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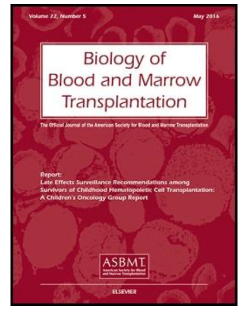

Title: Comparable Outcomes after HLA-Matched Sibling and Alternative Donor Hematopoietic Cell Transplantation for Children with Fanconi Anemia and Severe Aplastic Anemia

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PII: S1083-8791(17)30878-9
DOI: https://doi.org/10.1016/j.bbmt.2017.11.031
Reference: YBBMT 54888

To appear in: *Biology of Blood and Marrow Transplantation*

Received date: 27-9-2017
Accepted date: 28-11-2017

Title. Comparable outcomes after HLA-matched sibling and alternative donor hematopoietic cell transplantation for children with Fanconi Anemia and Severe Aplastic Anemia

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Short title: Comparable outcomes after MSD and AD HCT for FA SAA

HIGHLIGHTS

- MSD- and AD-HCT for FA associated SAA have largely equivalent outcomes
- 5-year OS is 88%, equivalent between MSD- and AD-HCT
- Neutrophil and platelet recovery are 96 and 92%, respectively, equivalent between MSD- and AD-HCT
- Severe acute and chronic GVHD rates are <10%, equivalent between MSD- and AD-HCT
- Viral infection incidence density is slightly higher for AD- compared to MSD-HCT

Abstract: Fanconi anemia (FA) associated severe aplastic anemia (SAA) requires allogeneic hematopoietic cell transplantation (HCT) for cure. With the evolution of conditioning regimens over time, alternative donor HCT (AD-HCT) outcomes have dramatically improved. We compared outcomes of HLA-matched sibling donor HCT (MSD-HCT, n=17) to AD-HCT (n=57) for FAassociated SAA at a single institution from 2001-2016. Overall survival at five years was 94 vs. 86%, neutrophil engraftment 100 vs. 95%, platelet recovery 100 vs. 89%, grade II-IV acute GVHD 6 vs. 12%, grade III-IV acute GVHD 6 vs. 4%, and chronic GVHD 0 vs. 7% for MSD- and ADHCT, respectively, with no statistically significant differences by type of transplant. Use of UCB was associated with decreased rates of neutrophil recovery (in AD-HCT) and platelet recovery (in both MSD- and AD-HCT). A trend toward higher serious infection density before day +100 post-HCT was observed in AD- as compared to MSD-HCT (p=0.02). These data demonstrate that AD-HCT should be considered at the same time as MSD-HCT for FA patients with SAA.

INTRODUCTION

Fanconi anemia (FA) is a rare disorder resulting from mutation in one of 22 genes[1, 2] encoding critical proteins for DNA damage repair during replication. One major consequence of faulty DNA repair is apoptosis and progressive contraction of the hematopoietic stem cell (HSC) pool, ultimately manifest as cytopenias and SAA. To date, the only curative therapy for FA-associated SAA is allogeneic hematopoietic cell transplantation (HCT). Historically, HCT outcomes were far superior for FA patients receiving bone marrow (BM) or umbilical cord blood (UCB) HSCs from an HLA-matched sibling donor (MSD)[3] as compared to an alternative donor (AD; HLA-matched or –mismatched non-sibling related donor or unrelated donor)[4-8]. However, recent efforts to optimize conditioning regimens and donor graft manipulation have greatly improved AD HCT for FA-associated SAA[9].

HCT for FA is uniquely challenging given the intrinsic difficulties of FA affected cells to tolerate DNA damage incurred by standard conditioning regimen chemotherapy[10] and radiation[11]. Recognizing these cellular sensitivities, Gluckman pioneered an effective, reduced toxicity HCT conditioning regimen combining low-dose alkylator cyclophosphamide with limited irradiation in the mid-1980's.[12-14] For the past 20 years, MSD recipients conditioned with low-dose cyclophosphamide, fludarabine and anti-thymocyte globulin experience graft failure or severe grade III/IV acute graft-versus-host disease (GVHD) at incidences <10%, with 5-year overall survival (OS) exceeding 90%.[3] These excellent outcomes prompted some families lacking a MSD for their child affected by FA-associated SAA to pursue preimplantation genetic diagnosis and in vitro fertilization

(PGD/IVF) to select an FA unaffected HLA-matched embryo to create a MSD while expanding their family[15, 16]. Alternatively, AD-HCT was delayed with use of transfusions and androgen therapy, both identified as independent risk factors for poor survival after HCT[4, 9, 17, 18]

While conditioning regimens for MSD recipients have remained largely unchanged for the past 2 decades, HCT conditioning regimens for AD recipients have evolved to reduce the comparatively high morbidity and mortality. Lympho-depleting fludarabine was incorporated to support donor engraftment/reduce graft rejection, thymic shielding was added during radiation to improve neo-thymopoiesis and reduce post-HCT infectious complications, radiation dosing was optimized, and bone marrow grafts were manipulated by CD34+ selection to control the donor T cell dose and reduce graft-versus-host disease (GVHD)[9, 19, 20]. The impact of these sequential changes on AD HCT in the FA population were recently published.[9] Here we directly compare the results of MSD to AD HCT in the modern era for FA-associated SAA at a single center.

