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A WEB-BASED SYSTEM SUPPORTING STRATEGICAL DECISIONS IN WORLD WIDE URELATED DONOR SEARCH

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Most active unrelated stem cell donor registries in the world are participating in the cooperation of Bone Marrow Donors Worldwide (BMDW). Formally, BMDW is a service provided by the Dutch registry Europdonor collecting all data, providing them back to the contributors and making them directly accessible via a web based matching program. Unfortunately, HLA information is limited for many donors, in particular where DRB1 alleles are missing or only tested at low or intermediate resolution. We are using our own copy of the BMDW data to generate haplotypes frequencies per registry for HLA-A,B-,DRB1. HLA-A and B are analyzed using serological nomenclature and DRB1 using a resolution of 2 and 4 digits as far as the data for each registry permits. These haplotypes frequencies are used for a web based system prototyped in our intranet, which calculates the probability of finding a donor who is a match for HLA-A and B (serology) and for DRB1 (allele level) by performing subsequent test on partially typed donors. In the calculation of the conditional match probabilities the program correctly interprets all broad serological designations and multiple allele codes using the individual frequencies for each registry. Where there are test candidates in several registries, the system can also indicate which donors according to their origin or partial typing status are most likely to turn out to be matches. This provides a highly intelligent sorting of BMDW match lists. The program is currently implemented as a CGI-script using PERL and typically takes 5–20 seconds on a 1.5 GHz XEON including a full molecular BMDW match run. The program has immediately become an indispensable tool for the analysis of all difficult donor searches.

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COMPARABLE RESULTS OF UMBILICAL CORD BLOOD AND HLA MATCHED SIBLING DONOR HEMATOPOIETIC STEM CELL TRANSPLANT AFTER REDUCED-INTENSITY PREPARATIVE REGIMEN FOR ADVANCED HODGKIN'S LYMPHOMA

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The lower regimen-related toxicity of reduced-intensity conditioning (RIC) preparative regimens has extended the opportunity for allogeneic stem cell transplantation (alloSCT) and its potential graft-versus-lymphoma effect to patients with advanced Hodgkin's lymphoma (HL). In this pilot study, we compared the safety and efficacy of RIC alloSCT in 21 adults with chemosensitive primary refractory or relapsed HL using either umbilical cord blood (UCB-9) or matched sibling donors (MSD-12). Indications for RIC alloSCT were age >55 years with HLA matched sibling donor (19%), age >45 years with UCB donor (5%), extensive prior therapy including previous autologous stem cell transplant (ASCT) (67%), or major comorbidity (22%). Of the 14 patients who had failed prior ASCT, median duration of post-transplant CR was 11 months (range, 3–54). Patient demographics, disease characteristics at initial diagnosis, and at relapse prior to alloSCT were comparable except for a younger age in the UCB cohort (median 28 years vs 42 years for MSD, $P = .04$). Results are shown in Table 1. Neutrophil recovery occurred earlier in the MSD group. All patients had sustained donor engraftment by day +60. Cumulative incidence of grade III/IV acute graft-versus-host disease and 180-day treatment-related mortality were comparable. Two patients who underwent MSD alloSCT received donor-lymphocyte infusion for post-transplant relapse with resultant partial responses lasting 3 and 6 months. With a median follow-up of 17 and 24 months for the UCB and MSD groups, respectively, the 2-year progression-free survival (PFS) for UCB is 25% compared to 20% for MSD alloSCT. The median time to disease progression was 4 months for the UCB group and 6 months for the MD group; all relapses occurred within 1 year of alloSCT. Patients with relapsed disease or longer post-ASCT CR (≥12 months) were more likely to be alive and free of progressive disease than patients with primary refractory disease or short CR. Our results suggest comparable outcomes for RIC alloSCT using UCB or MSD source in adults with high-risk, advanced HL. Since many patients lack a matched sibling or unrelated donor, UCB grafts can provide an effective and safe alternative. In addition, alloSCT using RIC is associated with durable PFS in a selected subgroup of patients. Further studies are ongoing to identify patients who would benefit the most by this approach (Table 1).

