Umbilical cord blood transplantation is a suitable option for consolidation of acute myeloid leukemia with FLT3-ITD

Internal tandem duplication (ITD) of the FMS-like tyrosine kinase (*FLT3*) gene (FLT3-ITD) is present in 10%-30% of AML.[1] FLT3-ITD$^+$ AML is associated with an increased risk of relapse and shorter overall survival.[1,2] Because of this, allogeneic hematopoietic cell transplantation (alloHCT) has been commonly used for consolidation of FLT3-ITD$^+$ AML patients. Although direct comparisons are limited, outcomes after alloHCT generally compare favorably to chemotherapy-based approaches with overall survival (OS) at two or more years as high as 60%, although results are variable.[2-5] Unfortunately, many patients lack a suitable HLA-matched donor, precluding alloHCT consolidation. Umbilical cord blood (UCB) transplantation provides an alternative donor source for alloHCT and has given promising results in many hematologic malignancies.[6-10] The delayed immune reconstitution, functional T-cell recovery, and lower incidence of graft-*versus*-host-disease (GvHD) following UCB transplantation raises concerns about a compromised graft- *versus*-leukemia effect and increased risk of disease relapse;[11] however, this has not translated into inferior outcomes for UCB transplantation in general.[12,13] How UCB transplantation performs for specific high-risk AML subsets, such as FLT3-ITD$^+$ AML, is not known. To address this, we analyzed the outcomes of UCB transplantation for FLT3-ITD$^+$ AML at our center.

We prospectively analyzed data collected from AML patients undergoing first alloHCT between 2008 and 2014 using the University of Minnesota Blood and Marrow Transplantation Database. Patients gave their consent and were treated according to protocols approved by our Institutional Review Board and registered at *clinicaltrials.gov*. Data on pre-transplantation comorbidities were collected using the HCT-specific comorbidity index (HCT-CI)[14] and were categorized as low-risk (score 0), intermediate-risk (score 1-2), or high-risk (score ≥3). Cytogenetic data (G-banded karyotype and/or FISH analyses) at diagnosis were classified according to the Southwest Oncology Group (SWOG) cytogenetic risk classification system.[15] *FLT3* mutation status was analyzed using DNA from blood or bone marrow using multiplex polymerase chain reaction (PCR), as previously described.[16] The PCR products were analyzed by capillary electrophoresis on an ABI 3130 (Foster City, CA, USA) before and after restriction enzyme digest. The *FLT3-ITD* mutation was identified by the presence of a peak size greater than the 330 base pair wild-type PCR product. Leukemia-free survival (LFS) and complete remission (CR) were defined according to the International Working Group criteria.[17] All patients were in CR at the time of alloHCT, as determined by a bone marrow biopsy performed within less than four weeks before alloHCT. The presence of minimal residual disease (MRD) at the time of transplantation was determined by flow cytometry, cytogenetic (FISH/G-banding), and *FLT3-ITD* mutation testing in some patients.

UCB grafts were matched at 4-6 of 6 HLA-A, -B (antigen level) and -DRB1 (allele level) loci to the recipient, and, in patients receiving two UCB units, were matched to each other. UCB units were required to have dose of at least 2.0x10^7 total nucleated cells (TNC)/kg with a target cell dose at least 3.0x10^7 TNC/kg. Reduced intensity conditioning (RIC) regimens included cyclophosphamide (50 mg/kg IV on day -6), fludarabine (30-40 mg/m^2 IV daily from days -6 through -2) and total body irradiation (TBI) (200 cGy on day -1) or fludarabine (30 mg/m^2 IV daily from days -6 through -2) and busulfan (3.2 mg/kg IV daily on days -5 and -4). Myeloablative conditioning (MAC) regimens included cyclophosphamide (60 mg/kg intravenously daily for 2 days) and 1320 cGy TBI in 8 fractions. Equine anti-thymocyte globulin (ATG, 15

Table 1. Patients', disease and donors' characteristics.