MATERIALS AND METHODS

Study design

Pediatric (age <18 years) patients with FA associated SAA undergoing HCT at the University of Minnesota between June 2001 and June 2016 were identified from a prospectively recorded Blood and Marrow Transplant Database of demographic, clinical and laboratory outcome measures, with analysis completed as of April 2017. All parents/guardians signed Institutional Review Board-approved informed consent, in accordance with the Declaration of Helsinki.

Patients

Patients eligible for HCT had confirmed diagnoses of FA by chromosomal breakage analysis and SAA defined as persistent absolute neutrophil count <5 x 10^7/L, and/or hemoglobin <8 g/dL and/or platelet count <20 x 10^9/L. Patients with advanced myelodysplastic syndrome[21], leukemia and/or BRCA2/FANCD1 mutations were excluded from this analysis, while patients with inadequate organ function (left ventricular ejection fraction < 45%, any liver function test >5x normal, oxygen saturation <92% in room air), active uncontrolled infection, poor performance status (Lansky <50%), or a personal history of squamous cell carcinoma within 2 years were ineligible for HCT. Thirty AD-HCT recipients reported here were included in an earlier publication.[9]

Transplant procedure

Patients with MSDs were conditioned as previously described[3] with fludarabine 175 mg/m^2 (35 mg/m^2 daily IV x 5 days, day -6 to -2), cyclophosphamide 20 mg/kg (5 mg/kg IV daily x 4 days, day -6 to -3), and equine anti-thymocyte globulin (ATG) 150 mg/kg (30 mg/kg IV daily x 5 days, day -6 to- 2, pre-medicated with methylprednisolone 2 mg/kg/d). Stem cell sources included either HLA-identical BM or UCB.

Patients with ADs were conditioned with a single 300 centigray (cGy) fraction of total body irradiation (TBI; day -6; administered with thymic shielding as previously described[9]), fludarabine 140 mg/m^2 (35 mg/m^2 daily IV x 4 days, day -5 to -2), and cyclophosphamide 40 mg/kg (10 mg/kg IV daily x 4 days, day -5 to -2). AD recipients additionally received equine ATG 150 mg/kg (30 mg/kg IV daily x 5 days, day -5 to -1, pre-medicated with methylprednisolone 2 mg/kg/d), methylprednisolone alone, or neither during conditioning. AD stem cell sources included non-genotypically identical related donor or unrelated donor BM or UCB.

For both MSD and AD recipients, BM grafts underwent T cell depletion (using elutriation prior to 2002, Isolex 300 from 2002-2010, and Miltenyi Biotec CliniMACs for CD34+ selection since 2010)[9]. An add back of T cells to achieve a fixed graft dose of 1 x 10^5 CD3+ T cells/kg was provided. Umbilical cord blood (UCB) stem cell sources were unmanipulated.

GVHD prophylaxis

For all patients, GVHD prophylaxis began on day -3 prior to transplant with the combination of cyclosporine (CSA) or sirolimus, and either mycophenylate mofetil (MMF) or methylprednisolone (MP). CSA was dosed either orally or intravenously (IV) targeting a goal trough of 200-400 mg/L, sirolimus was dosed orally targeting a goal trough of 4-12 mg/L. A protocol driven change from CSA to sirolimus was time-limited, affecting only 10 AD recipients, given the lack of an IV formulation and poor tolerance. Sirolimus use was only used in 10 patients, given the lack of an IV formulation. MMF was dosed 15 mg/kg/dose every 8 hours IV or orally with a maximum dose of 1 gm) and discontinued on day +30 or seven days following neutrophil engraftment, whichever occurred later. MP was dosed 2 mg/kg/day until day +15, then tapered off by day +24. A 10 week CSA taper began for MSD recipients on day +100 and for AD recipients on day +180, if no active GVHD or at least one month after control of acute GVHD.

Supportive care

Prior to admission, patients were placed on yeast/mold antifungal prophylaxis for one month. Upon admission, patients were hospitalized in single occupancy rooms with positive pressure high-efficiency particulate air filtration. Antibiotic prophylaxis was provided through neutrophil engraftment; anti-fungal prophylaxis until at least day +100. Patients seropositive for herpes simplex virus or cytomegalovirus (CMV), as well as those with a CMV seropositive donor, received acyclovir anti-viral prophylaxis until day +100. Prophylaxis against Pneumocystis juroveci pneumonia was provided with trimethoprim-sulfamethoxazole (or alternative in cases of medication allergy) after engraftment until 1 year post-HCT. Intravenous anti-infective coverage was broadened empirically for fever and subsequently adjusted per infection surveillance results. All patient received CMV-safe (CMV-seronegative or filtered) blood products. All patients received GCSF 5 mcg/kg IV daily from day +1 through neutrophil engraftment. Weekly blood CMV polymerase chain reaction (PCR) surveillance was prescribed until day +100 post-HCT with pre-emptive ganciclovir or foscarnet therapy upon identification.

End point definitions

Time to neutrophil recovery was the first of 3 consecutive days with absolute neutrophil count (ANC) $\geq 0.5 \times 10^9/L$ following ANC nadir. Inability to achieve ANC of $0.5 \times 10^9/L$ by day +42 defined primary graft failure. Secondary graft failure was defined as a decline in

ANC to <0.5 x 10^9/L x 3 consecutive days or 0% donor DNA by molecular analysis, having previously achieved an ANC of ≥0.5 x 10^9/L. Time to platelet recovery was the first of 3 consecutive days of platelet count >20 x 10^9/L without transfusion in the preceding 7 days. Bone marrow aspiration and biopsy was routinely performed at 21, 100, 180 days, 1 and 2 years after HCT. Additional evaluations were prompted by concern for graft failure or malignant transformation. Donor chimerism was assessed by molecular analysis at these time points as well. GVHD was scored using standard criteria.[22]

Infectious complication data were collected prospectively then audited retrospectively to ensure completeness and accuracy as previously described[23]. Overall survival (OS) was defined as time from transplant until death of any cause.

