Published in final edited form as:

Pediatr Transplant. 2011 September ; 15(6): 628–634. doi:10.1111/j.1399-3046.2011.01542.x.

Favorable preliminary results using TLI/ATG-based immunomodulatory conditioning for matched unrelated donor allogeneic hematopoietic stem cell transplantation in pediatric severe aplastic anemia

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Abstract

To assess whether a tolerance-induction regimen could be applied for unrelated (MUD) HCT in severe aplastic anemia (SAA), we retrospectively reviewed our HCT experience using unmanipulated 10/10 HLA-matched bone marrow grafts from matched sibling donors (MSD) versus MUD donors. Conditioning was cyclophosphamide 200 mg/kg (CTX) + rabbit anti-thymocyte globulin 10 mg/kg (ATG) for MSD (n = 9) and total lymphoid irradiation (TLI, 800 cGy) + CTX/ATG for MUD HCT (n = 5). Immunoprophylaxis was cyclosporine A (CSA) and short-course methotrexate. Median patient age was 14.7 years, median time to HCT 1.5 years, and median follow-up 3.0 years. Outcome measures included event-free survival (EFS), time to engraftment, and cumulative incidence of GVHD (CIN of GVHD) for MSD and MUD cohorts. EFS and stable engraftment rate were 100%. CIN of acute GVHD was: MSD, Grade I-II: 1 (11%), Grade III-IV: 0%; MUD, Grade I-II: 1 (20%), Grade III-IV: 1 (20%). CIN of chronic GVHD was: MSD, limited: 1 (11%), extensive: 0%; MUD, limited: 0%, extensive: 0%. All immunosuppressive-compliant patients successfully weaned immunosuppression. Though in limited patients, our results suggest that immunomodulatory TLI added to backbone CTX/ATG conditioning is a promising option for MUD HCT in SAA patients which we will examine in a prospective clinical trial.

Keywords

Hematopoietic stem cell transplantation; Pediatrics; Aplastic Anemia; Engraftment; Graft-versus-host disease; Non-myeloablative; Transplant Tolerance

INTRODUCTION

The current standard therapy for severe acquired aplastic anemia (SAA) patients is allogeneic matched related donor (MSD) hematopoietic cell transplantation (HCT) or, if no

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AUTHOR CONTRIBUTIONS: Concept/design: AP; Data Analysis/interpretation: AP, CH, JY; Patient care: AP, CH, AS, BT, MD, WL; Drafting/reviewing article: AP, JY, WL; Statistics: CW, DP, JY; Critical revision of manuscript: AP, WL.

MSD is available, immunosuppressive therapy (IST) with cyclosporine (CSA) and anti-thymocyte globulin (ATG) (1-5). Response rates of 60–80% and survival rates of 80–90% occur with IST (4–6), but roughly 40% of patients never achieve normalization of peripheral blood counts (4). In a recent long-term follow-up of patients treated with an alternative IST regimen of high-dose cyclophosphamide, the rate of hematologic response was 70% among the best responding IST-naïve patient subset, failure-free survival at 10 years was 58%, and there remained complications of late clonal evolution to myelodysplasias (7).

Among patients lacking MSDs in whom IST fails, most will require allogeneic HCT with an alternative donor source [matched unrelated donor (MUD), mismatched related donor (MMRD), or umbilical cord blood (UCB) stem cell source]. Alternative donor source HCT for SAA patients has included high transplant-related morbidity (TRM) including graft failure, infection, and/or graft-versus-host disease (GVHD) (8-12). The historical disparity between outcomes as well as TRM for MUD versus MSD HCT for SAA using conventionally accepted transplant conditioning regimens, has limited up-front HCT as a curative therapy for SAA patients lacking a MSD (9-15). Hence, development of therapies which reduce TRM are critical to allow broader access to early curative HCT for SAA patients.

