Conclusion: These demonstrated that safety and feasibility of third party UCB-derived MSCs use and co-infusion of UCB-derived MSCs can overcome graft dysfunction of UCBT.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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CORD BLOOD (CB) APGAR SCORE IS PREDICTIVE OF NEUTROPHIL ENGRAFTMENT AND GRAFT FAILURE PROBABILITIES FOR PLASMA DEPLETED/REDUCED CB PRODUCTS

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Nucleated cell (NC), CD34+ cell (CD34), and colony forming unit (CFU) doses have been proposed to measure CB potency - important for engraftment potential prediction and transplantation product selection. Though TNC is widely used for CB selection, its predictive value is not as robust as the progenitor cell measurements. CFU and CD34 suffer from high inter-laboratory coefficient of variance (CV) - decreasing the clinical utility as potency measures. Recently, the Duke Group proposed a CB APGAR scoring system composed of (a) a Pre-Cryopreserved Score (PCS) reflecting pre-freeze CFU, CD34, NC, and CB collected volume, as well as a (b) Composite Score (CS) which combines the PCS score with post-thaw NC, CD34, CFU and mononuclear cell dose. Based on single, myeloablative and first (SMF) transplants of largely pediatric patients performed at Duke and using mostly red cell reduced (RCR) CB, the PCS and CS scores were shown to be predictive of graft failure, neutrophil and platelet engraftment. With CIBMTR-audited outcome data of transplanted CB products from a multi-national CB bank, we sought to validate the CB APGAR system on a patient population with mostly adults, heavy representation of minority and international patients, and on both SMF transplants, and all transplants (All) using plasma depleted/reduced (PDR) CB products. The PCS and CS table below shows the day 42 neutrophil engraftment cumulative incidence (ANC500) and graft failure probability (GF) comparisons of the Duke data with PDR transplants for both SMF and All transplants. For each of the PCS and CS strata compared, ANC500 and GF appeared to be similar among the Duke SMF, StemCyte SMF and All cohorts. We conclude that the CB APGAR score, especially the PCS, is an easy-to-use and reproducible potency measurement for CB selection by transplant centers that is highly predictive of ANC500 engraftment and GF for (1) RCR as well as PDR CB, (2) for mostly pediatric patient population as well as for mixed populations of adults and children, and (3) for minority and international patients. Whether the method can be applied to double, non-myeloablative and repeat CB transplants remains to be seen. Lastly, for the same PCS or CS strata, PDR CB appear to have similar engraftment and GF probabilities as RCR CB; therefore, the Duke CB APGAR is applicable to CB products with or without RBC reduction and reflects potency of CB products processed and stored by various methods at different CB banks.

Table 1. ANC 500 Engraftment Cumulative Incidence & Graft Failure Probabilities

ANC 500	Duke SMF	PDR SMF	PDR All
PCS≥7.75	93% (86-100%)	100±18%	83±19%
PCS<7.75	75% (69-81%)	78±9%	76±4%
HR	2.44 (1.78 - 3.59)	2.43 (0.85 - 6.95)	1.92 (0.78 - 4.68)
CS≥13.5	90% (84 - 95%)	94±14%	84±11%
CS<13.5	69% (61 - 78%)	68±12%	77±6%
HR	2.31 (1.73 - 3.08)	1.54 (0.73 - 3.26)	1.19 (0.78 - 1.82)
Graft Failure Probability	**Duke SMF**	**PDR SMF**	**PDR All**
PCS≥7.75	7% (3-17%)	0±18%	17±19%
PCS≥5.5 & <7.75	19% (12-30%)	15±14%	18±10%
PCS≥4.25 & <5.5	26% (16-39%)	6±13%	14±9%
PCS<4.25	32% (22-45%)	38±12%	29±5%

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IN-VIVO EXPANSION OF T REGULATORY CELLS BY RAPAMYCIN IN A CALCINEURIN-INHIBITOR FREE GVHD PROPHYLAXIS IN UNMANIPULATED HAPLOIDENTICAL STEM CELL TRANSPLANTATION (SCT)

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Background: Tregs are attractive candidates for clinical modulation of excessive immune responses. In SCT mouse models, the adoptive transfer of purified natural Tregs has been shown to prevent GvHD, while sparing a significant GvL effect. Tregs' suppressor function has been demonstrated to be critically dependent on IL-2, therefore cyA significantly reduces the function of allostimulated Tregs.

Aim: To address the role of Tregs in human SCT, we focused on a calcineurin inhibitor-free GvHD prophylaxis. We tested this hypothesis in haploidentical peripheral blood stem cells SCT without any *in-vitro* manipulation.