Table 1. *Post-Transplant Outcomes*

		UCB (n = 9)	MSD (n = 12)	P-Value
Cell dose ($\times 10^7$ NC/kg)	Median (range)	3.8 (2.3–5.3)	10.0 (7.9–16.4)	<.01
HLA 1–2 antigen mismatch		9 (100%)	1 (8%)	<.01
Neutrophil engraftment (days)	Median (range)	10 (6–28)	7 (5–12)	.02
Complete donor chimerism	Day +21	6 (67%)	12 (100%)	.06
	Day +60	9 (100%)	12 (100%)	-
Acute GVHD	Grade II–IV	6 (67%)	7 (58%)	.70
	Grade III–IV	3 (33%)	4 (33%)	.99
Chronic GVHD		1 (11%)	4 (33%)	.24
CR after alloSCT		8 (89%)	9 (75%)	.42
Treatment related mortality	100 days	1 (11%)	2 (17%)	.80
	180 days	2 (22%)	3 (25%)	.88
Followup (months)	Median (range)	17 (4–51)	24 (9–53)	
2-year PFS (months)	(95% CI)	25% (0–55%)	20% (0–44%)	.67
2-year OS (months)	(95% CI)	51% (16–86%)	48% (19–77%)	.93

NC-nucleated cells; HLA-human leukocyte antigem; GVHD-graft-versus-host-disease; CR-complete remission; CI-confidence intervals.

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MOBILIZATION OF PERIPHERAL BLOOD CD34 STEM CELLS IN A HEAVILY PRE-TREATED PEDIATRIC MEDULLOBLASTOMA PATIENT USING AMD3100 AND G-CSF

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Autologous peripheral blood stem cell transplantation has been beneficial in the setting of recurrent medulloblastoma, however many of these patients are heavily pre-treated, making conventional mobilization of peripheral blood stem cells with G-CSF alone difficult. The recent development of AMD3100 as an inhibitor of the binding of SDF-1/CXCL12 to its receptor CXCR4 in the marrow stem cell compartment has resulted in significant enhancement in the mobilization of peripheral blood stem cells. This has met with considerable success in adults with phase III trials under way, however there is little pediatric experience with the use of AMD3100. We report here the use of AMD3100 to mobilize peripheral blood CD34 cells from a heavily pre-treated 11 year-old girl with recurrent medulloblastoma. DM was diagnosed with stage IV medulloblastoma in Feb 2004 and was treated with surgical resection, radiation, and maintenance chemotherapy including cisplatin, CCNU, and vincristine. Prior to her 5th cycle of chemotherapy she experi-

enced a (biopsy proven) relapse. She went on to receive salvage chemotherapy including carboplatin and cytoxan with stabilization of disease, but without shrinkage in her tumor. Attempts to collect peripheral blood stem cells upon recovery from chemotherapy using G-CSF at a dose of 24mcg/kg/day were unsuccessful with only 1×10^5CD-34 positive stem cells per kg collected over 3 days. In order to attempt to collect enough stem cells for an autologous stem cell transplant, we obtained consent from the AnorMED corporation and our institutional review board for a PBSC collection using 4 days G-CSF at 10 mcg/kg sc followed by 240 mcg/kg AMD3100 sc and subsequent apheresis 10 hours later. We collected PBSCs for three days and repeated this cycle a second time 30 days later, which allowed us to collect 1.3×10^6CD34 positive stem cells per kg, more than an order of magnitude greater than with G-CSF alone. There were no untoward toxicities including no GI upset, paresthesias, or injection site reactions, which have been previously reported with adult patients. Pharmacokinetics were performed and were similar to adults.

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IMPACT OF HIGH-RESOLUTION HLA MATCHING ON OUTCOMES OF UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective: We assessed the impact of high-resolution genotypic results of human leukocyte antigen (HLA) for all major class I and II loci between donors and recipients in the outcome of unrelated hematopoietic stem cell transplantation (HSCT). **Methods:** Between 1999 and 2005, high-resolution genotyping for HLA-A, -B, -C and -DRB1 was performed for 23 unrelated HSCT. All the patients were typed as HLA identical by serologic technique and then they were also typed HLA identical by high resolution technique. Unrelated bone marrow transplantation using DNA-based high resolution HLA compatibilities were considered in the analyses of clinical outcomes such as hematopoietic engraftment, acute GVHD, and survival. And then, we compared with patients who received related HSCT and also unrelated HSCT data from IBMTR. **Results:** Median follow up duration was 9 months (1–51). Fifteen patients were male and 8 were female. Median age was 22 years (range 6–52). Median time from diagnosis to transplantation was 7 months (range 4–63). Eight patients of acute myeloid leukemia (AML), 6 of chronic myeloid leukemia (CML, 2 of 6 were in blast crisis), 4 of acute lymphoid leukemia (ALL), 3 of severe aplastic anemia, and each case of juvenile myelomonocytic leukemia and myelodysplastic syndrome were enrolled. Median value of total nucleated cell and CD34 positive cell count was 3.51 (1.06–20.7) $\times 10^8$/kg and 4.88 (1.33–46.9) $\times 10^6$/kg, respectively. The conditioning regimen and prophylaxis for graft versus host disease (GVHD) were not different from conventional HSCT except one case of non-myeloablative transplantation. Median value of granulocytic (absolute granulocyte count $>500/mm^3$) and platelet ($>20{,}000/mm^3$) engraftment were D + 16, D + 17, respectively. Grade II acute GVHD developed in 4 patients (2 patients subsequently proceeded to chronic GVHD). Treatment related mortality was 8.7% (2 out of 23 patients). Median value of overall survival duration was 30 months. For AML patients, 3-year survival rate was 72.9%. **Conclusions:** Our survival data for unrelated HSCT based on high resolution genotyped HLA matching was inferior to related HSCT and superior to unrelated HSCT (of as expected). Although the sample size is small, the survival data of AML patients (CR1) was superior to the survivals of related HSCT as well as that of unrelated HSCT. We suggest that transplantation using unrelated donors selected by high-resolution genotype identity improves the transplantation outcomes.