To maintain durable donor cell engraftment, an aggressive myeloablative approach has historically remained the mainstay, both in SAA patients with MSD HCT and in those failing immunosuppression receiving MUD HCT (9-11, 14). The prevailing assumption has been that the high engraftment/alloreactivity barrier in SAA cannot be surmounted except by using myeloablative chemo- or radiation therapy to induce sufficient immunosuppression or clearance of reactive recipient T cell populations. However, more recently non-myeloablative regimens have been applied to HCT for a number of non-malignant disorders to reduce TRM, both for MSD HCT (9, 13, 15) and to facilitate alternative donor application when a MSD does not exist (13, 15). Our long-range goal is to adopt a novel immunoregulatory strategy including total lymphoid irradiation + anti-thymocyte globulin (TLI/ATG) (16-22) in combination with immunodepletive cyclophosphamide (CTX, 200 mg/kg) conditioning to mitigate non-engraftment while decreasing TRM, thus opening the field to allow curative MUD HCT for SAA and potentially for other marrow failure syndromes. In this context, we present preliminary outcome data of a stratified donor-based approach for pediatric patients using standard CTX/ATG conditioning for MSD HCT and immunomodulatory TLI added to CTX/ATG for MUD HCT. Our preliminary data demonstrate comparable outcomes between MSD and MUD HCT in a limited number of patients, a finding we will pursue in future prospective clinical trial using donor-adapted conditioning for MSD versus MUD donor HCT for pediatric and young adult SAA patients.

METHODS

Patients

A total of 14 patients underwent allotransplantation from minimally 10/10 high-resolution HLA matched related and unrelated donors for SAA at St. Jude Children's Research Hospital (St. Jude) from 01/01/1998 to 08/01/2009. All of these patients were included in the analysis. All patients had diagnostic bone marrow cytogenetics revealing no karyotypic abnormalities, and patients from 2004 onwards had routine screening to exclude major genetic syndromes associated with bone marrow failure, including Fanconi Anemia and Dyskeratosis Congenita.

Data Collection and Analysis

All data were collected retrospectively using the St Jude database with de-identified patient data. All data collection and analysis was performed according to the rules and regulations of the Health Information Portability and Accountability Act and St. Jude Institutional Review Board (IRB). A written protocol was submitted to and approval obtained from the St. Jude IRB prior to initiation of data collection.

Conditioning regimens

All MSD patients received conditioning of CTX 200 mg/kg divided in 4 daily doses from day -5 to day -2 and rabbit ATG 1 mg/kg on day -4 and 3 mg/kg/day from days -3 to -1 (total ATG dose 10 mg/kg). All MUD transplant recipients received the same CTX/ATG conditioning with the addition of TLI 200 cGy per day from days -9 to -6 (total TLI dose 800cGy). TLI field included the central lymphovascular tissues extending from the cervical region through the mediastinum and pulmonary hila, aorto-caval spaces to include the splenic trunk and spleen, and an inverted-Y field to encompass the common iliac trunk through the inguino-femoral branches terminating at the proximal aspect of the acetabula bilaterally. Supine positioning and an anterior-posterior treatment approach were used, with multi-leaf collimation blocking to spare normal tissues including head, lungs, and pelvic organs such as ovaries and urinary bladder.

Post-transplant immunosuppression

GVHD prophylaxis for both MSD and MUD recipients was methotrexate (MTX) short-course at a dose of 15 mg/m² intravenously on day 1 followed by 10 mg/m² intravenously on days 3, 6, and 11, and cyclosporin A (CSA) 5 mg/kg/day intravenously (transitioned to oral) beginning day -2, targeted to maintain trough levels in range 150-250 ng/mL. All MSD patients underwent CSA taper beginning at day +100, and all MUD patients began CSA taper at day +180, per standard clinical practice guidelines at our institution.

