PATIENT OUTCOMES BY CONDITIONING REGIMEN

	Median Day 7 TNFR1 Levels (pg/mL)	Mean Day 7 TNFR1 Levels (pg/mL)	Day 100 Grade 2-4 GVHD	Day 100 Grade 3-4 GVHD	Day 100 NRM	2 year NRM	2 year OS
Overall (n = 96)	2518	3053	53%	23%	16%	34%	55%
FluBu4 (n = 31)	1735	1897	39%	11%	0%	12%	70%
BCNU/Busulfan (n = 36)	2552	3009	61%	25%	14%	31%	58%
TBI (n = 29)	3907	4226	62%	32%	34%	55%	38%

first 100 days ($p < 0.001$), and improved 2 year survival rates (70% $p = 0.03$).

Our data suggest that etanercept effectively reduces TNFR1 levels at day 7 post-HCT, which has previously been correlated with transplant outcomes. The median day 7 TNFR1 levels were statistically different across conditioning regimens, and those with low TNFR1 levels had improved outcomes (FluBu4 > BCNU/Busulfan > TBI). The combination of FluBu4 with etanercept as a promising regimen for high-risk allogeneic HCT warrants further investigation.

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THE ROLE OF DONOR-SPECIFIC ANTI-HLA ANTIBODIES (DSA) IN HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM MATCHED UNRELATED DONORS

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We have previously identified a high risk of primary graft failure (PGF) in patients with DSA in T-cell depleted haploidentical transplantation (HaploSCT); 3/4 patients with DSA developed PGF compared with 1/20 patients without DSA. All patients with DSA against anti-HLA-A, -B and -DRB1 developed PGF, while 1 patient with anti-DP DSA did not. We hypothesized that anti-DP antibodies may have a significant but less deleterious impact on engraftment and evaluated the occurrence of PGF in 487 patients who received matched unrelated donor transplants (MUDT) at our institution after 9/2005. Approximately 80% of the transplants were mismatched in HLA-DPB1 in the HvG vector. The presence of DSA was determined by testing the patients' sera with a panel of fluorescent beads coated with single HLA antigen preparations using a Luminex™ platform; results were interpreted as fluorescence intensity (FI) against DSA mismatch as previously defined by us (see Table Footer). HLA-A,-B,-C,-DRB1,-DRB3/4/5,-DQB1 and -DPB1 were typed by high resolution methods. Specificity and intensity of antibodies is described in the Table. Eight patients had PGF, and 8 had early death. In each category one had DSA. Median time to ANC500 was 12 days. We found that 5 patients (1%) presented DSA in their pre-transplant specimens, all females with a median age of 49 years, and matched with their donors in 10/10 alleles. In all patients the DSA were directed against HLA-DP molecules with intermediate to strong intensities. One patient with DSA (#1) received rituximab/plasma exchange prior to transplant to decrease DSA levels and died on day 22 without engraftment. Excluding the patients with early death, we have found a moderate association between anti-DP antibodies and PGF ($p = 0.0654$). When early death was considered as a negative event associated with DSA, a significant association was identified between the presence of anti-DP DSA and graft failure/early death outcome ($p = 0.0108$, Fisher's Exact Test).

Number	Anti-DP DSA	DSA levels before 1st SCT	Engrafted (Y/N)	2nd SCT (Y/N)	DSA levels before 2nd SCT	Engrafted (Y/N)
1	DPB1*03DBZB	4+	N	N	N/A	N/A
2	DPB1*0401	1+ to 2+	Y	N	N/A	N/A
3	DPB1*0301	3+	N	Y	0	Y
4	DPA1*0201 and DPB1*0101	3+	Y	N	N/A	N/A
5	DPB1*0101 and DPB1*1101	3+ and 1+	Y	N	N/A	N/A

DSA-donor-specific anti-HLA antibodies; Y- yes, N-no; N/A- not applicable; 0 - no DSA, FI < 500 or negative; weak (500-1500 FI, 1+), intermediate (1500-3000 FI, 2+), strong (3000-7500 FI, 3+); very strong (>7500 FI, 4+).

Conclusions: These results, combined with our previous findings in HaploSCT, suggest that anti-DP DSA compared with DSA against anti-HLA-A, B or DRB1 may have a lower risk for PGF. The differences may reside in the lower levels of DP molecules expressed on cell surface. DSA screening is warranted when considering donors with HLA mismatches as strategies for donor selection and/or antibody level reduction may be needed to decrease the risk of PGF in allogeneic HSCT from partially HLA-matched donors.

