Regular Article

TRANSPLANTATION

Alternative donor hematopoietic cell transplantation for Fanconi anemia

Margaret L. MacMillan,[1,2] Todd E. DeFor,[1,3] Jo-Anne H. Young,[4] Kathryn E. Dusenbery,[5] Bruce R. Blazar,[1,2] Arne Slungaard,[1,4] Heather Zierhut,[6] Daniel J. Weisdorf,[1,4] and John E. Wagner[1,2]

[1]Blood and Marrow Transplant Program, [2]Department of Pediatrics, [3]Biostatistics and Informatics Core, Masonic Cancer Center, [4]Department of Medicine, [5]Department of Therapeutic Radiology, and [6]Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN

Key Points

- With no prior opportunistic infections/transfusions, 5-year survival after alternative donor HCT with TBI 300 cGy, CY, FLU, and ATG was 94%.

- Today, most FA patients with standard risk disease are cured of their bone marrow failure by HCT even without an HLA-matched sibling donor.

Historically, alternative donor hematopoietic cell transplantation (HCT) for Fanconi anemia (FA) patients resulted in excessive morbidity and mortality. To improve outcomes, we made sequential changes to the HCT conditioning regimen. A total of 130 FA patients (median age, 9.0 years; range, 1-48) underwent alternative donor HCT at the University of Minnesota between 1995 and 2012. All patients received cyclophosphamide (CY), single fraction total body irradiation (TBI), and antithymocyte globulin (ATG) with or without fludarabine (FLU), followed by T-cell–depleted bone marrow or unmanipulated umbilical cord blood transplantation. The addition of FLU enhanced engraftment 3-fold. The incidence of grades 2-4 acute and chronic graft-versus-host disease was 20% and 10%, respectively. Severe toxicity was highest in patients >10 years of age or those with a history of opportunistic infections or transfusions before HCT. Mortality was lowest in patients without a history of opportunistic infection or transfusions and who received conditioning with TBI 300 cGy, CY, FLU, and ATG. These patients had a probability of survival of 94% at 5 years. Alternative donor HCT is now associated with excellent survival for patients without prior opportunistic infections or transfusions and should be considered for all FA patients after the onset of marrow failure. These studies were registered at http://www.clinicaltrials.gov as NCT00005898, NCT00167206, and NCT00352976. (*Blood*. 2015;125(24):3798-3804)

Introduction

Fanconi anemia (FA) is a rare genetically and phenotypically heterogeneous inherited disorder characterized by congenital malformations, progressive bone marrow (BM) failure, and marked predisposition to malignancy.[1] Hematological abnormalities occur in at least 90% of FA patients at a median onset of 7 years,[2,3] and allogeneic hematopoietic cell transplantation (HCT) is the only proven potential curative therapy.[4] Most FA patients do not have an HLA-identical unaffected sibling donor and therefore require an alternative (HLA-matched related or unrelated) donor. Early experiences with alternative donor HCT for the treatment of the hematological complications of FA were discouraging, with long-term survival rates of approximately 30%.[5,6] Poor outcomes were often the result of graft failure in 25% to 30% patients, graft-versus-host disease (GVHD) in 50% to 70% patients, excessive regimen-related toxicities, and opportunistic infections.[5,6]

To improve outcomes, we made sequential changes to the HCT conditioning regimen in a series of 4 prospective clinical trials. The aim of this analysis was to identify patient-, treatment-, and graft-related factors associated with favorable outcomes.

Patient and methods

Study design

This is an analysis of all patients with FA undergoing alternative donor HCT at the University of Minnesota between February 1995 and June 2012. Clinical and laboratory data were prospectively collected in the University of Minnesota Blood and Marrow Transplant Database and analyzed as of April 2014. All patients and/or guardians signed institutional review board–approved informed consent in accordance with the Declaration of Helsinki.

Patients

Eligible patients had FA with severe marrow failure (defined as having either hemoglobin <8 g/dL, platelet count <20 × 10^9/L, and/or absolute neutrophil count <5 × 10^8/L), advanced myelodysplastic syndrome (MDS with ≥5% blasts), or acute leukemia.[7] Patients were excluded from transplant if organ function was inadequate (ie, left ventricular ejection fraction <45%, any liver function

---

Table 1. Conditioning regimens and stem cell source by trial

	Trial 1	Trial 2	Trial 3	Trial 4
No. patients	23	43	16	48
Time period	1995-1998	1999-2003	2004-2006	2006-2012
Conditioning regimen	TBI 450-600, CY,* ATG†	TBI 450, FLU,‡ CY,* ATG†	TBI 450 + TS, FLU,‡ CY,* ATG†	TBI 300§ + TS, FLU,‡ CY,* ATG†
Stem cell source				
1-Ag MM-related TCD BM	2	2	0	4
HLA-matched URD TCD BM	10	31	8	24
1-Ag MM URD TCD BM	8	5	1	4
6/6 URD UCB	1	2	1	3
4-5/6 URD UCB	2	3	5	11
6/6 + 5/6 double URD UCB	0	0	1	0
5/6 + 5/6 double URD UCB	0	0	0	2
Follow-up (median, range, y)	18 (17-18)	12 (10-14)	9 (7-10)	4 (2-8)

Ag, antigen; MM, mismatched; TCD, T-cell depleted; TS, thymic shielding; URD, unrelated donor.

*10 mg/kg per day × 4 d.

†30 mg/kg per day × 5 d.

‡35 mg/m^2 per day × 4 d.