Variable	Category	FLT3-ITD(+) (n=22)	FLT3-ITD(-) (n=44)
Age (years) at alloHCT	Median	40	59
	IQR	28-55	45-67
	Range	10-70	4-73
Sex	Male	10 (45%)	23 (52%)
	Female	12 (55%)	21 (48%)
WBC count at diagnosis	Median	47.6	4.9
	IQR	14.7-89.7	1.7-30.0
	Range	0.6-319.8	0.9-145.0
Cytogenetics*	Favorable	1 (5%)	3 (7%)
	Intermediate	17 (77%)	26 (59%)
	Unfavorable	3 (14%)	12 (27%)
	Unknown	1 (5%)	3 (7%)
Disease status	CR1	17 (77%)	35 (80%)
	CR2 or CR3	5 (23%)	9 (20%)
Minimal residual disease	Positive	5 (23%)	4 (9%)
by flow cytometry	Negative	17 (77%)	37 (84%)
	Unknown	0 (0%)	3 (7%)
by cytogenetics/FISH	Positive	1 (5%)	2 (5%)
	Negative	5 (23%)	17 (39%)
	Unknown	6 (27%)	9 (20%)
	Normal karyotype	10 (45%)	16 (36%)
Months from diagnosis	Median	4.7	4.4
to alloHCT	IQR	3.5-5.8	3.5-7.9
	Range	2.1-28.9	2.3-26.1
Karnofsky score	< 90	3 (14%)	8 (18%)
	90-100	19 (86%)	36 (82%)
HCT-comorbidity index	0	12 (55%)	17 (39%)
	1-2	5 (23%)	13 (30%)
	3+	3 (14%)	12 (27%)
	Unknown	2 (9%)	2 (5%)
Conditioning intensity	Myeloablative	11 (50%)	11 (25%)
	Non-myeloablative	11 (50%)	33 (75%)
ATG in conditioning		4 (18%)	7 (16%)
HLA	4/6 – 5/6	11 (50%)	16 (36%)
	6/6	11 (50%)	28 (64%)
CMV serostatus	R+	16 (73%)	23 (52%)
	R-/D-	6 (27%)	20 (45%)
	R-/D+		
	Unknown		1 (2%)
GvHD prophylaxis	CSA/MMF	17 (77%)	31 (70%)
	MMF/SIROLIMUS	3 (14%)	10 (23%)
	Other	2 (9%)	3 (7%)
Number of UCB units	Single	1 (5%)	4 (9%)
	Double	21 (95%)	40 (91%)
Years of follow up	Median (range)	2.3 (1.0-6.3)	2.0 (0.7-6.3)

*alloHCT: allogeneic hematopoietic cell transplant; ATG: anti-thymocyte globulin; CMV: cytomegalovirus; CR: complete remission; CSA: cyclosporine A; D: donor; GvHD: graft-*versus*-host disease; HCT-CI: HLA: human leukocyte antigen; MMF: mycophenylate mofetil; R: recipient; UCB: umbilical cord blood; WBC: white blood cell. *Southwest Oncology Group classification.*

mg/kg) was administered every 12 hours for six doses for patients who had not received chemotherapy within three months of transplantation. GvHD prophylaxis consisted of cyclosporine A (CSA) and mycophenolate mofetil (MMF) or sirolimus plus MMF. MMF was discontinued on day +30. Granulocyte-colony stimulating factor (G-CSF) was administered to all patients from day +1 until the absolute neutrophil count was more than $2.5x10^9$/L for two days. Patients received institutional standard antimicrobial prophylaxis with fungal, bacterial, and viral directed antibiotics. One FLT3-ITD$^+$ AML patient received a tyrosine kinase inhibitor (TKI) before alloHCT for remission induction. No patient received TKI maintenance therapy after alloHCT.

The Kaplan-Meier method was used to estimate overall and disease-free survival from day of HCT. The cumulative incidence function with competing risks was used to estimate cumulative incidence of relapse (CIR) and non-relapse mortality (NRM). Statistics were calculated using R software v.3.0.2.

Patients' characteristics are summarized in Table 1. UCB transplantation was performed for 22 FLT3-ITD$^+$ and 44 FLT3-ITD$^-$ AML patients. The FLT3-ITD$^+$ and FLT3-ITD$^-$ groups were well matched for sex, disease status, HLA-matching, GvHD prophylaxis regimen, performance status, and comorbidities. The FLT3-ITD$^+$ group had higher white blood cell (WBC) counts at diagnosis and more intermediate and fewer unfavorable risk cytogenetics than FLT3-ITD$^-$ patients. FLT3-ITD$^+$ patients were younger than FLT3-ITD$^-$ patients and were more likely to receive a myeloablative conditioning regimen. The *FLT3-ITD* mutation was not detected in any of the 15 FLT3-ITD$^+$ patients who were tested at the time of transplantation. Flow cytometric evidence of MRD seemed more common in the FLT3-ITD$^+$ group. Two of the 5 FLT3-ITD$^+$ patients with flow cytometric evidence of MRD at transplant tested negative for FLT3-ITD by molecular testing.

Our analysis revealed that the 2-year CIR was similar in the FLT3-ITD$^+$ (29%, 95%CI: 8%-50%), FLT3-ITD$^-$ (36%, 95%CI: 20%-51%) (Figure 1A). NRM was 23% (95%CI: 4%-41%) and 18% (95%CI: 6%-30%) for the FLT3-ITD$^+$ and FLT3-ITD$^-$ groups at one year, respectively. Two-year LFS years was similar for FLT3-ITD$^+$ (48%, 95%CI: 31%-7%) and FLT3-ITD$^-$ (37%, 95%CI: 25%-56%) groups (Figure 1B). OS at two years was also similar for the FLT3-ITD$^+$ (47%, 95%CI: 29-75%) and FLT3-ITD$^-$ (42%, 95%CI: 29%-61%) groups (Figure 1C). OS at two years for patients with MRD$^+$ (n=9) and MRD$^-$ (n=54) by flow cytometry was 13% (95%CI: 1-43, n=9) and 49% (95%CI: 35-62), respectively.