IMMUNE RECONSTITUTION

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TRANSCRIPTIONAL MAPPING OF THE ANTI-VIRAL T CELL RESPONSE SHOWS THAT IMPAIRED MEMORY DIFFERENTIATION CAUSES T CELL DYSFUNCTION

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Human T cells responding to viral pathogens after HSCT can be dysfunctional and ineffective even when present in adequate numbers. The basis for this qualitative, rather than quantitative, T cell defect is not known. We hypothesized that impaired differentiation of naive T cells into memory T cells causes a qualitative T cell defect. We, therefore, studied CD8 T cell memory differentiation in 2 murine models of LCMV infection: an acute infection in which virus-specific T cells are fully functional and a chronic infection model in which T cells are present in normal numbers but are dysfunctional. In order to define how the differentiation state of dysfunctional T cells in chronic infection differ from those in acute infection, we generated gene expression profiles from naive (day 0), effector (day 7 [d7]), and memory (day 30 [d30]) T cells using oligonucleotide microarrays. In acute infection, T cell states were so distinct that 100% of naive, effector, and memory samples could be correctly classified, using a k-Nearest-Neighbor prediction classifier (kNN). Next, we hypothesized that the signature of normal memory T cells would contain gene-sets known to be important in memory differentiation. We found that Stat-5 target genes were the most enriched gene-sets in memory cells relative to naive cells of >400 gene-sets tested ($P = .004$ by permutation testing). This is consistent with the central role of Stat-5 signaling in memory development. Lastly, we determined where dysfunctional d30 T cells from chronic infection lie relative to the "differentiation-space" of acute infection. None of the chronic d30 T cell samples were classified as memory by kNN prediction; all were misclassified as effectors. However, chronic d30 T cells were not simply persistent effectors because chronic d30 T cells showed marked differences in gene expression pattern compared to d7 effectors. Thus, dysfunctional T cells in chronic viral infection fail to complete normal memory differentiation and are arrested in a distinct, effector-like phase. Our results show that the disruption of memory differentiation causes qualitative T cell dysfunction, and that this altered differentiation can be detected by gene-expression profiling. We are now generating a global map of antigen-specific T cell differentiation in humans. This gene-expression-based approach to evaluating immune reconstitution will identify the mechanisms limiting the recovery of T cell memory after HSCT.

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EXPRESSION OF INDIVIDUAL KILLER IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) GENES MEASURED BY QUANTITATIVE REAL-TIME-PCR DIVIDES BLOOD NATURAL KILLER (NK) CELLS INTO THREE DEVELOPMENTALLY DISTINCT POPULATIONS

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Although hematopoietic cell transplant strategies that use KIR-ligand mismatching to generate alloreactive NK cells have been associated with improved clinical outcomes, the contributing effects of the fundamental processes regulating individual KIR expression are poorly understood. To study KIR emergence during NK cell reconstitution, we created and validated a novel Quantitative Real Time PCR (Q-PCR) expression typing assay to measure comparative KIR mRNA expression at the single gene level. TaqMan primers and probes were designed for 13 KIR genes and compared to genotyping. Analysis of 435 reactions run on mononuclear cells (5–15% NK cells) from 36 normal volunteers showed excellent concordance between expression typing and genotyping: