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ORIGINAL ARTICLE

Pharmacokinetically-targeted BU and fludarabine as conditioning before allogeneic hematopoietic cell transplantation for adults with ALL in first remission

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Allogeneic hematopoietic cell transplantation offers improved survival in patients with ALL, but with regimens containing TBI, the nonrelapse mortality is 20–40%. Efforts to lessen transplant toxicities by reducing conditioning regimen intensity have led to increased relapse risk. Therefore, there is a need for less toxic regimens that maintain an anti-leukemia effect. We report here a retrospective review of 65 patients with ALL in first remission receiving grafts from allogeneic donors after fludarabine 40 mg/m^2/day for 4 days and i.v. BU targeted to a median daily area under the concentration–time curve below 6000 μmoles min/L. At 2 years after transplantation, OS was 65% (95% confidence interval (CI): 52–77%), relapse-free survival was 61% (95% CI: 48–73%), cumulative incidence of relapse was 26% (95% CI: 17–39%) and cumulative incidence of nonrelapse mortality was 14% (95% CI: 8–26%). Age over 35 years, Ph chromosome positivity and minimal residual disease at transplant did not adversely affect outcomes. Pharmacokinetically targeted BU and fludarabine can provide intensive pre-transplant conditioning for adults with ALL in first remission, with promising relapse-free and OS rates.

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INTRODUCTION

CR is attainable with chemotherapy in most adults with ALL, but less than half of standard-risk patients are alive and free of relapse 5 years after treatment with chemotherapy alone.[1] Outcomes are worse for patients with Ph chromosome-positive disease, with a 5-year relapse-free survival of 10% in the pre-imatinib era.[2,3] Although the addition of imatinib to induction therapy has improved CR rates of patients with Ph-positive ALL, relapse rates remain high.[3] Allogeneic hematopoietic cell transplantation offers improved survival regardless of Ph chromosome status, but with regimens containing TBI, the nonrelapse mortality at 2 years ranges from 20–40%.[1,2] Efforts to lessen transplant toxicities by reducing conditioning regimen intensity have been successful especially in older patients and in those with comorbidities, but have also led to increased relapse in adult ALL.[4,5] Therefore, there is a need for less-toxic regimens that maintain an anti-leukemia effect.

BU is a potent inducer of apoptosis in ALL cells[6] and has been evaluated in myeloablative doses with CY in order to avoid TBI-related toxicity in ALL patients.[7–9] In pediatric patients, BU was inferior to TBI; however, pharmacokinetic dose adjustment was not done and, because of a higher clearance of BU in children, subtherapeutic BU exposures may have accounted for the outcome. In addition, poor results with BU may be explained by erratic oral absorption that results in as much as a 10-fold range of systemic exposures, with excess toxicity at high exposure and lack of efficacy at low exposure.[10,11] Intravenous administration of BU results in less variability in blood concentrations, but is still associated with a three-fold range of systemic exposure.[12,13] As relationships between BU systemic exposures and safety and efficacy outcomes have been defined,[11,12,14] use of pharmacokinetic (PK) dose-targeting of BU may reduce toxicity and optimize efficacy by limiting the variation of systemic exposure.[15] Replacing CY with fludarabine has also contributed to reduced toxicity.[16–18] Based on these data, we began using the combination of fludarabine with PK-targeting of BU as our standard allogeneic preparative regimen for all patients with hematologic malignancies; no other myeloablative regimen was used in patients with ALL. We previously reported the early safety of this approach in adult ALL patients with a nonrelapse mortality of 18% at 1 year after transplant.[19] Since then we have completed a prospective trial identifying the maximally tolerated area under the concentration–time curve (AUC) for BU.[20] Herein, we report more mature results showing potential efficacy with this approach in a cohort of 65 adult ALL patients in first remission, the majority of whom were treated near the maximally tolerated AUC.

PATIENTS AND METHODS

Patient selection

We conducted retrospective review of 65 consecutive patients with ALL in first remission treated with fludarabine and PK-targeted i.v. BU followed by allo-SCT between July 2004 and June 2012. Twenty-seven patients (41%) of

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this series were included in a prior publication.[19] By more than doubling the sample size and extending the follow-up, this report provides further support for multicenter phase II studies of fludarabine and targeted BU in ALL in first remission.