Statistical analysis

Statistical comparisons by donor type (MSD versus AD) for categorical factors were completed with the chi-square test or Fisher's exact test, in cases of limited expected counts, and for continuous factors by the general Wilcoxon rank test for non-parametric data. Unadjusted estimates of OS were calculated by Kaplan-Meier curves[24]. Unadjusted estimates of neutrophil engraftment, platelet engraftment, and GVHD were analyzed using cumulate incidence treating non-event mortality as a competing risk[25]. While primary comparisons in univariate analysis were outcomes by donor type, additional factors examined included patient gender (male vs female), patient-donor gender match, (match vs mismatch), age (0-9 vs 10-17 years), number of congenital malformations (0-2 vs 3+), pre-HCT transfusions (none vs any), pre-HCT renal function by glomerular filtrations rate (GFR;

normal vs abnormal vs <40 ml/min/1.73 m^2), diepoxybutane (DEB) chromosomal breakage mosaicism (≥90-100% affected peripheral blood lymphocytes vs <90%), pre-HCT GCSF use (no vs yes), and year of HCT (2001-2005 vs 2006-2010 vs 2011-2015). The independent effect of donor type on OS was assessed by Cox regression[26] and on engraftment and GVHD by Fine and Gray proportional hazards regression[27]. Visual plots and plots and Martingdale residuals were used to test for violations of the proportional hazards assumption[28]. Rates of serious infections were reported from time of transplant to day +100 as infections per 1000 patient-days, allowing for multiple infections per patient, and compared across donor type by the Mantel-Haentsel chi-square test for incidence densities. Given multiple comparisons, only p-values <0.01 are considered significant in analysis of serious infections. All reported p-values were two-sided. SAS 9.3 (SAS Institute, Cary, NC) and R 3.0.2 (R foundation for Statistical Computing, Vienna, Austria) were used for all statistical analyses.

RESULTS

Patient, donor, and HCT characteristics

Seventy-four patients with FA-associated SAA underwent HCT, MSD (n=17) and AD (n=57). There were no differences between MSD and AD recipients in regards to age at time of transplant, gender, recipient-donor gender mismatch, bone marrow versus umbilical cord blood donor source, time from diagnosis to HCT, complementation group, diepoxybutane (DEB) sensitivity (mean number of chromosome breaks/cell *in vitro*) or mosaicism (defined as present if >10% of cells revealed resistance to DEB), number of congenital malformations, incidence of androgen use or number of blood product transfusions prior to

Table 1. Patient, donor, and HCT characteristics

	All Patients (n=74)	MSD-HCT (n=17)	AD-HCT (n=57)	P-value
PRE-HCT CHARACTERISTICS				
Patient sex (male)	42 (57%)	8 (47%)	34 (60%)	0.36
Patient-donor sex mismatch	34 (47%)	9 (53%)	25 (45%)	0.59
Age at HCT in years, median (range)	8 (2-15)	8 (3-13)	8 (2-15)	0.74
Months from diagnosis to HCT, median (range)	27 (2-143)	23 (2-131)	27 (3-143)	0.29
Complementation group				NA
FANCA	50 (68%)	12 (71%)	38 (67%)	
FANCC	10 (14%)	1 (6%)	9 (16%)	
FANCD2	3 (4%)	3 (18%)	0	
FANCF	1 (1%)	0	1 (2%)	
FANCG	3 (4%)	0	3 (5%)	
FANCJ	2 (3%)	0	2 (4%)	
FANCP	1 (1%)	0	1 (2%)	
Unknown	4 (5%)	1 (6%)	3 (5%)	
DEB sensitivity* median (range)	7.0 (0.4-16.0)	7.0 (1.2-13.5)	6.9 (0.4-16.0)	0.89
DEB mosaicism**	23 (31%)	6 (35%)	17 (30%)	0.73
Congenital malformations				0.91
0-2	27 (36%)	6 (35%)	21 (37%)	
3+	47 (64%)	11 (65%)	36 (63%)	
Prior therapies				
Blood product transfusions (≥1)	40 (54%)	6 (35%)	34 (60%)	0.08
Androgen use	3 (4%)	0	3 (5%)	0.33
GCSF use	32 (43%)	2 (12%)	30 (53%)	**<0.01**
Organ function and performance status				
Serious infection	0	0	0	--
Patient CMV IgG positive	26 (35%)	7 (41%)	19 (33%)	0.55
Glomerular filtration rate				>0.99
≥40 ml/min/1.73 m^2	69 (93%)	16 (94%)	53 (93%)	
<40 ml/min/1.73 m^2	5 (7%)	1 (6%)	4 (7%)	
Lansky performance scale score (<100%)	13 (18%)	4 (24%)	9 (16%)	0.48
HCT CHARACTERISTICS				