Patients and Methods: Since 2007, 68 pts underwent allo-SCT for AML (43), ALL (9), MDS (3), MPD (4), NHL (4) or HD (5). Median age was 48 years (range 14-69). At SCT all but 8 pts were in advanced phase. Conditioning included Treosulfan (14 g/m^2 for 3), Fludara (30 mg/m^2 for 5) and an *in-vivo* T and B-cell depletion, by ATG-Fresenius (10 mg/kg for 3) and Mabthera (a single 500 mg dose). All pts received allogeneic PBSC from an HLA-haploidentical related donor without any *in-vitro* positive selection. GvHD prophylaxis consisted of Rapamycin (target level 8-15 ng/ml, till day +60) and MMF (15 mg/kg tid till day +30).

Results: All pts but 3 had neutrophil engraftment. CI of grade 2-4, grade 3-4 aGvHD and cGvHD were 22%, 11% and 26%. 100 days TRM and relapse incidence at 1 year were 17% and 44%. Projected OS at 1 year is 39%. Immunoreconstitution was fast and sustained with a median 220 circulating CD3+cells/μL on day +30. We detected high levels of CD4+CD25+CD127- FOXP3+ Tregs (up to 30% of circulating CD4+ T lymphocytes) on day +30. These cells were able to suppress in vitro proliferation of autologous effector cells. This observation was further reinforced at a molecular level. We applied a quantitative RT-PCR based methylation assay that enables a specific and sensitive determination of T regs numbers by measuring demethylated FOXP3 at T reg specific demethyleted region (TSDR). An expansion of cells carrying FOXP3 demethylation was evident in our pts, but not in a control group of pts receiving mismatched SCT and cyclosporine.

Conclusions: Rapamycin-Mycophenolate-ATG are effective as GvHD prophylaxis in unmanipulated haploidentical peripheral SCT and are associated with an early T-cell immunoreconstitution characterized by the *in-vivo* expansion of Tregs. Further studies are warranted to gain insight correlations between Tregs expansion and SCT outcome.

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8/8 HIGH-RESOLUTION HLA MATCH RATE: THE IMPACT OF RACE

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Aim: Calculation of the 8/8 (HLA-A, B, C, DRB1) high-resolution (HR) match rate using real patient unrelated donor (URD) searches presents a biased sample for reasons including access to treatment, financial barriers and incomplete donor testing. A study was designed to estimate the true match rate for Caucasian (CAU), Hispanic (HIS), Asian/Pacific Islander (API), and African American (AFA) groups, representing the four largest race groups in the US population.

Methods: 1344 URD searches were performed for pseudopatients (PP) who were randomly selected, previously HR tested donors in the NMDP's Be The Match Registry (BTMR). Searches were based on a fixed BTMR file as of January 2009. Search results from CAU, HIS, API, and AFA PP were classified as follows:

1) At least one 8/8 HR matched donor exists on BTMR
2) No potential 8/8 HR donors exist
3) Potential 8/8 HR donors exist

PP searches falling into category 3 (accrued until N = 200 per race) then had an HLA search strategy expert rank potential donors within BTMR in order of their matching likelihood. Previously stored donor samples were HR HLA tested in order of ranking and evaluated to determine match status. Consecutive rounds of donor sample testing were performed until either an 8/8 matched donor was identified or no potential donors with stored samples remained.

Results: The table below shows the 8/8 HR match rate of cases to be 68% for CAU, 42% for HIS, 45% for API, and 27% for AFA. Careful review of the cases "Pending further testing; no stored sample" suggests that few additional cases would yield 8/8 HR matches.

	CAU PP	HIS PP	API PP	AFA PP
8/8 HR Matched	258 (68%)	128 (42%)	122 (45%)	105 (27%)
Pending Further Testing; No Stored Sample	48 (13%)	65 (21%)	57 (21%)	54 (14%)
No 8/8 HR Match	71 (19%)	114 (37%)	91 (34%)	231 (59%)
TOTAL	377	307	270	390

Conclusions: This study provides a true 8/8 HR match rate estimate for CAU, HIS, API, and AFA patients through BTMR, which has not been accomplished previously. These results demonstrate the racial disparity in HLA match rates and can be used to inform patients searching BTMR. This study also provides vital information for donor recruitment and availability efforts. Results provide a baseline match rate that can be further supplemented using the additional worldwide URD inventory.

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A 2 STEP APPROACH TO MYELOABLATIVE HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANATION (HSCT): REPORT OF A PHASE II TRIAL WITH 18 MONTHS OF FOLLOW-UP FOR ALL PATIENTS

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Haploidentical HSCT using post transplant cyclophosphamide (CY) for elimination of alloreactive lymphocytes has been reported as a safe option for patients lacking an HLA identical donor. We report an alternate approach with the following salient differences: myeloablative vs non-myeloablative conditioning, peripheral blood rather than marrow stem cell source, no exposure vs exposure of HSC to cyclophosphamide, higher fixed number of CD3 cells versus a lower variable number of CD3 cells in each graft. Results are reported now with a followed up of 18-46 months.