All patients provided consent for long-term follow-up after transplantation on a protocol approved by the University of South Florida Institutional Review Board. The same Institutional Review Board approved this study with waiver of consent. First remission was defined by standard morphological criteria of $<5\%$ marrow blasts and absence of extramedullary leukemia. Minimal residual disease was defined by positive flow cytometry for the leukemia phenotype, or positive PCR for the breakpoint cluster region-Ableson murine leukemia fusion transcripts (Ph chromosome) or clonal T cell receptor or Ig gene rearrangements.

Treatment

Patients received fludarabine (Fludara; Bayer HealthCare, Wayne, NJ, USA) 40 mg/m^2 intravenously once daily for 4 days with dose reduction for renal insufficiency, followed daily by i.v. BU 130 mg/m^2 (Busulfex; Otsuka America Pharmaceutical, Rockville, MD, USA) daily over 3 hours on the first and second day of the regimen. Blood samples were drawn 15 min after the end of the first BU dose then at 2, 4, 6 and 9 h after the end of the infusion. BU steady-state AUC was estimated from concentration data using the single-compartment first-order elimination model. BU doses for the third and fourth days of the regimen were adjusted based on a linear relationship between dose and AUC to achieve the average daily AUC target over the 4 days of administration. Further details of PK assay methodology, AUC determination and BU dosing have been previously published.[15,19,20] All patients received lorazepam for seizure prophylaxis. Ursodiol was given to prevent hepatotoxicity.

Supportive care

All patients received granulocyte-colony-stimulating factor-mobilized, T-replete PBSC grafts from HLA-identical siblings or unrelated donors compatible for 8/8 or 7/8 HLA-A, -B, -C and -DRB1 alleles. For GVHD prophylaxis, patients received tacrolimus in combination with MTX, mycophenolate mofetil or sirolimus as previously published.[21,22] Patients with HLA-mismatched unrelated donor grafts were also treated with rabbit antithymocyte globulin (Thymoglobulin; Genzyme Corporation, Cambridge, MA, USA) 1 mg/kg intravenously on day 3 followed by 3.25 mg/kg/day on day 2 and day 1. Antimicrobial prophylaxis was given as previously described.[15,19,20]

Endpoint definition

Neutrophil recovery was defined as the first of 3 consecutive days when neutrophil counts exceeded 0.5×10^9/L, and platelet recovery was defined as the first of 3 consecutive days when platelets exceeded 20×10^9/L without transfusion in the previous 7 days. Chimerism was tested by PCR of DNA from unsorted BM samples, sorted blood T cells or sorted blood granulocytes.[23] BU-related toxicity was evaluated in the first 100 days after transplantation by the Common Terminology Criteria for Adverse Events, version 3 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Hepatic veno-occlusive disease was diagnosed and staged according McDonald *et al.*[24] Acute and chronic GVHD were graded using consensus criteria.[25,26] Relapse was defined by morphological criteria. Nonrelapse death was defined as death from any cause without evidence of leukemia.

Statistical analysis

OS and relapse-free survival was estimated using the Kaplan–Meier method. Survival curves among subgroups were compared using the log-rank test. Cumulative incidence was estimated and compared using standard techniques.[27] Relapse and nonrelapse death were treated as competing risk events when estimating the incidence of GVHD, relapse and nonrelapse death. The 95% CI for cumulative incidence point estimates were estimated from the logarithmic transformation.[28] *P*-values were not adjusted for multiple comparisons. Multivariable analyses were not attempted due to the low number of informative events.

RESULTS

Patient and disease characteristics

A total of 65 consecutive patients with ALL in CR1 were included in this evaluation (Table 1). Median patient age was 42 (range

Table 1. Patient and transplant characteristics

	N	%
Number of patients	65	
Age, median in years (range)	42 (20–65)	
Immunophenotype		
B cell	57	88
T cell	8	12
Cytogenetics		
Ph + [a]	29	45
Other unfavorable[b]	8	12
Normal	17	26
Other	10	16
Missing	1	1
WBC at diagnosis		
B cell type		
$\geqslant 30 \times 10^9$/L	18/57	32
T cell type		
$\geqslant 100 \times 10^9$/L	1/8	13
Extramedullary disease at diagnosis		
None	56	86
CNS or other	9	14
Median time in months from diagnosis to transplant (range)	6.5 (2.9–15.2)	
Induction regimen		
HyperCVAD[c]	55	85
Other	10	15
Minimal residual disease at transplant[d]	21	33
Karnofsky performance status		
100%	14	22
90%	37	57
$<90\%$	14	21
HCT-CI scores[e]		
0	26	40
1 or 2	26	40
>2	13	20
Donor		
Matched related	29	45
Matched unrelated	29	45
Mismatched unrelated	7	10
Median donor age in years (range)	34 (18–68)	
Donor/recipient gender		
F/F	11	17
F/M	18	28
M/F	19	29
M/M	17	
Donor/recipient CMV serology		
N/N	14	22
N/P	20	31
P/N	10	15
P/P	21	32
Median number ($\times 10^6$/kg) CD34+ cells/kg infused (range)	7.88 (3.12–10)	
Daily BU AUC target		
5300 ± 530 μmoles min/L	53	82
>6000 μmoles min/L	12	18
GVHD prophylaxis		
Tacrolimus + mtx	47	72
Tacrolimus + other	18	28

Abbreviations: AUC = area under the concentration–time curve; CNS = central nervous system; F = female; HCT-CI = hematopoietic cell transplantation comorbidity index; M = male; N = negative; P = positive. [a]Includes patients with *t*(9;22) alone or with additional abnormalities. [b]Includes −7, +8 or 11q23 rearrangements [c]Fractionated CY, vincristine, doxorubicin and dexamethasone.[39] [d]By cytogenetics, PCR, flow cytometry or FISH. [e]Assessed retrospectively according to Sorror *et al.*[40]

20–65) years. At diagnosis, 29 (45%) patients were positive for the Ph chromosome and an additional 10 (16%) patients had other unfavorable cytogenetics. All Ph-positive patients received a tyrosine kinase inhibitor prior to transplant. The median duration of tyrosine kinase inhibitor administration was 4 (range, 0.5–10) months. None of the patients with a history of extramedullary disease received radiation therapy prior to transplant. Twenty-one (33%) patients had evidence of minimal residual disease at the time of transplant: 12 were positive by PCR for BCR/abl (three of these were also positive by other measures), four for T-cell receptor rearrangements, four for Ig chain rearrangements and one had evidence of leukemic phenotype by flow cytometry. When evaluating the differences in characteristics between patients younger and older than 35 years, younger patients were more likely to have Ph-negative disease ($P = 0.04$) and receive a transplant from a matched-unrelated donor ($P = 0.04$); 6/25 (24%) younger patients had mismatched unrelated donors, compared with 2/40 (5%) older patients. There were no significant differences in the targeted BU AUC, Karnofsky performance status, hematopoietic cell transplant comorbidity index, or presence of minimal residual disease between the age groups.

Treatment

Fifty-three patients (82%) received fludarabine followed by PK-targeted i.v. BU to a daily average of AUC of 5300 ± 530 μmoles min/L and the remainder were enrolled onto a prospective clinical trial evaluating higher AUCs.[20] The eligibility criteria for that trial excluded major comorbidities, poor performance status, active infections and poor organ function. For those patients targeted to 5300 μmoles min/L, the median average daily BU dose after adjustment was 120 (range, 65–214) mg/m^2. Twenty-four Ph-positive patients received tyrosine kinase inhibitors for the maintenance of remission starting a median of 2 (range 1–11) months after transplantation, for a median of 8 (range, 0.5–31) months.

BU–related toxicities and causes of death

Within the first 100 days after transplant, the most common grade 3 or 4 BU-related toxicities were oro-pharyngeal mucositis (68%), diarrhea (5%) and non-VOD hepatotoxicity (5%). Three cases of veno-occlusive disease occurred, one of which was fatal. Other causes of death included relapse ($n = 15$), GVHD ($n = 5$), diffuse alveolar hemorrhage ($n = 1$), infection ($n = 2$) and suicide ($n = 1$). An additional patient died of unknown causes.