	All Patients (n=74)	MSD-HCT (n=17)	AD-HCT (n=57)	P-value
Year of transplant				**<0.01**
2001-2005	7 (9%)	7 (41%)	0	
2006-2010	37 (50%)	7 (41%)	30 (53%)	
2011-2015	30 (41%)	3 (18%)	27 (47%)	
Stem cell source				NE
8/8 HLA MSD BM	9 (12%)	9 (53%)		
6/6 HLA MSD UCB	8 (11%)	8 (47%)		
8/8 HLA parental BM	1 (1%)		1 (2%)	
7/8 HLA mMSD BM	1 (1%)		1 (2%)	
7/8 HLA mm parental BM	2 (3%)		2 (4%)	
5/6 HLA mMSD sUCB + BM boost	1 (1%)		1 (2%)	
5-6/6 HLA (m)MUD BM			32 (56%)	
4-6/6 HLA (m)MUD sUCB	52 (70%)		20 (35%)	
Stem cell dose				
TNC ($x10^7$/kg), median (range)	2.7 (0.1-24.8)	3.9 (0.3-17.4)	2.4 (0.1-24.8)	0.73
BM	0.7 (0.1-24.8)	0.5 (0.3-17.4)	0.7 (0.1-24.8)	
UCB	4.7 (2.2-12.0)	4.6 (2.2-12.0)	5.3 (2.2-10.2)	
CD34 ($x10^6$/kg), median (range)	1.8 (0.04-15.4)	2.7 (0.2-10.1)	1.8 (0.04-15.4)	0.87
BM	3.1 (0.04-15.4)	4.1 (1.7-10.1)	2.6 (0.04-15.4)	
UCB	0.7 (0.1-2.7)	0.5 (0.2-2.7)	0.7 (0.1-1.8)	
GVHD prophylaxis				NE
MP alone	1 (1%)	1 (6%)		
CSA + MP	41 (55%)	13 (76%)	28 (49%)	
CSA + MMF	22 (30%)	3 (18%)	19 (33%)	
Sirolimus + MMF	10 (14%)		10 (18%)	
Length of HCT hospitalization				
Median days (range)	28 (11-211)	24 (20-52)	28 (11-211)	**0.02**
Years of Follow-Up, median (IQR)	7.0 (3.9-9.6)	10.3 (8.0-14.0)	6.2 (3.3-8.7)	**<0.01**

* Diepoxybutane sensitivity = mean chromosome breaks/cell

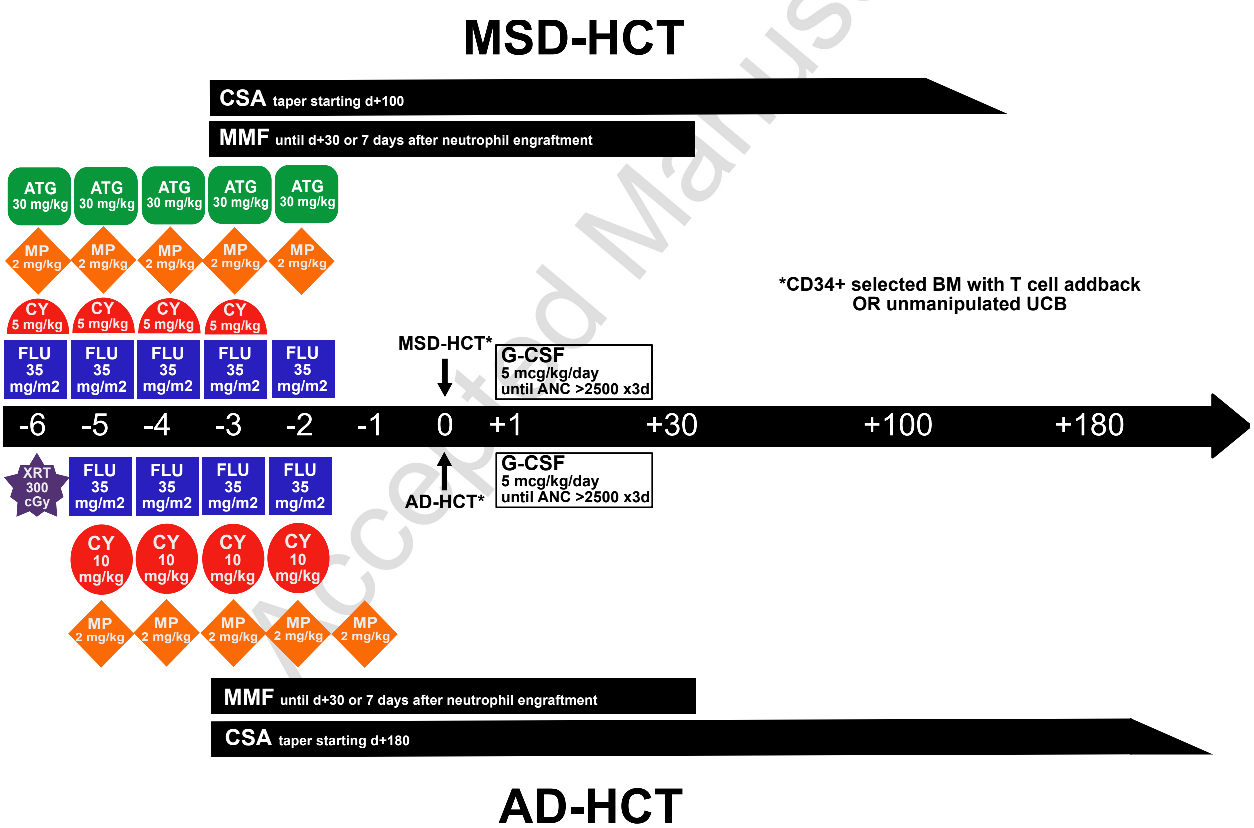
** Diepoxybutane mosaicism = presence of >10% DEB-resistant lymphocytes