This is the first study providing results of UCB transplantation specifically for FLT3-ITD$^+$ AML patients. The 2-year CIR of 29% and OS of 47% for UCB transplantation for FLT3-ITD$^+$ AML in our series were similar to that of FLT3-ITD$^-$ patients. Moreover, our results are similar to published outcomes following transplantation for FLT3-ITD$^+$ AML using HLA-matched sibling, HLA-haploidentical, or HLA-matched unrelated donors and superior to the 20%-30% survival that has been reported with chemotherapy consolidation alone.[3,4,18,19] Therefore, UCB transplantation seems to overcome the adverse effects of FLT3-ITD$^+$ on AML. This is consistent with standard/conventional donor (sibling or unrelated donor) alloHCT studies that have demonstrated not only lower rates of disease relapse,[18] but also improved OS for FLT3-ITD$^+$ AML who undergo alloHCT, particularly patients with a higher FLT3-ITD to wild-type FLT3 allelic ratio.[3,4,19] The patient population and transplantation characteristics were homogenous, data were detailed [e.g. tyrosine kinase inhibitor (TKI) use] FLT3-ITD and the size of the study was reasonable (given frequency of *FLT-ITD3* mutations and UCB transplantation) for a single center study. Moreover, the encouraging results seen for FLT3-ITD$^+$ AML patients in this analysis are consistent with our previous experience with UCB transplantation for a variety of high-risk hematologic malignancies.[6-9] While very few patients in our cohort received FLT3-targeted TKI therapy, the role of TKI therapy before and/or after transplantation is a topic of intense interest that warrants further investigation.[20,21] Using FLT3 mutation as an MRD marker has not been standardized or demonstrated in large studies; however, several small studies have suggested that *FLT3* mutations may be useful as an MRD marker, especially when sensitive methods are employed.[22-25] In our limited numbers, *FLT3-ITD* mutations was negative in all tested patients while some of them had residual disease by other meth-

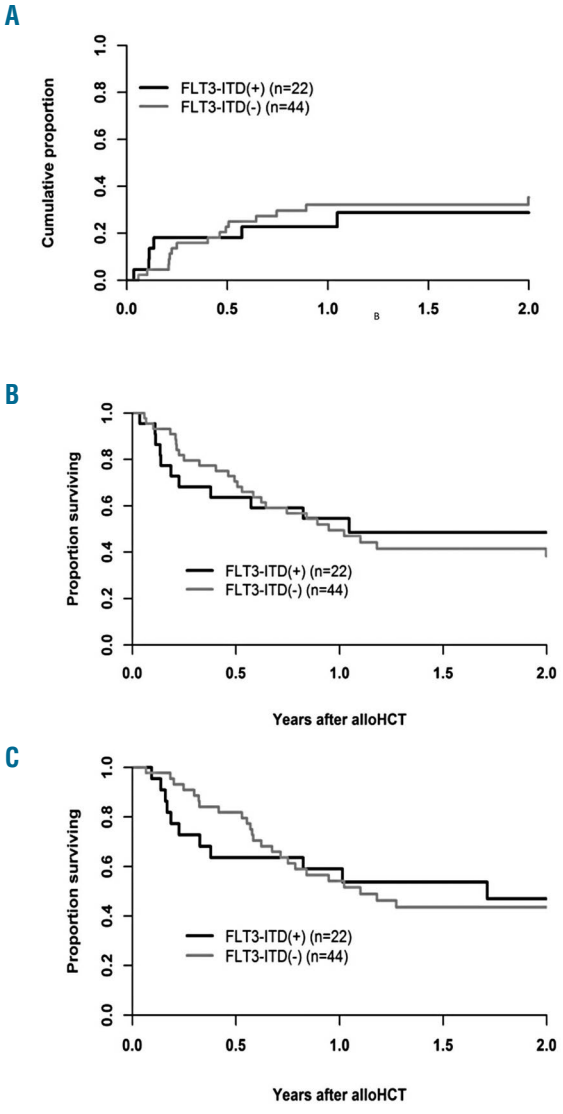

Figure 1. Two-year (A) cumulative incidence of relapse (CIR), (B) leukemia-free survival (LFS) and (C) overall survival (OS) for FLT3-ITD$^+$ and FLT3-ITD$^-$ AML cohorts.

ods, possibly due to loss of the FLT3-ITD. Change in *FLT3* mutations status at relapse (including either gain or loss of mutation) is a well-known phenomenon that occurs in a relatively small subset of patients. *FLT3* mutation testing will not be useful as an MRD marker in patients who lose their *FLT3* mutation; however, these patients have better OS and a longer time to relapse than patients who gain or retain *FLT3* mutations.[26,27] In line with reported studies by us and others,[28-30] the presence of MRD by flow cytometry might be associated with a lower OS after alloHCT regardless of *FLT3-ITD* mutation status; however, considerable caution should be exercised given that only a very small number of patients had MRD positivity.

Overall, our findings support the view that UCB is a safe and effective donor source for transplantation of FLT3-ITD$^+$ AML, thereby expanding the donor options for these high-risk patients. This is particularly important for FLT3-ITD$^+$ AML patients who have no suitable sibling donor and who, during the search for an unrelated donor, will probably experience rapid and frequent relapses that would preclude alloHCT.

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