Engraftment and GVHD

There were no primary graft failures. The median time to neutrophil recovery was 15 (range, 11–23) days and platelet recovery was 16 (range, 9–22) days; 10 patients never had counts of $< 20 \times 10^9$/L. At day 90 after transplantation, the median donor DNA chimerism was 98% (range, 47–100%) in the marrow, 91% (range, 6–100%) in the T cells and 100% (range, 98–100%) in the granulocytes. Low donor chimerism in the marrow occurred in patients immediately prior to relapse of the disease. Low donor T cell chimerism appeared in patients receiving ATG as part of their conditioning. These latter patients eventually reached full donor T cell chimerism without further intervention.

In the 47 patients who received tacrolimus and MTX, the cumulative incidence values of grades II–IV and III–IV acute GVHD were 85% (95% CI: 76–96%) and 11% (95% CI: 5–24%), respectively. The cumulative incidence of moderate–to-severe chronic GVHD at 2 years was 55% (95% CI: 41–73%).

Survival and relapse outcomes

The cumulative incidence of nonrelapse mortality was 5% (95% CI: 2–14%) at 100 days and 14% (95% CI: 8–26%) at 2 years (Figure 1a and Table 2). The 2-year cumulative incidence of relapse was 26% (95% CI: 17–39%) and the median time to relapse was 4 (range, 1–33) months (Figure 1b). With a median follow-up of 31 (range, 6–71) months, the 2-year relapse-free survival was 61% (95% CI: 48–73%) (Figure 1c) and OS was 65% (95% CI: 52–77%) (Figure 1d).

Patients who had BU targeted to an AUC of 5300 ± 530 μmoles min/L had a better OS ($P = 0.04$) compared with those who had BU targeted to a higher AUC, likely due to a trend in lower non-relapse mortality. Patients with a lower hematopoietic cell transplantation–comorbidity index score had improved OS and relapse-free survival, reflecting trends in lower nonrelapse

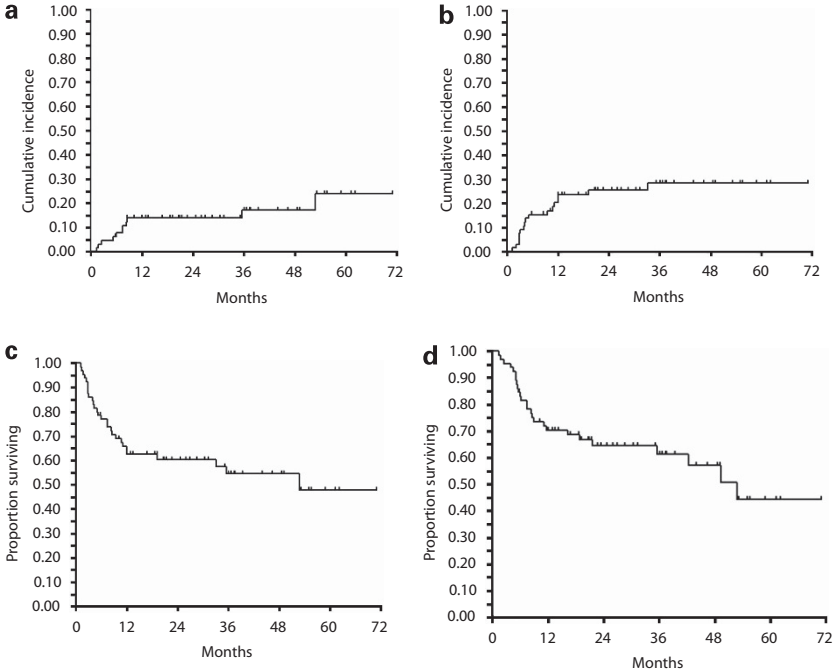

Figure 1. (**a**) Nonrelapse mortality. (**b**) Relapse. (**c**) Relapse-free survival. (**d**) Overall survival.