Table 1. Patient Characteristics-2 Step Approach

Age		52 (19-67)
AML		16
	Remission	7
	Resistant/PIF	9
Biphenotypic Leukemia (Active Disease)		1
ALL		4
	CR2 (ph-)	3
	Persistent Disease (PH+)	1
MDS		2
NHL Resistant		3
SAA		1
HLA MM (GVH Direction)		
	4	13
	3	11
	2	2
	0	1

Patients received 12 Gy of total body irradiation (TBI), followed by a donor lymphocyte product (DLI) containing 2 × 10e8 CD3+ cells/kg (Step 1). This large dose of haploidentical lymphocytes resulted in fever (median temperature 103.8°f), diarrhea and rash. CY 60 mg/kg was given on days -3 and -2 resulting in resolution of symptoms. Tacrolimus and MMF were begun on day-1. A CD 34 selected donor product was infused on day 0 (Step 2). Two of the 27 patients died of toxicity and infection before day 14. Of the remaining 25 patients, 23 had complete engraftment while two with pre-existing anti-donor HLA antibodies failed to engraft. Only 2 of 25 (8%) patients developed severe acute GVHD, 3 of 25 (12%) developed limited chronic GVHD, and no patient died of GVHD. Only two of 25 patients (8%) died of infection. Of 16 disease-free patients surviving 6 months from HSCT, median CD4+ count at day 100 was 105 cells/μl (range 10-403). Eight of 25 (32%) patients relapsed after HSCT. Probability of survival (OS) at 1 and 3 years post transplant is 52% and 48% respectively. All surviving patients are disease-free. OS at 3 years is 75% for patients transplanted in CR, but only 27% for patients transplanted with active disease. KIR mismatching was not correlated with relapse rates. In contrast, child to mother transplants for AML appear to be relapsing at higher rates than other combinations (66% vs 14%). In the context of CY tolerization, a dose of 2 × 10e8/kg T-cells resulted in consistent engraftment, prompt immune reconstitution, little severe GVHD, acceptable toxicity, and encouraging overall survival, particularly in patients transplanted in CR. Using this 2-step platform allows us to explore the use of alternate agents for the elimination of alloreactive lymphocytes, increase the length of time between DLI and CY, and to employ two donor strategies to improve outcomes in high risk patients.

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UNMANIPULATED HAPLOIDENTICAL STEM CELL TRANSPLANTATION USING MYELOABLATIVE OR REDUCED-INTENSITY PRECONDITIONING REGIMEN

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Background: Related haploidentical donors, as cord blood, can be alternative donor sources in stem cell transplantation (SCT). Severe GVHD, however, has interfered the progress of haploidentical SCT (haploSCT). To deal with this strong GVHD, T cell depletion has usually been used in US and European countries. In order to pursue the controllable GVL effect by T cells, we have performed unmanipulated haploSCT using myeloablative or reduced intensity preconditioning regimen accompanied with intensified GVHD prophylaxis. In this meeting, we will summarize our experience of haploSCT for more than ten years.

Patients: From August 1998 to September 2010, we have performed 351 cases of haploSCT (all cases were HLA 2-3 antigen mismatched in GVH direction). Patients' characteristics are sex: male 186, female 168, age: 16-65 years old (median 39), disease: AML/MDS 149, ALL 81, ML 67, others 54. 83% of cases underwent SCT in non-complete remission (non-CR) state. Patients under 45 years old underwent myeloablative preconditioning regimen consisting of FLU/CA/CY/TBI8Gy (haplo-full, n = 100), and patients over 45 years old or with comorbidities or repetitive SCT (including second to fifth SCT) underwent reduced intensity preconditioning regimen consisting of FLU/(CA)/BU/ATG or FLU/(CA)/MEL/ATG (haplo-mini, n = 251). High dose Ara-C (CA) was optional to reduce tumor burden. As ATG, ATG (Fresenius) 8mg/kg, or thymoglubulin (genzyme) 2-4mg/kg were used. GVHD prophylaxis consisted of taclolimus (TAC), methylprednisolone (mPSL) 2mg/kg/day, short term MTX, and mycophenolate mofetil (MMF) 15mg/kg/day in haplo-full, and TAC, mPSL 1mg/kg/day in haplo-mini, respectively. For elderly patients over 50 years old in haplo-mini, MMF was added.

Results: Hematopoietic engraftment in haploSCT was as rapid as that in HLA-identical SCT, except ten cases of graft rejection. Acute GVHD (grade II-IV) was observed in 30%. Overall survival in five years is 30% in haplo-full and 40% in haplo-mini, respectively. If limited to CR cases, overall survival reached over 60% in haplo-mini. There is no difference in survival rate among patients' diseases.

Discussion: Unmanipulated haploSCT is feasible and effective for refractory diseases. ATG dose used in haplo-mini is critical, and rather low compared with that of European cases reported so far.