HCT, hematopoietic cell transplantation; MSD, matched sibling donor; AD, alternative donor; GCSF, granulocyte-colony stimulating factor; HLA, human leukocyte antigen; BM, bone marrow; UCB, umbilical cord blood; mMSD, mismatched sibling donor; mm, mismatched; sUCB, single umbilical cord blood; GVHD, graft-versus-host disease; MP, methylprednisolone; CSA, cyclosporine; MMF, mycophenylate mofetil; IQR, interquartile range

HCT (Table 1). AD recipients did demonstrate statistically significant greater use of GCSF prior to HCT (MSD 12%, AD 53% GCSF use, p<0.01). Evaluations immediately prior to HCT revealed no difference between MSD and AD recipients in regards to renal function, performance scale scoring, or current serious infection (the latter absent in all recipients).

Conditioning regimens and GVHD prophylaxis were as described in the Methods as well as Figure 1. Stem cell sources for MSD recipients included BM in 9 (53%) and UCB in 8 (47%),

Figure 1. MSD- and AD-HCT conditioning regimens and GVHD prophylaxis for FA-associated SAA

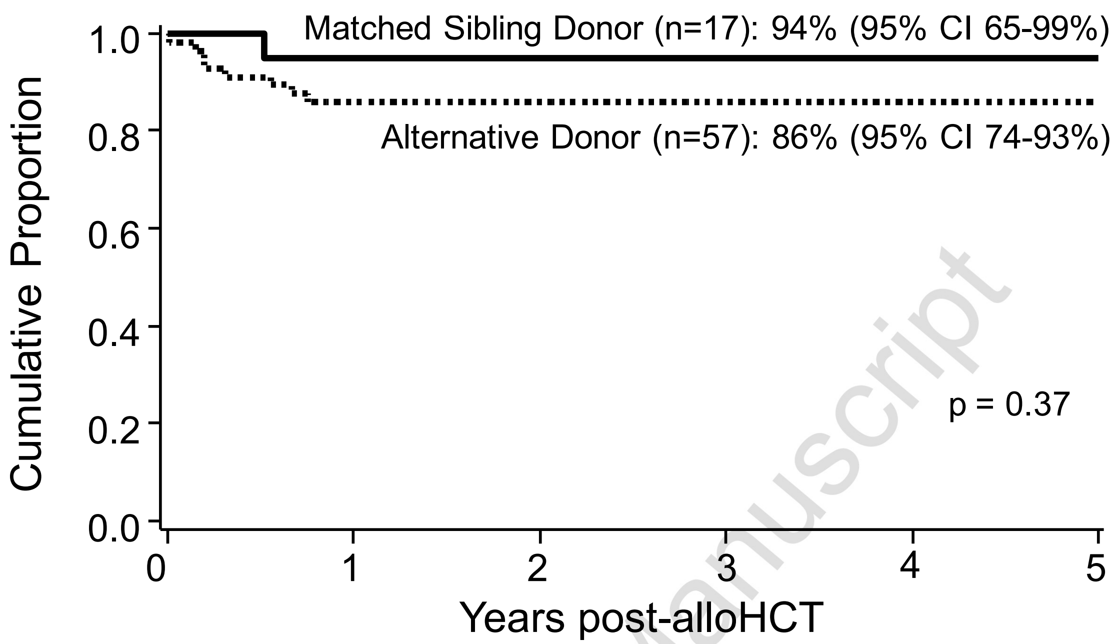


MSD-HCT, matched sibling donor hematopoietic cell transplant; CSA, cyclosporine; MMF, mycophenylate mofetil; MP, methylprednisolone; ATG, equine anti-thymocyte globulin; CY, cyclophosphamide; FLU, fludarabine; G-CSF, granulocyte-colony stimulating factor; ANC, absolute neutrophil count; XRT, irradiation (with thymic shielding); cGy, centigray; SIRO, sirolimus; AD-HCT, alternative donor hematopoietic cell transplant with median cell dose of 3.9 x 10^7 total nucleated cells(TNC)/kg patient weight and 0.5 x 10^6 CD34+ cells/kg. For AD recipients, stem cell sources were heterogeneous in regards to donor and degree of HLA-match as shown in Table 1, with the majority of patients receiving BM [n=36 (63%); UCB n=20 (35%), combination of BM and UCB n=1 (2%)]. Median cell dose for AD-HCT recipients was 2.2 x 10^7 TNC/kg and 0.7 x 10^6 CD34+ cells/kg. Cell doses were not statistically different between MSD- and AD-HCT. Length of HCT hospitalization (including those patients who died prior to discharge) was a median of four days longer for AD-HCT recipients (28 days, range of 11-211 days) compared to MSD-HCT recipients (24 days, range of 20-52 days), p=0.02.

Survival

Overall survival at 5 years post-HCT was not statistically different for the 2 groups, being 94% for MSD-HCT recipients (95% CI 65-99%) and 86% for AD-HCT recipients (95% CI 74-93%), (p=0.37; Figure 2). Median follow-up for MSD- and AD-HCT recipients is 10.3 and 6.2 years, respectively, with all deaths occurring within the 1st year after HCT. Risk of death increased with poor pre-HCT renal function, with only 60% OS for the 5 patients with GFR <40 ml/min/1.73 m^2, compared to 90% for 69 patients with better renal function (univariate analysis, p=0.02; multiple regression HR 5.4, p=0.04). Pre-HCT androgen use was also associated with decreased 5-year OS, with survival estimate of 33% for the 3 patients requiring androgen and 90% for the 71 patients without exposure (univariate analysis, p<0.01; multiple regression HR 10.1, p<0.01). However, for both of these factors, the low patient numbers reduce confidence in the reported significance.