Table 2. Two-year nonrelapse mortality, relapse, relapse-free survival and Overall survival (95% CI)

	Cumulative incidence of NRM	*Cumulative incidence of relapse*	*Relapse-free survival*	*OS*
All patients ($n=65$)	14% (8–26%)	26% (17–39%)	61% (48–73%)	65% (52–77%)
Ph cytogenetics at diagnosis				
Ph-negative ($n=36$)	11% (5–29%)	26% (15–45%)	63% (47–79%)	66% (50–81%)
Ph-positive ($n=29$)	17% (7–38%)	25% (13–47%)	58% (40–76%)	63% (44–82%)
P-value	0.44	0.92	0.53	0.73
Age				
Age <35 ($n=25$)	8% (2–30%)	37% (22–62%)	55% (35–75%)	59% (42–83%)
Age $\geqslant 35$ ($n=40$)	18% (9–35%)	18% (9–35%)	64% (49–79%)	69% (54–84%)
P-value	0.4	0.15	0.48	0.43
BU target AUC				
AUC 5300 ($n=53$)	10% (4–22%)	26% (16–41%)	65% (51–78%)	70% (57–83%)
AUC >5300 ($n=12$)	33% (15–74%)	25% (9–67%)	42% (14–70%)	42% (14–70%)
P-value	0.11	0.65	0.06	0.04
Minimal residual disease[a]				
No ($n=43$)	14% (7–30%)	25% (15–43%)	61% (46–76%)	68% (53–82%)
Yes ($n=21$)	14% (5–41%)	29% (15–56%)	57% (36–78%)	57% (35–78%)
P-value	0.64	0.67	0.42	0.22
Donor				
Matched related ($n=29$)	7% (2–26%)	25% (13–48%)	68% (50–85%)	71% (54–88%)
Unrelated ($n=36$)	20% (10–38%)	26% (15–45%)	54% (38–71%)	59% (42–76%)
P-value	0.17	0.87	0.35	0.53
KPS				
100% ($n=14$)	14% (4–52%)	7% (1–47%)	79% (43–95%)	79% (57–100%)
90% ($n=37$)	14% (6–31%)	23% (13–43%)	63% (47–79%)	71% (56–87%)
$<90\%$ ($n=14$)	14% (4–51%)	51% (30–87%)	34% (9–60%)	32% (7–58%)
P-value	0.92	0.02	0.07	0.08
HCT-CI				
0 ($n=26$)	4% (1–26%)	17% (7–41%)	80% (64–96%)	84% (70–98%)
1 or 2 ($n=26$)	15% (6–38%)	27% (14–51%)	58% (39–77%)	60% (41–80%)
>2 ($n=13$)	33% (15–73%)	41% (21–81%)	26% (1–51%)	35% (8–62%)
P-value	0.14	0.40	0.02	0.03

Abbreviations: AUC = area under the concentration–time curve; HCT-CI = hematopoietic cell transplant comorbidity index; KPS = Karnofsky performance status; NRM = nonrelapse mortality. [a]One patient with missing data.

mortality and relapse rate. Ph-positive and other high-risk ALL at diagnosis, minimal residual disease at transplant, patient age over 35 and unrelated donor transplants were not associated with an increased risk of treatment failure.

DISCUSSION

In adults with ALL in CR1, we found that a conditioning regimen of fludarabine followed by PK-based targeting of i.v. BU is safe, based on the observed 14% 2-year incidence of nonrelapse mortality, and shows promising efficacy, based on the 26% incidence of relapse and the rates of 2-year OS and relapse-free survival over 60%. Our results are not unlike previously reported outcomes for allogeneic transplantation in similar populations. Investigators at the Fred Hutchinson Cancer Research Center have recently updated their results of high dose TBI-containing regimens ($\geqslant 1200$ cGy) for transplantation in 76 patients with ALL in CR1. The 100-day incidence of nonrelapse mortality was 19%, the 2-year incidence of relapse was 27% and the 2-year relapse-free survival was 54%.[29] Using a regimen of TBI (1320 cGy in 6 fractions twice daily) and etoposide (60 mg/kg), investigators from the MRC UKALLXII/ECOG 2993 study[1] reported a 5-year OS of 53% in Ph-negative patients with an available donor, a 2-year incidence of nonrelapse mortality of 36% in high-risk patients and 20% in standard-risk patients. With the same regimen in Ph-positive patients, these investigators reported a 5-year OS of 44% in recipients of a HLA-matched sibling donor transplant and 35% for those receiving grafts from HLA-matched unrelated donors.[2]

