete remission had 3-year OS and event-free survival rates of 78 and 74%, respectively. The overall incidence of grade II-IV aGVHD was 15.8% and that of extensive cGVHD was 34.1%. Thus, i.v. BuFlu was found to be a well-tolerated and efficacious myeloablative conditioning regimen with reduced toxicity. Russell et al (24) reported that high-dose fludarabine 250 mg/m² and busulfan 12.8 mg/kg plus thymoglobulin was also a well-tolerated and effective regimen, with a particularly low incidence of grade III-IV aGVHD (3%) and cGVHD (38%) at 2 years.

In our study, we analyzed the clinical data of Chinese Han patients with acute leukemia who underwent HLA-matched allo-HSCT with the BuFlu conditioning regimen. As in most other studies, we did not observe graft failure. Neutrophil and platelet engraftment occurred on days +11 and +13 for patients who received sibling donor transplants, and on days +13 and +14 for patients who received unrelated donor transplants, respectively. There were no significant differences with the BuCy conditioning regimen regarding the time to hematopoietic reconstitution. Grade III-IV RRT was observed in 30% of the patients. The most common and serious RRTs were mucositis (86.7%) and HC (3.3%, respectively). CMV infection was common (80%). Mucositis and HC were resolved with drug adjustment and supportive treatment. Our results were similar to those of Iravani et al (5). Grade III-IV aGVHD was detected in 13.3% of the patients, without significant differences in the incidence of aGVHD according to donor type. The rate of cGVHD in all patients (20%) was lower compared with that observed with BuCy (25,26). A probable reason for this is the strong immune inhibitory effect and alkylator-induced DNA damage repair with fludarabine.

Several studies have reported an association of the BuFlu conditioning regimen with a decreased relapse rate following allo-HSCT (27,28). However, other studies have reported greater relapse or progression in the BuFlu regimen arm compared with that in the BuCy arm (10). In our study, the rate of overall relapse was 30%. The majority of the relapsed patients had a high risk or advanced status prior to the transplant, indicating that the rate of relapse is also associated with the pre-transplant disease status and post-transplant adjustment of immune inhibitors.

In conclusion, our study indicated that BuFlu was an acceptable regimen, due to its low rates of RRT, GVHD and morbidity in the Chinese population. BuFlu may replace BuCy, with the aim to decrease regimen-related side effects, without compromising the efficacy. However, further comparative studies on BuFlu and standard regimens with subgroup analyses according to standard- vs. high-risk leukemia are required.

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References