§2 patients received TBI 150.

test >5× normal, oxygen saturation <92% in room air), for poor performance status (Karnofsky <70% or Lansky <50%), active uncontrolled infection, or a history of squamous cell carcinoma within 2 years.

Transplant procedure

All patients received a conditioning regimen containing a single fraction total body irradiation (TBI), cyclophosphamide (CY) 10 mg/kg per day IV with divided dose mesna 10 mg/kg per day IV for 4 days, and equine antithymocyte globulin (ATG), with cyclosporine A as the principal agent for GVHD prophylaxis. Modifications were made over time including the addition of fludarabine (FLU) 35 mg/m^2 per day IV ×4 days (1999), the use of thymic shielding[8] during TBI (2003), a reduction of TBI to 300 cGy (2006), and the use of mycophenolate mofetil instead of methylprednisolone for GVHD prophylaxis along with cyclosporine A (2010), as shown in Table 1.

Marrow from a 7/8 allele level HLA-matched related or 7 to 8/8 allele level HLA-matched unrelated donor was ex vivo T-cell–depleted with an add back of T cells to achieve a fixed graft T-cell dose of 1×10^5 CD3/kg recipient. Unmanipulated 4 to 6/6 HLA-matched unrelated umbilical cord blood (UCB) was used based on standard matching criteria.[9]

In all groups, TBI was administered in a single fraction delivered with anteroposterior and posteroanterior fields at extended distance (dose rate was 26 cGy/min) on day –6 before HCT. The TBI dose was prescribed at the midplane of the patient at the midpelvis. Lung compensators were designed to maintain prescribed dose (±5%) to the lungs. For thymic shielding, a contrast enhanced treatment planning computed tomography scan was performed to locate the thymus. 5 half value layer Cerrobend blocks were fabricated and placed to block the thymus with a 1-cm margin from both the anterior and posterior TBI fields.

Patients were hospitalized in single rooms with high-efficiency particulate air filtration with positive pressure until neutrophil recovery. Patients received antibiotic prophylaxis at least until engraftment and antifungal antimicrobials until at least day 100. Patients received acyclovir prophylaxis if they were seropositive for herpes simplex virus and/or cytomegalovirus (CMV). Oral trimethoprim-sulfamethoxazole was given for *Pneumocystis* prophylaxis after engraftment for 1 year. Broad-spectrum IV antibacterial and as-indicated additional antifungal and/or antiviral antimicrobials were administered for fevers. CMV-seronegative recipients received CMV-safe (CMV-seronegative

or filtered) blood products. Additionally, patients received antifungal prophylaxis, including itraconazole (2000-2003) and later voriconazole (2003-2012) 1 month before admission. All patients received granulocyte colony-stimulating factor (G-CSF, Neupogen) 5 μg/kg per day IV from the day after transplant until neutrophil recovery. CMV reactivation was monitored weekly until at least day 100 following transplant and preemptively treated with ganciclovir or foscarnet.

End point definitions

Time to neutrophil recovery was the first of 3 consecutive days on which the absolute neutrophil count (ANC) was ≥0.5×10^9/L. Primary graft failure was defined as failure to achieve an ANC of 0.5×10^9/L by day 42 and secondary graft failure as an ANC <0.5×10^9/L for 3 consecutive days or 0% donor DNA by molecular analysis having previously achieved an ANC of ≥0.5×10^9/L. Time to platelet recovery was the first of 3 consecutive days (or laboratory measurements) on which the platelet count was >20×10^9/L without transfusions for 7 days before the first measurement. BM aspirations and biopsies were performed routinely at 21, 100, and 180 days and 1 and 2 years after HCT and more frequently if graft failure or relapse was suspected. Donor origin of reconstituted cells was documented by molecular analysis. GVHD was graded by standard criteria.[10]

Infection data were collected prospectively and then audited for completeness and accuracy by retrospective review of the outpatient
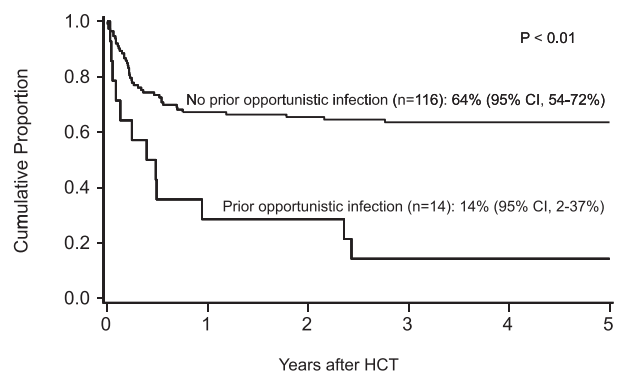
Figure 1. Probability of survival after HCT by history of opportunistic infection before HCT.
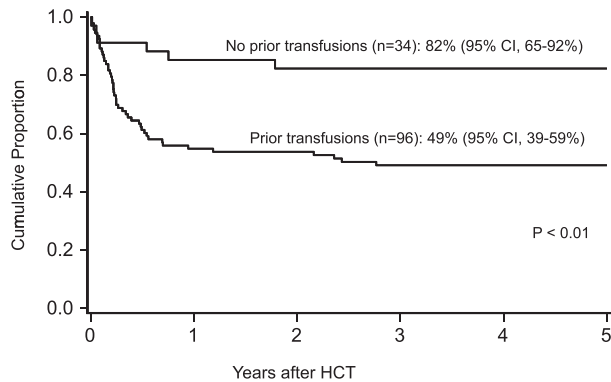
Figure 2. Probability of survival after HCT by history of transfusions before HCT.

and inpatient records of patients transplanted from 1999 and beyond, as described previously.[11] Infections were categorized as being either serious viral infections including CMV, Epstein-Barr virus, adenovirus, influenza, parainfluenza and respiratory syncytial virus, fungal infections caused by mold or yeast infections, or bacterial infections. Infections caused by *Clostridium difficile* or herpes zoster were not included. MDS or acute leukemia relapse was disease recurrence at any site and supported by cytogenetic and molecular analyses. Survival was time from transplant to death from any cause. Transplant-related mortality was death due to any cause other than recurrent MDS or leukemia.