Figure 2. No difference in 5-year overall survival between MSD-HCT and AD-HCT



Donor type had no association with overall survival in multiple regression (AD-HCT HR 1.9, 95% CI 0.2-16.2, p=0.54, compared to the MSD-HCT reference group). However, a statistically significant difference was observed in overall survival by stem cell source with UCB associated with an increased risk of death compared to BM in multiple regression (UCB HR 6.3, 95% CI 1.3-31.0, p=0.02). There were too few deaths to further investigate the interaction between donor type (MSD vs. AD) and stem cell source (BM vs. UCB). Of the 9 patients who died following HCT, one was a MSD-HCT recipient who died of GVHD. Eight deaths were AD-HCT recipients with varied attributable causes including graft failure (n=3), GVHD (n=2), infection (n=1), regimen related toxicity (n=1), and multi-organ failure (n=1).

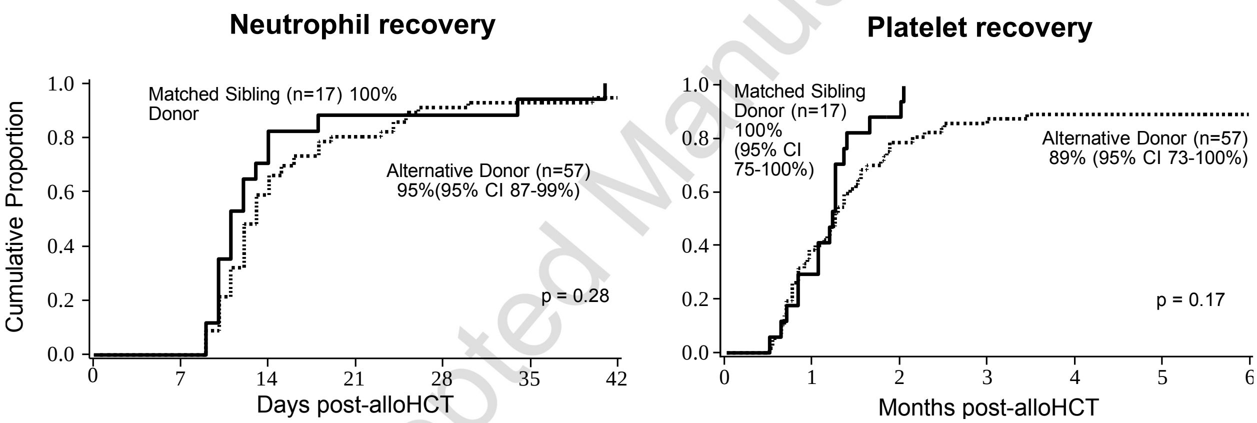
Engraftment

Neutrophil engraftment was achieved by day +42 after HCT in 96% of patients, with no statistically significant differences observed between MSD-HCT and AD-HCT recipients ($p=0.28$, Figure 3). The median day of neutrophil recovery for MSD-HCT recipients was day +11 (9-41) and AD-HCT recipients day +13 (9-40) after HCT. In multiple regression, no factors were associated with neutrophil recovery, including donor type (AD-HCT HR 0.8, 95% CI 0.5-1.4, $p=0.42$, compared to MSD-HCT as the reference group). In a separate multiple regression model investigating the interaction between donor type (MSD vs. AD) and stem cell source (BM vs. UCB) with MSD BM as the reference group, both MSD UCB (HR 0.1, 95% CI 0.04-0.3, $p<0.01$) and AD UCB (HR 0.1, 95% CI 0.03-0.2, $p<0.01$) recipients had increased risk of failed neutrophil engraftment. However, AD UCB did not convey an increased risk of failed neutrophil engraftment when compared to MSD UCB (HR 0.9, 95% CI 0.3-1.7, $p=0.49$). There were too few graft failures to further evaluate risk factors. Briefly, the three affected patients received mismatched unrelated UCB prior to routine assessment of donor specific anti-HLA antibodies. Cell doses were within the expected range for high probability of engraftment (3.1-3.7 x 10^7 TNC/kg and 0.3-0.7 x 10^6 CD34/kg recipient weight).

Platelet recovery was achieved by 6 months after HCT in 92% of patients, with no statistically significant differences observed between MSD-HCT (100% platelet engraftment, 95% CI 75-100%) and AD-HCT recipients (89% platelet engraftment, 95% CI 73-100%, $p=0.17$, Figure 3). In multiple regression, only the need for blood product transfusions prior to HCT was associated with failed platelet recovery (HR 0.5, 95% CI 0.3-

0.9, p=0.01). In a separate multiple regression model investigating the interaction between donor type (MSD vs. AD) and stem cell source (BM vs. UCB) with MSD BM as the reference group, risk of failed platelet recovery was statistically significantly higher in all other groups: AD BM (HR 0.4, 95% CI 0.2-0.8, p=0.01), MSD UCB (HR 0.4, 95% CI 0.2-0.8), p<0.01), and AD UCB (HR 0.3, 95% CI 0.1-0.5, p<0.01). However, as with neutrophil engraftment, no increased risk was seen for failed platelet recovery comparing AD UCB to MSD UCB (HR 0.6, 95% CI 0.3-1.2, p=0.14).