Two recent registry studies of adult ALL compared reduced intensity regimens that included BU at $\leqslant 8$ mg/kg without PK targeting or melphalan at $\leqslant 150$ mg/m^2, with fully intense conditioning regimens that include predominantly high-dose TBI.[3,4] In patients with ALL in CR1, the European Bone Marrow Transplant registry reported a 2-year incidence of nonrelapse mortality of 17% with reduced intensity and 32% with full-intensity regimens.[4] The 2-year incidence of relapse, however, was higher in ALL CR1 patients receiving reduced intensity at 48% vs 28% for full intensity, resulting in 2-year leukemia-free survival of 35% vs 40%, respectively. The Center for International Blood and Marrow Transplant Research registry reported similar outcomes in patients with ALL in CR1, resulting in a 3-year leukemia-free survival of 36% for reduced intensity vs 49% for full-intensity regimens.[4] The higher median age of patients treated with reduced intensity regimens in both studies may in part account for the higher incidence of nonrelapse mortality compared with our series. However, the results we report here with a myeloablative regimen are similar to reduced-intensity regimens with respect to nonrelapse mortality, but are also similar to full-intensity regimens in the incidence of relapse after transplantation.[3,4]

The combination of fludarabine and pharmacokinetically targeted BU has also been given with low-dose TBI and ATG in

adults with high-risk ALL.[30] Low-dose (400 cGy) TBI may add to the efficacy of fludarabine/BU in ALL, but a controlled study would be needed to prove its added value in this combination. In addition, clofarabine in combination with BU has also been evaluated with intriguing preliminary results in ALL.[31] The relative contribution of clofarabine vs fludarabine in the efficacious treatment of ALL when used in combination with BU before allogeneic transplantation remains to be fully assessed.

Comparing results of different publications is difficult owing to the differences in patient selection, follow-up time and other variables. However, we believe that using fludarabine with PK-targeted intravenous BU, as we describe here, may result in lower mortality compared with TBI-containing regimens. It is unlikely that the difference in mortality in our study compared with myeloablative, TBI-containing regimens was due to patient selection, as our patients were a mix of standard and high-risk as defined by Goldstone *et al.*[1] The use of i.v. BU and pharmacokinetic dose-targeting may reduce relapse and mortality by reducing wide variations in exposure, thus providing a more individualized approach to conditioning. We found that BU exposures over 5300 μmoles min/L are not beneficial in this population as nonrelapse death was increased without a detectable effect on preventing relapse. The same finding was previously reported by Geddes *et al.*[32]

We saw no significant increase in the risk of relapse with Ph-positive (25%) vs Ph-negative (26%) disease. The use of tyrosine kinase inhibitors both before and after transplantation in Ph-positive ALL likely had a beneficial effect in preventing relapse as others also have reported.[33,34] However, leukemic relapse remains the main cause of death in ALL and continued effort is needed to identify effective strategies to prevent relapse following transplant.

Increasing age has been associated with poorer outcomes.[35] We did not see a significant difference in survival outcomes with age over 35 years, either because of our sample size or using an individualized dosing strategy with BU counteracted the potential negative effect of age on death. When evaluating different characteristics between the two age groups, younger patients were more likely to have Ph-negative disease that would bias toward better survival, but also more likely to receive their graft from an unrelated donor that could have biased towards a worse survival.

The detection of minimal residual disease has provided insight for overall response to induction chemotherapy, risk of relapse and need for further intensification.[36] It has been suggested that sensitive techniques such as quantitative PCR for leukemia-specific sequences may predict relapse in some settings, but validated tests that can reliably predict post-transplant relapse are not yet available.[37] In a subgroup analysis of non-T-lineage Ph-negative patients enrolled on the MRC UKALL XII/ECOG2993 trial, minimal residual disease measured by PCR of clone-specific Ig or T-cell receptor rearrangements before allogeneic transplantation did not adversely affect relapse-free survival.[38] Using perhaps a less-sensitive measure of minimal residual disease in both B- and T-cell lineage disease, neither were we able to show a difference in relapse or survival outcomes. Again, while this could be an issue of sample size in our exploratory analysis, an alternative explanation is that allogeneic transplantation in CR1 overcomes the negative prognostic impact of minimal residual disease.

Collectively, known poor prognostic factors for adult ALL did not adversely affect our patients' outcomes. However, our small sample size limited the power of the analysis, so these results should be interpreted with caution. We conclude that fludarabine with PK-guided targeting of i.v. BU can be given safely in this population and that this combination has promising activity as an allogeneic transplant conditioning regimen for adults with ALL in first CR.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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