Patient monitoring assessments, prophylaxis, and routine supportive care

Peripheral blood monitoring of CMV, EBV, and adenovirus by PCR assay was performed routinely on a weekly basis on all patients and pre-emptive directed anti-viral therapy initiated for rising PCR levels in appropriate clinical contexts. Engraftment was monitored by weekly peripheral blood chimerism through day +100 or until criteria for platelet engraftment were fulfilled in the case of MUD HCT, including subset chimerism in cases of decreasing overall chimerism. Bone marrow aspiration and biopsy to assess morphology and chimerism was obtained by day 30, following achievement of criteria for absolute neutrophil count (ANC) engraftment. Renal, hepatic, and nutritional parameters were assessed daily by peripheral blood monitoring, and patients with mucositis or otherwise unable to maintain oral nutrition received intravenous total parenteral nutrition (TPN). All patients received anaerobic antibiotic prophylaxis of oral metronidazole, *Pneumocystis jirovecii* prophylaxis of trimethoprim/sulfamethoxazole or pentamidine, and antifungal prophylaxis with micafungin or voriconazole. All patients who were HSV or CMV seropositive or had a CMV seropositive donor received acyclovir prophylaxis throughout the peri-transplant period until the peripheral blood CD3 cell count was $> 0.4 \times 10^9/L$. All patients received intravenous immune globulin (IVIg) weekly until serum Ig > 400 ng/dL one month following last IVIg infusion.

Outcome Measures

The main outcome measures estimated time to engraftment of ANC and platelets, cumulative incidence of GVHD within one year (CIN of GVHD, Year 1), and event-free survival (EFS). ANC engraftment was defined as achievement of a peripheral blood ANC $>$

$0.5 \times 10^9/L$. Platelet engraftment was defined as achievement of an unsupported peripheral blood platelet count $> 20 \times 10^9/L$. Time to CD3 count $> 0.4 \times 10^9/L$ was also determined by records review of monthly T cell subset analyses on peripheral blood. Overall survival (OS) was defined as the time from the date of transplant to death due to any cause or to the last follow-up date. Patients who were still alive without experiencing an event at their last follow-up date were considered censored in OS estimates. CIN of GVHD, Year 1 was defined as the time from date of transplant to GVHD or to the last follow-up date within year 1, with death prior to GVHD within year 1 as a competing event for GVHD. Patients who survived greater than one year from date of transplant without having GVHD were treated as censored with 365 days as time at risk. This category included: (a) patients who had GVHD only after year 1 ($n = 0$), and (b) patients who did not have GVHD and survived longer than one year from the date of transplant ($n = 11$). Acute and chronic GVHD were graded by modified Seattle criteria, which was the clinical standard during this period (23). EFS was defined as the time from date of post-transplant transfusion independence to the occurrence of any event. An event was defined as transplant-related death or recurrence of transfusion dependence. Patients who did not experience an event at their latest follow-up date were considered censored in EFS estimates.

Statistical Analysis

EFS distributions according to various subgroups were estimated by the method of Kaplan and Meier (24). CIN of GVHD, Year 1 was estimated as described by Kalbfleisch and Prentice (25). Wilcoxon rank-sum test was used to compare the time to ANC and platelet engraftment between MSD and MUD groups. SAS version 9.2 (SAS Institute, Cary, NC) and StatXact (Cytel Corporation, Cambridge, MA) Windows version 8 were used for statistical analysis.

RESULTS

Patient characteristics

Patient characteristics are summarized in Table 1. All patients fulfilled clinical criteria for SAA, including bone marrow cellularity $\leq 25\%$ plus at least 2 of the following additional criteria: ANC $< 0.5 \times 10^9/L$, platelet count $< 20 \times 10^9/L$, and reticulocyte count $< 20 \times 10^9/L$ or $< 1\%$ corrected for patient hemoglobin (Hb). All patients were chronically platelet and erythrocyte (PRBC) transfusion-dependent at time of transplantation.

Serologic status

Five of 9 patients in the MSD group and 4 of 5 patients in the MUD group were CMV sero-positive. Donors were CMV sero-positive in 4 of 9 MSD transplants and in 2 of 5 MUD transplants. Donor-recipient pairs were CMV sero-negative in 2 of 9 MSD transplants and 1 of 5 MUD transplants.

Transplant characteristics

All patients received a bone marrow stem cell source that was high-resolution matched by molecular typing at 10/10 HLA loci (HLA-A, -B, -C, -DRB1, -DQB1) to the patient. All 14 patients received an unmanipulated marrow product. Nine patients received MSD transplants, and 5 patients received MUD transplants. Mean total nucleated cell (TNC) dose, CD34⁺ cell dose, and CD3⁺ cell dose are shown in Table 2.