Figure 3. No difference in neutrophil or platelet count recovery between MSD-HCT and AD-HCT

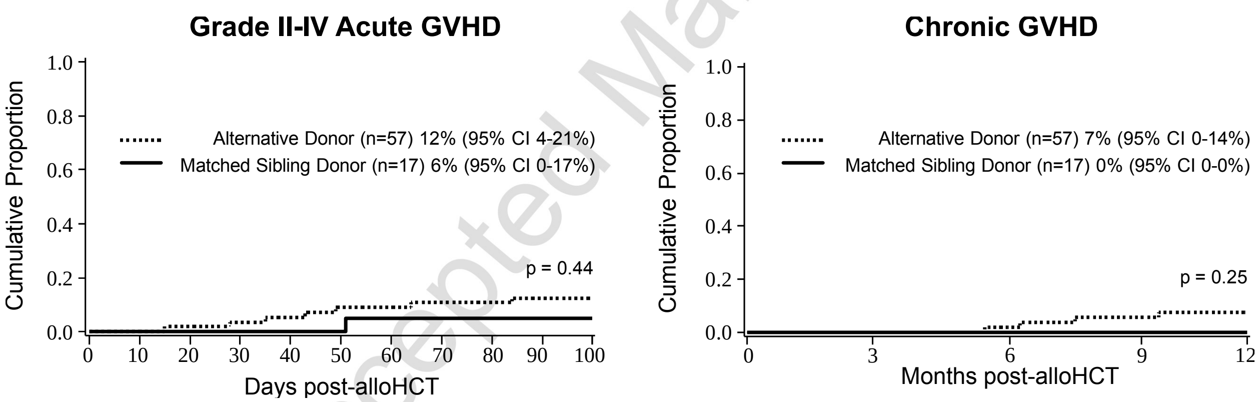


GVHD

The incidence of grade II-IV acute GVHD by day 100 in all patients was 11% (95%CI 4-18%). Univariate analysis revealed no difference by donor type [MSD-HCT 6% (95% CI 0-17%) versus AD-HCT 12% (95% CI 4-21%), p=0.44, figure 4], but an increased risk with UCB as a stem cell source compared to BM [UCB 21% (95% CI 6-35%) versus BM 4% (95% CI 0-10%), p=0.02)]. In multiple regression, UCB was associated with grade II-IV acute GVHD (HR 5.1, 95% CI 1.1-24.3, p=0.04). However, the incidence of acute GVHD was too rare to further evaluate an interaction between donor type (MSD vs. AD) and stem cell type (BM vs. UCB) and this complication.

Incidence of grade III-IV acute GVHD by day 100 was 4% (95% CI 0-9%, Figure 2), occurring in one MSD and two AD recipients. No patient or transplant factors were associated with severe acute GVHD. The incidence of chronic GVHD by 12 months post-HCT was 5% (95% CI 0-11%), occurring only in four AD recipients with no associated patient or transplant risk factors (Figure 4).

Figure 4. No difference in incidence of acute or chronic GVHD between MSD-HCT and AD-HCT



Infectious complications

No significant differences in infection density were identified between MSD-HCT and AD-HCT recipients (Table 2). AD-HCT recipients trended toward more infections overall, with 10.9 serious infections per 1000 patient days compared to 4.71 for MSD-HCT recipients (p=0.02). Specifically, AD-HCT recipients demonstrated higher rates of non-Clostridium difficile serious bacterial infections (infection density of 7.63 in AD-HCT compared to 2.35

in MSD-HCT per 1000 patient day, p=0.02), though this difference failed to reach statistical significance. There were no differences in of candida or aspergillus fungal infections, CMV, EBV, or other viral infections. Neither CD4+ T cell count nor IgG level at day 100 was associated with incidence of bacterial, fungal, or viral infection (data not shown).

Table 2. Infection density. Number of infections per 1000 patient days occurring prior to day +100 post-HCT

Serious Infection	All Patients (n=74)	MSD-HCT (n=17)	AD-HCT (n=57)	P-value*
Fungal				
Aspergillus	0.42	0	0.55	0.34
Candida	0.28	0.59	0.18	0.38
Bacterial				
Non-C. diff	6.39	2.35	7.63	0.02
Viral				
CMV	1.11	1.17	1.09	0.93
EBV	0.42	0	0.55	0.34
Other**	0.83	0.59	0.91	0.69
Total Serious Infections per 1000 patient days	9.44	4.71	10.90	0.02

*Only p-values <0.01 should be considered significant due to multiple comparisons
** Other viral infections include: adenoviremia, HHV-6 pneumonitis and viremia, HSV viremia, BK viremia with associated hemorrhagic cystitis requiring anti-viral therapy, varicella, and influenza A

MSD-HCT, matched sibling donor hematopoietic cell transplantation; AD-HCT, alternative donor hematopoietic cell transplantation; C. diff., clostridium difficile; CMV, cytomegalovirus; EBV, Epstein-barr virus

DISCUSSION

Using modern strategies of conditioning and GVHD prophylaxis, we have demonstrated that children with FA undergoing HCT for SAA have excellent survival, neutrophil and platelet engraftment and low rates of acute and chronic GVHD, with equivalent outcomes using MSD and AD stem cell sources. Current recommendations for monitoring of bone marrow function in FA include complete blood count with differential every three months and annual bone marrow biopsies with aspirate for cellularity, screening for myelodysplastic changes, cytogenetic evolution, and leukemia by flow cytometry[20]. HCT is indicated for SAA, defined as persistent absolute neutrophil count <5 x 10^7/L, and/or hemoglobin <8 g/dL and/or platelet count <20 x 10^9/L. Optimal timing for HCT and donor selection is best accomplished at a blood and marrow transplant center with FA expertise.