Statistical analysis

Kaplan-Meier curves were used to estimate the probability of survival.[12] The log-rank test was used to compare outcomes between groups. Cox regression was used to examine the independent effect of factors on survival.[13] Factors considered in regression models included preparative therapy (no FLU + TBI [450 or 600 cGy] vs FLU + TBI 450 cGy vs FLU + TBI 300 cGy), donor type (HLA-mismatched related BM vs HLA-matched unrelated-donor BM vs HLA-mismatched unrelated BM vs UCB), age (<10 vs ≥10), disease status (aplastic anemia/early MDS [<5% blasts] vs late MDS [≥5% blasts]/acute myeloid leukemia), clonal cytogenetic abnormalities (yes vs no), number of malformations (<3 vs ≥3),[6] gender (male vs female), gender donor/recipient match (match vs mismatch), performance score at baseline (Lansky/Karnofsky score 60-80 vs 90-100), prior opportunistic infection (yes vs no), CMV serostatus (recipient negative/donor negative vs recipient negative/donor positive vs recipient positive), prior transfusions (none vs any), diepoxybutane (DEB) T-cell mosaicism (presence of resistant cells in ≥10% vs < 10%), DEB sensitivity (mean chromosomal breaks per cell), time-dependent acute GVHD, and prior androgen therapy (yes vs no).

All factors were tested for violations of the proportional hazards assumption using Martingale residuals. Neutrophil recovery, platelet recovery, acute and chronic GVHD, regimen-related toxicities, opportunistic infections, and mortality were estimated with cumulative incidence treating nonevent deaths as a competing risk.[14] Fine and Gray regression was used to examine the independent effect of factors for these end points.[15] The adjusted overall survival and engraftment curves of different types of conditioning in Figures 1 and 2 were computed as average estimates of the pooled sample, weighted by the proportions of the significant variables in the regression models.[16,17] Multivariate analysis of the incidence of opportunistic infections was completed by using a Cox regression–type analysis to calculate the

relative risks. However, the correlation of multiple events within each subject was taken into account by an appropriate correction to the variance estimate, and an appropriate risk set was defined for each infection. The model that performed this task was the conditional model of Prentice et al for multiple infections of a similar type.[18] Logistic regression analysis was used to determine risk factors for grade 4 or 5 toxicities using National Cancer Institute Common

Table 2. Patient and donor characteristics

Factor	N = 130 (%)
Age, y, median (range)	9 (1-48)
Male sex	68 (52)
Complementation group	
FANCA	68
FANCC	20
FANCD1 (BRCA2)	1
FANCE	1
FANCF	2
FANCG	8
Not available	30
Diepoxybutane sensitivity,* median (range)	8.9 (0.4-23.9)
Diepoxybutane mosaicism†	
Present	47
Absent	83
<3 malformations	65 (50)
Transfusions before start of conditioning therapy	
None	34 (26%)
≥1	96 (74%)
Androgen use before HCT	49 (38%)
G-CSF use before HCT	62 (48%)
Disease state before HCT	
Aplastic anemia/early MDS (<5% blasts)	120 (92%)
Late MDS (≥5% blasts)/acute leukemia	10 (8%)
Clonal abnormality before HCT	37 (28%)
Serious infection before HCT	14 (11%)
Estimated glomerular filtration rate before HCT	
≥40 mL/min	94 (72%)
<40 mL/min	5 (9%)
Not available	22 (17%)
Performance score (Lansky or Karnofsky) at the time of HCT	
60%-80%	16 (14%)
90%-100%	111 (86%)
Hematopoietic stem cell source	
HLA-matched unrelated TCD donor BM	73 (56%)
1-Ag HLA-mismatched unrelated TCD donor BM	18 (14%)
1-2 Ag HLA-mismatched single UCB	28 (22%)
1-2 Ag HLA-mismatched double UCB	3 (3%)
1-Ag HLA-mismatched TCD related donor BM	8 (6%)
Cell doses	
Median (range) BM nucleated cells (×10⁷/kg)	0.7 (0.1-14.2)
Median (range) BM CD34⁺ cells (×10⁶/kg)	2.5 (0.6-86.6)
Median (range) BM CD3⁺ cells (×10⁵/kg)	2.6 (0.6-8.6)
Median (range) UCB nucleated cells (×10⁷/kg)	3.5 (0.5-18.4)
Median (range) UCB CD34⁺ cells (×10⁶/kg)	0.35 (0.05-1.84)
Median (range) UCB CD3⁺ cells (×10⁵/kg)	110 (30-310)
Donor/recipient sex mismatch, no.	68 (52%)
Cytomegalovirus serostatus	
Patient positive	43 (33%)
Patient negative/donor positive	30 (23%)
Patient negative/donor negative	57 (44%)
Follow-up, y, median (range)	7.7 (1.8-18.8)

*Diepoxybutane sensitivity = mean chromosome breaks/cell.