Engraftment

The incidence of sustained donor engraftment was 100% in both MSD and MUD transplants. Notably, neutrophil engraftment occurred at a median 22 days for both MSD

and MUD transplants, with no statistical difference between these 2 groups ($p = 0.87$) (Table 3). Median time to CD3 count $> 0.4 \times 10^9/L$ was 8.5 months for MSD and 10.8 months for MUD transplants. Platelet engraftment occurred at a median of 26 days for MSD and 43 days for MUD transplants. The difference at all platelet engraftment timepoints was not statistically significant between MSD and MUD subsets ($p = 0.28$) (Table 3). In the MUD group, 3 of 5 patients achieved platelet engraftment by 45 days post-HCT, and 1 patient by 189 days. A fifth patient had ANC engraftment at 22 days but required 449 days to meet platelet engraftment criteria due to effects of anti-epileptic medications for antecedent seizure disorder. This patient recovered platelet count rapidly and has maintained a platelet count $> 100 \times 10^9/L$ following alteration of anti-epileptic medications. All patients remain packed red blood cell (PRBC) and platelet transfusion-independent as of point of last follow-up. Median time to achievement of transfusion independence was 26 days for the MSD group and 43 days for the MUD group. This difference was not statistically significant ($p = 0.10$) (Table 3).

Donor Chimerism

Following both MSD and MUD transplants, 13 of 14 patients achieved sustained 100% donor chimerism by restriction fragment length polymorphism (RFLP) analysis of the peripheral blood at day 28 and weekly thereafter (data not shown). One patient in the MSD group was non-compliant with immunosuppression and developed rapidly downtrending split donor chimerism (88-89% donor total peripheral blood chimerism, with 8.3% CD3 cell chimerism) at day +56 post-HCT; this patient received 5 bi-weekly donor leukocyte infusions (DLI) in escalating doses from $2.5 \times 10^4/kg$ to $2.5 \times 10^5/kg$ donor CD3⁺ cells and converted to stable 100% donor chimerism. DLIs were well-tolerated in this patient without associated GVHD. All patients remained at stable 100% peripheral blood donor chimerism at their latest follow-up.

Regimen-related complications

No patients experienced serious infectious complications following HCT. One patient in the MUD HCT group developed adenoviral hemorrhagic cystitis and low-level blood copy number for adenovirus detected on routine weekly monitoring, both of which resolved with cidofovir therapy and supportive care. There were no episodes of veno-occlusive disease (VOD) or microangiopathy. There were no significant bleeding events, cardiopulmonary compromise, or chronic renal dysfunction in either MSD or MUD HCT groups.

Graft-versus-host disease (GVHD)

CIN of acute GVHD was: MSD, Grade I-II: 1 patient (11%), Grade III-IV: 0%; MUD, Grade I-II: 1 patient (20%), Grade III-IV: 1 patient (20%). CIN of chronic GVHD was: MSD, limited: 1 patient (11%), extensive: 0%; MUD, limited: 0%, extensive: 0%. Acute GVHD was responsive to increase of primary immunosuppression, addition of methylprednisolone to a maximum dose of 2 mg/kg/day with wean of steroid immunosuppression by day +180, and/or ultraviolet-B (UV-B 411-413 nm) "narrow-band" therapy in the case of cutaneous GVHD, allowing wean of steroids by 9 months post-transplant. All but one non-compliant patient with persistent chronic GVHD were weaned from immunosuppression by 9 months post-HCT and remain off of immunosuppression as of their latest date of follow-up.

Survival

There was no transplant-related mortality (TRM) among the 14 patients in this study. EFS was 100% for the overall cohort.

DISCUSSION

Expanding the pool of allogeneic donors and control of TRM are the major requirements for broader application of allo-HCT for patients with SAA. Dominant obstacles to extending the allogeneic donor pool for HCT in SAA are the prohibitive risks of non-engraftment and GVHD with increasing mismatch of donor and host. Extended molecular HLA typing and the practice of screening for serosensitization in chronically transfused recipients has greatly reduced the burden of non-engraftment, but TRM including GVHD remains a significant obstacle.