Historically, FA patients with SAA lacking a MSD received androgen therapy and/or blood product transfusions to delay AD-HCT, given inferior outcomes. However, we now have evidence of comparable outcomes with MSD- and AD-HCT and additional evidence that such delay strategies are deleterious. Here we report pre-HCT androgen exposure is associated with decreased 5-year overall survival. While our confidence in these findings is low given the small number of patients falling into these exposure groups (only 3 of 74 with androgen use), pre-HCT androgen use has been identified as an independent risk factor for poor survival in several studies of HCT for FA[4, 17, 29]. We also found platelet engraftment to be negatively correlated with pre-HCT blood product transfusion(s). While not directly assessed, this association is suggestive of alloimmunization interfering with platelet recovery. With evidence of equivalent outcomes with MSD and AD-HCT for FA associated SAA, patients lacking a MSD should proceed expeditiously to AD-HCT before exposure to androgens and/or blood product transfusions.

Of the 17 FA patients undergoing MSD-HCT, four (24%) sibling donors were products of PGD/IVF. This complex process involves genetic testing to ensure a selected embryo is both free of FA and an HLA-match to the existing child with FA prior to pregnancy[30]. With dramatic improvements in the outcomes of AD-HCT for FA as demonstrated here, families may no longer feel obligated to undergo such costly and emotionally taxing measures to find a reliable donor source for their affected child.

UCB appears to be a less favorable stem cell source compared to BM, here associated with delayed neutrophil recovery, increased incidence of grade II-IV acute GVHD, and poorer survival, though we were unable to further associate this latter finding with donor type given the small number of deaths. HLA-mismatch of UCB is a known risk factor for transplant-related mortality in patients with non-malignant disease. Of the 5 AD-HCT deaths after UCBT, examination of high-resolution HLA-typing of HLA-A, -B, -C, and -DRB1 revealed donor-recipient pairs were mismatched at 2 of 8 alleles in two patients and 3, 4, and 6 of 8 alleles in one patient each. MacMillan et al.[9] recently reported on a larger cohort of FA AD-HCT recipients from the University of Minnesota (n=130, not limited to SAA as HCT indication) again showing delayed neutrophil recovery with UCB, but no statistically significant difference in OS between stem cell source (BM vs. UCB). The association of increased grade II-IV acute GVHD and UCB likely reflects the heterogeneity within the AD UCB recipient group, encompassing HLA-matching ranging from 4-6/6, as well as the extremely low rates of GVHD realized with TCD of BM. In the literature on HCT for FA, T cell depleted BM is the optimal stem cell source for this population, demonstrating low rates of GVHD, <10% for severe grades III-IV acute GVHD and <5% for chronic GVHD[9, 31]. Minimization of GVHD is critical in this patient population where this post-HCT complication contributes to development of head and neck cancers[7, 32, 33].

Recipients of AD grafts trend toward higher incidence of infections and demonstrate statistically significantly longer duration of hospitalization after HCT compared to MSD recipients, highlighting future targets for improvement. Considerations for differences between AD and MSD recipient rates of infection include the role of serotherapy and/or UCB use in delayed T cell reconstitution, and the lack of virus specific T cells available in the donor inoculum when utilizing UCB stem cell sources. Higher rates of infection may also contribute to the slightly longer duration of hospitalization for AD-HCT recipients. The lack of association between day 100 CD4+ T cell count or IgG level with infection density is not surprising. While lower cell counts and immunoglobulin levels might be expected to correlate with increased infection, increases in these values can be a reflection of appropriate immune response to infection making these measures poor biomarkers of infection risk.

Limitations to this report include small number of subjects, particularly in the MSD-HCT group, and restriction to a single center. However, this latter limitation allows for homogeneity in transplant eligibility between MSD- and AD-HCT groups and consistent data collection and reporting.

In summary, patients with FA-associated SAA today have equivalent chance of long-term survival with low risk of GVHD with either MSD or AD stem cell sources for HCT. Transplant should not be delayed for lack of a MSD. To further improve upon these AD-HCT outcomes, we are considering methods to reduce the incidence of post-HCT infections and subsequently improve hospital length-of-stay and overall survival. Such methods may include replacement of CD34+ selection of BM with TCR-αβ depletion, allowing for infusion of innate type γδ-T cells with strong anti-viral effects[34] and prompt utilization of adoptive immunotherapy with virus-specific T cells to supplement current anti-viral pharmacotherapies. T cell depleted BM continues to show superior outcomes compared to UCB as a stem cell source, in regards to overall survival, neutrophil recovery, and rates of GVHD, and is preferred when available. Methods of ex-vivo stem cell expansion may be utilized to bolster stem cell numbers and improve hematopoietic recovery following UCBT[35].

ACKNOWLEDGEMENTS

This work was partially supported by the Fanconi Anemia Research Fund, Children's Cancer Research Fund and Kidz1stFund. We would like to thank our patients and families affected by Fanconi Anemia.

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