†Diepoxybutane mosaicism = presence of >10% diepoxybutane-resistant T lymphoblasts).

Table 3. Factors associated with 5-year mortality: multivariate analysis

Factors	RR of mortality (95% CI)	P value compared with reference group	P value compared with second reference group
Conditioning regimen			
No FLU + TBI*	1.0		<.01
FLU + TBI 450 cGy†	0.3 (0.1-0.6)	<.01	
FLU + TBI 300 cGy	0.1 (0.03-0.2)	<.01	<.01
Hematopoietic stem cell source			
HLA-matched TCD URD BM*	1.0		
1-Ag HLA-mismatched TCD URD BM	0.3 (0.1-0.8)	.02	
1-Ag HLA-mismatched TCD RD BM	0.3 (0.03-2.1)	.21	
URD UCB	2.0 (0.9-4.4)	.08	
Age at transplant			
<10 y*	1.0		
10-17 y	2.2 (1.1-4.5)	.03	
≥18 y	2.7 (1.2-5.8)	.01	
Opportunistic infections before HCT			
No*	1.0		
Yes	3.5 (1.6-7.5)	<.01	
Transfusions before HCT			
None*	1.0		
Any	2.3 (1.0-5.6)	.06	
CMV serostatus			
Patient negative/donor negative*	1.0		
Patient negative/donor positive	1.7 (0.8-3.9)	.19	
Patient positive	2.3 (1.2-4.5)	.02	

RD, related donor.
*Reference group.
†Second reference group.

Terminology Criteria for Adverse Events, version 4.03. Analyses were performed with SAS 9.3 (SAS Institute, Cary, NC) and R 3.0.2 (R Project for Statistical Computing, http://www.r-project.org/).

Results

Patient and donor characteristics

One hundred and thirty patients with FA, median age 9.0 years (range, 1-48) with 19 (15%) >18 years), underwent alternative donor HCT (Table 2). The population reflects the expected distribution of FA gene mutations (68% FA complementation group A [FANCA], 20% FANCC, 1% FANCD1, 1% FANCE, 2% FANCF, and 8% FANCG of the 100 patients with known mutations). DEB mosaicism (ie, presence of ≥10% DEB-resistant cells) was present in 36% patients. Before HCT, patients were treated for opportunistic infections[11] (11%) or cytopenias with blood products (74%), hematopoietic growth factors (48%), or androgens (38%). Median follow-up was 7.7 years (range, 1.8-18.8).

Survival

For the entire cohort, the probability of survival at 1 year was 63% (95% confidence interval [CI], 54-71), at 5 years 58% (95% CI, 49-59), and at

10 years 57% (95% CI, 47-65-%). In regression analysis, recipients of FLU-containing regimens had a lower risk of mortality at 5 years (Table 3), especially those who received FLU and TBI 300 cGy (relative risk [RR], 0.1; 95% CI, 0.03-0.2; P < .01). Higher mortality at 5 years was associated with older age (10-17 years, RR, 2.2; 95% CI 1.1-4.5; P = .03; 18+ years, RR, 2.7; 95% CI, 1.2-5.8; P = .01), prior opportunistic infection (RR, 3.5; 95% CI, 1.6-7.5; P < .01), positive recipient CMV serostatus (RR, 2.3; 95% CI, 1.2-4.5; P = .02) and a trend toward higher mortality for patients receiving transfusions of packed red blood cells or platelets before the beginning of the conditioning regimen (RR, 2.3; 95% CI, 1.0-5.6; P = .06) (Figures 1 and 2). Factors that did not influence the risk of survival included gender, number of malformations, DEB mosaicism, complementation group, androgen or G-CSF use before transplant, disease status, clonal cytogenetic abnormalities, performance score, and renal function before transplant. For 17 patients (including 2 patients >18 years of age) without a prior history of opportunistic infection or transfusions who received a conditioning regimen of TBI 300 cGy, CY, FLU, and ATG, probability of survival at 5 years was 94% (95% CI, 65-99; Figure 3).

Given that the trials were conducted consecutively, the period of transplant and trials had a statistically collinear relationship, making it not possible to include both factors in the regression model. Notwithstanding, we investigated year of transplant within trials that spanned a timeframe of 4 to 8 years. The relative risk of mortality did not change over time within each trial, being 1.0 (95% CI, 0.5-1.8; P = .98) for trial 1 (FLU + TBI), 0.9 (95% CI, 0.7-1.1; P = .24) for trials 2 and 3 (FLU + TBI 450 cGy), and 0.8 (95% CI, 0.5-1.1; P = .18) for trial 4 (FLU + TBI 300 cGy).

Causes of death were graft failure (n = 5), GVHD (n = 10), regimen-related toxicity (n = 13), opportunistic infection (n = 17), relapse (n = 4), posttransplant lymphoproliferative disease (n = 2), and new malignancy (n = 2).

Hematopoietic recovery

The incidence of neutrophil recovery was 90% (95% CI, 84-95) at a median of 12 days (range, 8-40). Factors associated with neutrophil recovery were use of FLU in the conditioning regimen and hematopoietic stem cell source (Table 4; Figure 4). The use of UCB was associated with a lower likelihood of neutrophil recovery (RR, 0.2; 95% CI, 0.1-0.4; P < .01) compared with HLA-matched unrelated-donor BM, but was strongly influenced by the conditioning regimen. For 46 recipients of the FLU/TBI 300 cGy–based conditioning
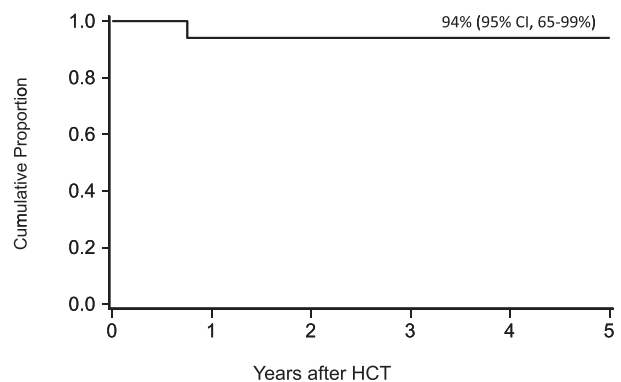
Figure 3. Probability of survival after HCT in patients without a prior history of opportunistic infection or transfusions who received conditioning with TBI 300 cGy and thymic shielding, CY, FLU, and ATG.

Table 4. Factors associated with neutrophil recovery: multivariate analysis

Factors	RR (95% CI)	P value compared with reference group	P value compared with second reference group
Conditioning regimen			
No FLU + TBI*	1.0		<.01
FLU + TBI 450 cGy†	2.6 (1.3-5.1)	<.01	
FLU + TBI 300 cGy	2.9 (1.4-6.2)	<.01	.60
Hematopoietic stem cell source			
HLA-matched TCD URD BM*	1.0		
1-Ag HLA-mismatched TCD URD BM	0.7 (0.4-1.3)	.27	
1-Ag HLA-mismatched TCD RD BM	0.6 (0.2-1.7)	.35	
URD UCB	0.2 (0.1-0.4)	<.01	
Androgen use			
No*	1.0		
Yes	0.7 (0.4-1.1)	.15	

*Reference group.
†Second reference group.

regimen, neutrophil recovery was similar in recipients of BM and UCB, occurring in 28 of 29 T-cell–depleted BM recipients and in 15 of 17 UCB recipients. Chimerism data from BM were available after 1999. All patients who achieved neutrophil recovery had complete marrow chimerism by day 100.

Platelet recovery data were not collected for patients transplanted before 2000, restricting this analysis to 105 patients. For these patients, all of whom received a FLU-containing regimen, the incidence of platelet recovery by 6 months was 77% (95% CI, 65-89) at a median of 40.5 days (range, 16-238). In regression analysis, factors associated with platelet recovery were use of androgens before transplant and hematopoietic stem cell source. Patients who received androgens before transplant (RR, 0.5; 95% CI, 0.3-0.9; P = .02) or transplanted with UCB (RR, 0.5; 95% CI, 0.3-0.8; P = .01) were less likely to achieve platelet recovery by 6 months. Importantly, no other factors, including DEB mosaicism, TBI dose, use of thymic shielding, or cell dose, adversely affect neutrophil or platelet recovery engraftment.

GVHD

The incidence of grade 2-4 and grade 3-4 acute GVHD was 20% (95% CI, 13-27%) and 9% (95% CI, 4-14), respectively. In regression analysis, the only factor associated with acute GVHD was hematopoietic stem cell source. Recipients of unrelated donor 7/8 HLA-matched T-cell–depleted BM (RR, 2.8; 95% CI, 1.1-7.4; P = .03) or 4-6/6 HLA-matched UCB (RR, 2.9; 95% CI, 1.2-7.0; P = .02) were more likely to have grade 2-4 acute GVHD than recipients of HLA-matched T-cell–depleted BM. Similarly, recipients of unrelated-donor HLA-mismatched T-cell–depleted BM (RR, 4.5; 95% CI, 1.1-19.1; P = .04) or UCB (RR, 5.5; 95% CI, 1.4-22.1; P = .02) were more likely to have severe (grade 3-4) acute GVHD than recipients of HLA-matched T-cell–depleted BM. The overall incidence of chronic GVHD was 10% (95% CI, 5-15) at 2 years. In regression analysis, the only factor associated with chronic GVHD was the use of androgens before transplant (RR, 3.4; 95% CI, 1.1-10.6; P = .03). No other factor was associated with developing chronic GVHD, although there was a trend toward higher chronic GVHD in patients with prior acute GVHD (RR, 3.8; 95% CI, 0.8-17.9; P = .09).

Regimen-related toxicity

Detailed toxicity data were available for patients transplanted after 1999 (Table 5). For these patients, the overall incidence of any nonhematological severe (grade 4 or 5) toxicity was 43% (95% CI, 34-52). In regression analysis, factors associated with the development of severe regimen-related toxicities were older age (≥10 years [odds ratio [OR], 2.9; 95% CI, 1.2-7.0; P = .02]), prior opportunistic infections (OR, 5.7; 95% CI, 1.1-29.7; P = .04), and history of any prior transfusions (OR, 5.0; 95% CI, 1.7-15.3; P < .01). No other factor, including preparative therapy or graft source, showed significant association with severe regimen-related toxicities.

Opportunistic infections

One hundred and seventy-six severe opportunistic infection events were reported in the 105 patients transplanted after 1999. Forty-five patients did not develop any opportunistic infections. Among severe opportunistic infections, 58% were bacterial, 31% were viral, and 11% were fungal. Among the disseminated fungal infections, yeast infections by *Candida* (n = 12) occurred more often than mold infections caused by *Aspergillus* (n = 7).

The frequency of all opportunistic infections per patient was higher in patients with a history of opportunistic infection before transplant (RR, 1.8; 95% CI, 1.3-2.5; P < .01) or who were CMV-seropositive (RR, 2.0; 95% CI, 1.4-2.7; P < .01). Stem cell source was not a significant factor for overall risk of opportunistic infections. Risk factors for serious bacterial infections included prior opportunistic infections (RR, 1.9; 95% CI, 1.3-3.5; P = .04), prior transfusions (RR, 1.9; 95% CI, 1.1-3.4; P = .03), and HLA-mismatched BM (RR, 2.9; 95% CI, 1.2-7.1; P = .02). Invasive fungal infections were more likely in patients who were CMV-seropositive (RR, 6.7; 95% CI, 1.6-29.0; P = .01) or received TBI ≥450 (RR, 3.7; 95% CI, 1.0-14.3; P = .06). Viral infections were more common in patients who were CMV-seropositive (RR, 4.0; 95% CI, 1.8-8.8; P < .01).

Relapse and new malignancies

Two of 10 patients with advanced MDS or acute leukemia relapsed at 32 days and 175 days after transplant for an incidence of 20% (95% CI, 1-45). Twelve patients had Epstein-Barr virus reactivation, with 8 developing posttransplant lymphoproliferative disease (PTLD), of whom 7 received unrelated donor BM and one UCB as grafts. Four patients died of PTLD or complications of therapy. Three patients developed non–lympho-hematological malignancies after transplant for an overall incidence of 3% (95% CI, 0-6). All 3 had squamous cell
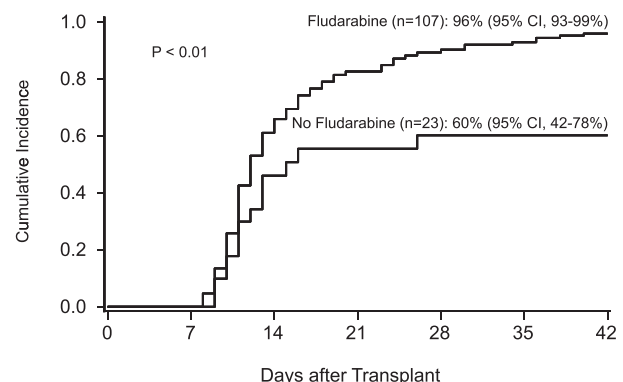
Figure 4. Probability of neutrophil recovery by use of fludarabine in the conditioning regimen.

Table 5. Severe regimen-related toxicities by trial

	Trial 2 N = 43	Trial 3 N = 16	Trial 4 N = 46	*P* value
Pneumonia	13 (30%)	3 (19%)	11 (24%)	.62
Respiratory failure	0	0	2 (4%)	.27
Renal failure	0	1 (6%)	1 (2%)	.29
Multisystem organ failure	21 (49%)	5 (31%)	9 (20%)	.01
Gastrointestinal hemorrhage	1 (2%)	1 (2%)	1 (2%)	.68
Hemorrhagic cystitis	14 (30%)	8 (50%)	17 (37%)	.47
Infectious	4	2	3	
Noninfectious	10	6	14	
Posterior reversible encephalopathy syndrome	7 (16%)	1 (6%)	2 (4%)	.14
Death before day 100	11 (26%)	4 (25%)	3 (7%)	<.01

carcinoma (mouth in 2, lung in 1) at 1, 12.4, and 14 years after transplant. None of these patients had a history of acute or chronic GVHD.

Discussion

This is the largest single-center experience of alternative donor HCT for the treatment of the hematological manifestations of FA. Establishing the optimal approach to achieve engraftment without excessive toxicity has been particularly challenging for FA patients because of their inherent DNA repair defect and intolerance to conventional doses of chemotherapy and radiation.[19-21] In 1984, based upon in vitro laboratory studies that confirmed hypersensitivity of FA cells to chemotherapy[19,20] and radiation,[22] Gluckman et al proposed the use of low-dose chemotherapy and unfractionated radiation in conditioning regimens,[21] which led to less toxicity and an improved yet suboptimal 2-year survival rate of 29%.[5] Graft failure occurred in approximately 1/3 of FA patients,[5,23] especially in those with T-cell mosaicism,[23] suggesting that the presence of DEB-resistant T cells increased the risk of rejection. In 1999, FLU, an antimetabolite with profound immunosuppressive qualities, was added to the conditioning regimen, leading to higher rates of engraftment, even in patients with mosaicism. This beneficial effect of FLU on engraftment has also been reported by the Center for International Blood and Marrow Transplant Registry[24] and the European Group for Blood and Marrow Transplantation,[25] together establishing the importance of FLU in the conditioning regimens for FA patients undergoing alternative donor HCT. Importantly, this effect was sustained even when the TBI dose was decreased to 300 cGy.

Although the trials were conducted consecutively, we observed a similar risk of mortality within a given trial over time, suggesting that the conditioning regimen change with each trial was the primary factor responsible for improved outcomes in our cohort. Importantly, we have demonstrated that successful alternative donor HCT for FA patients can be achieved with less conditioning therapy, which is particularly pertinent for this patient population with DNA repair defects.

Thymic shielding was employed to minimize thymic damage from TBI to potentially hasten immune recovery.[26-28] Importantly, there was no deleterious effect of thymic shielding on engraftment. Although there was a trend toward lower rates of opportunistic infections in patients who had thymic shielding, its role on enhancing immune recovery requires larger patient numbers.

Although UCB has historically been associated with inferior engraftment rates, the conditioning regimen, degree of HLA mismatch,

and cell dose are key factors.[29,30] Our results demonstrate that UCB is a suitable hematopoietic stem cell source in conjunction with our FLU- and TBI 300–based conditioning regimen, increasing the likelihood of identifying a suitable graft for most FA patients. New methods of ex vivo expansion are under investigation, potentially changing the pace of hematopoietic recovery and engraftment after UCB transplantation in the future. Despite an increased incidence of GVHD, it is encouraging that 1 antigen-mismatched related and unrelated T-cell–depleted marrow was not associated with higher mortality; however, the number of patients is limited to fully evaluate these stem cell sources.

Reducing the risk of GVHD is particularly important because the inherently high risk for malignancies, particularly squamous cell carcinomas of the head and neck, is magnified in FA patients who develop GVHD.[25,31,32] The risk of acute and chronic GVHD rates was low in this cohort.[24,25,32] Importantly, we observed a 3-fold increased risk of acute GVHD in recipients of T-cell–depleted HLA-mismatched BM or UCB compared with recipients of HLA-matched BM, demonstrating that HLA-matched BM is still the preferred alternative donor graft source. Additional strategies are needed to overcome GVHD in patients with HLA-mismatched BM or UCB donors.

Few patients in our series developed malignancies compared with other large series in the literature,[25,32] which may be at least partly due to their low incidence of acute and GVHD. However, follow-up in our most recent cohort is insufficient for evaluating malignancy risk. Eight patients developed PTLD, all of whom received ATG, a known risk factor for developing PTLD.[33,34] To potentially decrease the risk of PTLD, we have eliminated ATG in our current conditioning regimen for FA patients receiving alternative donor HCT.

Although age <10 years was associated with better outcomes, a finding in other disease groups undergoing HCT was that FA patients should only undergo HCT when necessary for severe marrow failure, advanced MDS, or acute leukemia.[35-37] Additional prognostic factors observed by us and others include donor source, CMV serostatus, prior transfusions, and opportunistic infections but not number of malformations.[6,24,25,29]

Over the past 20 years, historical obstacles of excessive regimen-related toxicities, immunological graft rejection, and GVHD have been circumvented with lower dose conditioning, the addition of FLU and graft T-cell depletion. As a result, heightened surveillance is required to minimize transplant risk factors such as opportunistic infections, need for transfusions, or advanced MDS/leukemia. Although there is renewed interest in the use of androgens in this patient population, there are deleterious side effects that should be considered because they may adversely affect transplant outcomes.[38-40] Alternative therapies such as androgens or hematopoietic cell gene correction may best be reserved for patients without donors or those who have risk factors for high mortality (eg, older age, poor organ function). Today, most FA patients with standard risk disease are cured of their BM failure by HCT even if an HLA-matched sibling donor is not available.

Acknowledgments

Authorship

Contribution: M.L.M., T.E.D., B.R.B., K.E.D., and J.E.W. designed the research; all authors performed the research; T.E.D. performed the statistical analysis; all authors analyzed and interpreted the data; M.L.M. wrote the initial draft of the manuscript and edited the final draft; and all authors reviewed the draft manuscript and approved the final version for submission.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Margaret L. MacMillan, Blood and Marrow Transplant Program, Department of Pediatrics, University of Minnesota, MMC 484, 420 Delaware St, SE, Minneapolis, MN 55455; e-mail: macmi002@umn.edu.

References

1. Fanconi G. Familial constitutional panmyelocytopathy, Fanconi's anemia (F.A.). I. Clinical aspects. *Semin Hematol.* 1967;4(3):233-240.

2. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. *Blood.* 1994;84(5):1650-1655.

3. Kutler DI, Singh B, Satagopan J, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood.* 2003;101(4):1249-1256.

4. Wagner JE, MacMillan ML, Auerbach AD. Hematopoietic Cell Transplantation for Fanconi's Anemia. In: Appelbaum FR, Forman SJ, Negrin RS, Blume KG, eds. Thomas' Hematopoietic Cell Transplantation, 4th ed. Oxford: Blackwell Publishing Ltd; 2008:1178-1199.

5. Gluckman E, Auerbach AD, Horowitz MM, et al. Bone marrow transplantation for Fanconi anemia. *Blood.* 1995;86(7):2856-2862.

6. Guardiola P, Pasquini R, Dokal I, et al. Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. *Blood.* 2000;95(2):422-429.

7. Cioc AM, Wagner JE, MacMillan ML, DeFor T, Hirsch B. Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with fanconi anemia: morphologic and cytogenetic characteristics. *Am J Clin Pathol.* 2010;133(1):92-100.

8. Dusenbery K, Gerbi B. Total body irradiation conditioning regimens in stem cell transplantation. In: Levitt SH, Purdy JA, Perez CA, Vijayakumar S, eds. Technical Basis of Radiation Therapy: Practical Clinical Applications. 5th ed. Berlin: Springer; 2012.

9. Brunstein CG, Barker JN, Weisdorf DJ, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. *Blood.* 2007;110(8):3064-3070.

10. MacMillan ML, Weisdorf DJ, Wagner JE, et al. Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. *Biol Blood Marrow Transplant.* 2002;8(7):387-394.

11. Barker JN, Hough RE, van Burik JA, et al. Serious infections after unrelated donor transplantation in 136 children: impact of stem cell source. *Biol Blood Marrow Transplant.* 2005;11(5):362-370.

12. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.

13. Cox DR. Regression models and life tables. *J R Stat Soc.* 1972;34:187-220.

14. Lin DY. Non-parametric inference for cumulative incidence functions in competing risks studies. *Stat Med.* 1997;16(8):901-910.

15. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc.* 1999;94(446):496-509.

16. Zhang X, Loberiza FR, Klein JP, Zhang MJ. A SAS macro for estimation of direct adjusted survival curves based on a stratified Cox regression model. *Comput Methods Programs Biomed.* 2007;88(2):95-101.

17. Gail MH, Byar DP. Variance calculations for direct adjusted survival curves, with applications to testing for no treatment effect. *Biom J.* 1986;28(5):587-599.

18. Prentice R, Williams B, Peterson A. On the regression analysis of multivariate failure time data. *Biometrika.* 1981;68(2):373-379.

19. Berger R, Bernheim A, Gluckman E, Gisselbrecht C. In vitro effect of cyclophosphamide metabolites on chromosomes of Fanconi anaemia patients. *Br J Haematol.* 1980;45(4):565-568.

20. Auerbach AD, Adler B, O'Reilly RJ, Kirkpatrick D, Chaganti RS. Effect of procarbazine and cyclophosphamide on chromosome breakage in Fanconi anemia cells: relevance to bone marrow transplantation. *Cancer Genet Cytogenet.* 1983;9(1):25-36.

21. Gluckman E, Berger R, Dutreix J. Bone marrow transplantation for Fanconi anemia. *Semin Hematol.* 1984;21(1):20-26.

22. Gluckman E, Devergie A, Dutreix J. Radiosensitivity in Fanconi anaemia: application to the conditioning regimen for bone marrow transplantation. *Br J Haematol.* 1983;54(3):431-440.

23. MacMillan ML, Auerbach AD, Davies SM, et al. Haematopoietic cell transplantation in patients with Fanconi anaemia using alternate donors: results of a total body irradiation dose escalation trial. *Br J Haematol.* 2000;109(1):121-129.

24. Wagner JE, Eapen M, MacMillan ML, et al. Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. *Blood.* 2007;109(5):2256-2262.

25. Peffault de Latour R, Porcher R, Dalle JH, et al; FA Committee of the Severe Aplastic Anemia Working Party; Pediatric Working Party of the European Group for Blood and Marrow Transplantation. Allogeneic hematopoietic stem cell transplantation in Fanconi anemia: the European Group for Blood and Marrow Transplantation experience. *Blood.* 2013;122(26):4279-4286.

26. Järplid B. Thymic shielding in irradiated mice. *Acta Radiol Ther Phys Biol.* 1972;11(6):536-544.

27. Vávrová J, Petýrek P. Shielding of the thymic region during X-irradiation: effect on haemopoiesis in mice. *Folia Biol (Praha).* 1982;28(6):415-425.

28. Amagai T, Kina T, Hirokawa K, Nishikawa S, Imanishi J, Katsura Y. Dysfunction of irradiated thymus for the development of helper T cells. *J Immunol.* 1987;139(2):358-364.

29. Gluckman E, Rocha V, Ionescu I, et al; Eurocord-Netcord and EBMT. Results of unrelated cord blood transplant in Fanconi anemia patients: risk factor analysis for engraftment and survival. *Biol Blood Marrow Transplant.* 2007;13(9):1073-1082.

30. Ayas M, Al-Seraihi A, El-Solh H, et al. The Saudi experience in fludarabine-based conditioning regimens in patients with Fanconi anemia undergoing stem cell transplantation: excellent outcome in recipients of matched related stem cells but not in recipients of unrelated cord blood stem cells. *Biol Blood Marrow Transplant.* 2012;18(4):627-632.

31. Deeg HJ, Socié G, Schoch G, et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. *Blood.* 1996;87(1):386-392.

32. Guardiola P, Socié G, Li X, et al. Acute graft-versus-host disease in patients with Fanconi anemia or acquired aplastic anemia undergoing bone marrow transplantation from HLA-identical sibling donors: risk factors and influence on outcome. *Blood.* 2004;103(1):73-77.

33. Brunstein CG, Weisdorf DJ, DeFor T, et al. Marked increased risk of Epstein-Barr virus-related complications with the addition of antithymocyte globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. *Blood.* 2006;108(8):2874-2880.

34. Landgren O, Gilbert ES, Rizzo JD, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. *Blood.* 2009;113(20):4992-5001.

35. MacMillan ML, Wagner JE. Haematopoeitic cell transplantation for Fanconi anaemia - when and how? *Br J Haematol.* 2010;149(1):14-21.

36. Mitchell R, Wagner JE, Hirsch B, DeFor TE, Zierhut H, MacMillan ML. Haematopoietic cell transplantation for acute leukaemia and advanced myelodysplastic syndrome in Fanconi anaemia. *Br J Haematol.* 2014;164(3):384-395.

37. Ayas M, Saber W, Davies SM, et al. Allogeneic hematopoietic cell transplantation for fanconi anemia in patients with pretransplantation cytogenetic abnormalities, myelodysplastic syndrome, or acute leukemia. *J Clin Oncol.* 2013;31(13):1669-1676.

38. Kumar AR, Wagner JE, Auerbach AD, et al. Fatal hemorrhage from androgen-related hepatic adenoma after hematopoietic cell transplantation. *J Pediatr Hematol Oncol.* 2004;26(1):16-18.

39. Touraine RL, Bertrand Y, Foray P, Gilly J, Philippe N. Hepatic tumours during androgen therapy in Fanconi anaemia. *Eur J Pediatr.* 1993;152(8):691-693.

40. Velazquez I, Alter BP. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. *Am J Hematol.* 2004;77(3):257-267.