The cumulative incidence of acute GVHD in our case series was low, with 1 case of Grade 3-4 GVHD which resolved fully with immunosuppressive treatment, and no graft rejection despite all MUD HCT patients weaning permanently from immunosuppression by 9 months post-transplant. The incidence and severity of chronic GVHD was also limited and easily managed. EFS was 100% for both MSD and MUD HCT, and the conditioning and transplant regimens were overall very well-tolerated, without infectious mortality.

Our preliminary results correlate well with current published rates of success for MSD HCT for SAA using similar regimens (8-9, 12-13). Though very few patients are reported in the current MUD HCT cohort, outcomes appear at least consistent with larger published reports for MUD HCT in pediatric SAA (14, 15). A recent series by Kennedy-Nasser and colleagues (26) retrospectively reported favorable outcomes for MUD HCT patients received 200 cGy total body irradiation (TBI) with either ATG or humanized anti-CD52 antibody (CAMPATH-1H). Time to ANC and platelet engraftment was not significantly different than that reported here, and overall survival at 52 months was 93% after MSD HCT and 89% for all alternative donor HCT combined. In the past several years, Fludarabine (FLU)-based reduced intensity conditioning regimens for SAA have gained favor following initial encouraging reports in pediatric and adult Fanconi Anemia patients, who exhibit exquisite sensitivity to DNA-damaging agents and TBI conditioning regimens. Favorable results have recently been described for MUD HCT in both pediatric and adult SAA patients using low-dose TBI/FLU/CTX or FLU/CTX/ATG (27-30). Though very high stable engraftment rates were seen, significant TRM was reported in each of these studies, mainly in form of chronic GVHD and/or infectious complications including EBV-PTLD, which may be related to Fludarabine and/or higher doses of ATG used in these reports.

TLI/ATG regimens have been successfully applied as a non-myeloablative strategy in adults with hematolymphoid malignancies, as well as for induction of tolerance in combined marrow/renal transplantation (16-19). TLI-based regimens have also been applied in children following primary or secondary non-engraftment in MSD and MUD HCT, prior to repeat HCT from the primary donor (20). In murine modeling and patient studies, we and others have shown that TLI/ATG regimens induce an anti-inflammatory IL-4 and IL-10 dominated (Th2) milieu amongst both host and donor cells (21-22), which has been shown to protect against donor-host alloreactivity and GVHD (17, 21-22, 31-32) and theoretically could further ameliorate the pro-inflammatory/Th1 marrow environment pre-disposing to host-versus-graft (HVG) allo-reactivity in SAA patients (33-34). Moreover, SAA is characterized by a deficiency of regulatory T cell (Treg) function which may either predispose to or result from the pro-inflammatory cytokine milieu within the marrow (35). We have shown that TLI/ATG regimens enhance key regulatory T cell subset function on both host and donor sides of the transplant (21-22, 36) which could directly address the relative deficiency of Treg function shown to be associated with disease in SAA patients and facilitate transplantation tolerance (21-22, 36-39).

We postulate that the beneficial effect of TLI immunotolerance induction prior to CTX + ATG for SAA patients functions through induction of regulatory T cells and conversion of the immunoreactive milieu in SAA marrow to one of immunoregulation, such that both donor and host immune tolerance are facilitated. This postulate merits study and is planned in the context of a clinical trial. Issues that also remain to be clarified include whether the use of a radiation-based conditioning regimen can be tolerated in pediatric SAA patients, a sub-fraction of whom may have previously undetected congenital bone marrow failure (BMF) syndromes such as Fanconi anemia. Overall, TLI regimens have been a well-tolerated means of tolerance induction in children (20, 40-41). Though there is theoretical reason for concern regarding increased potential for secondary malignancies following TLI regimens in SAA patients, data to date does not support increased risk outside the setting of DNA damage repair defects (42-43); head/neck carcinomas and secondary myeloid leukemias have been reported in studies using thoraco-abdominal radiation (a variation of TLI) only in Fanconi anemia patients (42-44). Our current preliminary results and future trial exclude patients with detectable congenital BMF syndromes.

Acknowledged limitations of our report include single-center experience, a very small sample size, no data on total pre-transplant transfusion burden or regulatory cell number/function available in our data set, and the long time interval (11 years) over which outcomes are reported. These are largely limitations of a small retrospective review.

We propose a pilot clinical trial of MUD HCT for pediatric SAA patients lacking MSD, using TLI/CTX/ATG versus FLU/CTX/ATG conditioning, ideally within 6 months of initiating immunosuppression. This will include a detailed assessment of parameters of donor-host regulatory cell-mediated tolerance. Outcomes will be compared against outcomes for MSD HSCT using similar conditioning. We hope that favorable outcomes in such a pilot study will allow broader application of curative HCT to address not only the primary disease, but also the ongoing risk of secondary malignant transformation in IST responsive patients with SAA.

Acknowledgments

We thank Drs. Neal Young (NHLBI), Winfred Wang and Ulrike Reiss (St Jude), and Michael Jeng (Stanford University) for critical review of the manuscript, and Ms. Nancy Wright and Mr. Richard Lovins for data collection. This work was supported in part by the American Lebanese Syrian Christian Association Charities (ALSAC). AP is supported by Grant #5K08HL088260 (NHLBI).

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Table 1

Summary of patient demographics and period of follow-up (years) * (N=14)

Variable	Median	Range
Age at HCT (years)	14.7	4.6 - 19.3
Time from diagnosis to HCT (years)	1.5	0.1 - 8.7
Period of follow-up (years) *	3.0	1.1 - 10.3
	Subgroup	Number (%)
Race	Caucasian	10 (71.4)
	Black	3 (21.4)
	Other	1 (7.1)
Gender	Male	7 (50.0)
	Female	7 (50.0)
Donor	Sibling	9 (64.3)
	Unrelated	5 (35.7)

* defined as interval from date of HCT to date of last contact.

Table 2

Summary of graft characteristics

Graft Source	Mean +/- SEM TNC/kg (range)	Mean +/- SEM CD34 ⁺ /kg (range)	Mean +/- SEM CD3 ⁺ /kg (range)
MSD	6.5×10^8 ($2.2 - 8.3 \times 10^8$)	$6.2 \pm 4.2 \times 10^6$ ($2.1 - 15.3 \times 10^6$)	$28.3 \pm 16.2 \times 10^6$ ($20.2 - 47.3 \times 10^6$)
MUD		$4.4 \pm 6.6 \times 10^6$ ($2.9 - 6.5 \times 10^6$)	$27.8 \pm 23.9 \times 10^6$ ($26.5 - 40.3 \times 10^6$)

MSD, matched sibling donor; *MUD*, matched unrelated donor

Table 3

Summary of days to primary ANC and platelet engraftment

Variable	Donor Type				P value [#]
	MSD (N = 9)		MUD (N = 5)		
	Median	Range	Median	Range	
Days to ANC > 0.5 × 10 ⁹ /L *	22	15 - 32	22	18 - 26	0.87
Days to Platelets > 20 × 10 ⁹ /L *	26	18 - 59	43	17 - 449	0.28
Days to Platelets > 50 × 10 ⁹ /L *	26	20 - 70	43	18 - 458	0.28
Days to Transfusion Independence *	26	18 - 59	43	21 - 462	0.10
ANC (× 10 ⁹ /L) **	3.0	2.1 - 5.2	2.9	1.2 - 5.7	0.49
Platelet Count (× 10 ⁹ /L) **	182	57 - 289	146	90 - 226	0.52
Hemoglobin (gm/dL) **	15.0	12.8 - 16.4	14.5	11.8 - 16.8	0.82

MSD, matched sibling donor; MUD, matched unrelated donor.

* defined as number of days from transplant date to variable

** value as of latest follow-up evaluation

[#] P value using Wilcoxon rank-sum test indicates comparisons between MSD and MUD groups for listed variable.