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EVALUATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) UP TO AGE SEVENTY-FIVE, REFERRED FOR ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (HCT) INCLUDING DONOR AVAILABILITY AND HCT OUTCOMES

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Allogeneic HCT remains the only curative treatment strategy for patients (pts) with MDS. Moffitt Cancer Center (MCC) has implemented a targeted IV busulfan plus fludarabine (tBuFlu) induction regimen that is myeloablative, with reduced treatment related toxicity, permitting the expansion of empiric age limitations for HCT up to 75 years (yrs). From January 2004 on, 243 new pts (NP) with a diagnosis of MDS or CMML were evaluated for HCT. An allogeneic donor was identified in 170 pts and 112 received an HCT. The median age of the 112 transplant pts was 55.6 yrs (24.8 – 73.5). Sixty pts were older than 55 yrs. At the NP visit, IPSS risk was Low (11), Int-1 (35), Int-2 (42), and High (21) or not evaluable (3) (NE). Donors included 49 sibling donors (SIB), 48 matched unrelated donors (MUD) and 15 mismatched unrelated donors (mMUD). Median follow-up is 19.3 months (0 – 61.4) and the Kaplan-Meier overall survival (OS) from NP visit is 77% at one year and 56% at two years. Overall survival is not statistically different between SIB, MUD or mMUD HCT ($P = 0.39$). In 58 pts who did not receive a HCT, a donor was identified. **(Yes donor, No HCT)** Median age was 57 yrs (19 – 69) with 28 pts older than 55yrs. At the NP visit, IPSS was Low (5), Int-1 (19), Int-2 (17), High (16) and NE (1). Donors included SIB (14), MUD (27), mMUD (15), and umbilical cord blood (2). Median follow up is 10.3 months (1.7 – 66.3). Seventy-three pts did not obtain a donor **(No Donor, No HCT)**. Median age was 64.4 yrs (32.6 – 72.1) with 65 pts older than 55yrs. IPSS at NP visit was Low (8), Int-1 (16), Int-2 (25), High (16) and NE (8). Median follow up of these pts is 6.7 months (0.9 – 61.3). For the entire cohort (243 pts), pts with a donor had significantly improved overall survival vs pts with no donor ($P = 0.003$); there was no difference in OS between pts with a donor who did not proceed to HCT and everyone without a donor ($P = 0.27$). Twenty-eight of the 73 pts without a donor did not have donor searches. If these are excluded, in the remaining 215pts OS for pts with an identified donor was improved compared to OS of those without a donor ($P = 0.009$) and pts with a donor and no HCT had similar OS to those without an identified donor ($P = 0.3$). These data demonstrate that 70% of patients can acquire a suitable allogeneic donor and 47% of patients seen as a HCT new

patient consult can proceed to HCT for MDS with a significant overall survival advantage using a SIB, MUD or mMUD.

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HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MAJOR THALASSEMIA: NINETEEN YEARS EXPERIENCE IN IRAN

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Objective: Hematopoietic Stem Cell Transplantation (HSCT) still remains the only cure available for Major Thalassemia. Below there are the results of 19 years using HSCT as treatment in our centre.

Patients and Methods: 425 Major Thalassemia Patients, (242 male and 183 female) with a median age of 7 (Range: 2-28 years), have received HSCT from July 1991 through October, 2009. From 425 patients, 113 patients were in class I, 146 in class II, 142 in class III and 24 in Thalassemia Intermediate. They received allogeneic transplantation from 407 HLA full matched siblings, nine HLA mismatched siblings or other relatives, nine HLA full matched other relatives. The sources of HSCT were 158 bone marrow, 194 peripheral blood, 9 cord blood and one bone marrow combined with peripheral blood. Since three years ago 58 patients with class III thalassemia were randomized for double blind study with two groups; one with co-transplantation (mesenchymal cells) and another without mesenchymal cells.

Results: The median time to Absolute Neutrophil Count $\geq 0.5 \times 10^9$/L and platelet count $\geq 20 \times 10^9$/L were +15 and +22, respectively. Acute GvHD occurred in 281 (66%) and chronic GvHD in 91 (21.4%) patients. 356 (83.8%) patients are still living and 69 (16.2%) are deceased. The most common cause of death was GvHD. 5-year overall survival (OS) and disease-free survival (DFS) were 79.8% and 70.2%, respectively. The 5-year OS of patients with peripheral blood and bone marrow stem cell were 75.5% and 84.2%, respectively ($p = .141$). The 5-year DFS of peripheral blood and bone marrow recipients were 69.3% and 73.1%, respectively ($p = .773$). Up to now the results of HSCT for both groups in double blind randomized study is same and we have to wait for future analysis.

Conclusion: HSCT, based on our experience and other documented studies, is an acceptable treatment for Major Thalassemia with better results in younger patients.

KEY WORDS Hematopoietic Stem Cell Transplantation, Major Thalassemia

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IL-15 ENHANCES IN-VITRO EXPANSION AND FUNCTIONAL ACTIVITY OF ANTIGEN-SPECIFIC EFFECTOR MEMORY T CELLS (T_{EM}) WHILE CO-EXPRESSION OF IL-15 AND IL-15 R ON ANTIGEN PRESENTING CELLS ALSO PROMOTES ENRICHMENT AND PREFERENTIAL EXPANSION OF CENTRAL MEMORY T-CELLS (T_{CM})

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Adoptive immunotherapy with in-vitro expanded antigen-specific T cells (TC) is often hampered by extended culture times and limited persistence of infused cells in-vivo. IL-2 predominantly supports the generation of short-lived effector memory (T_{EM}) and effector (T_E) $CD8^+$ TC without expanding the $CD62L^+$ and $CCR7^+$ central memory TC (T_{CM}), which may persist longer in- vivo. We examined if IL-15 could foster the growth of CMVpp65-specific T_{EM} and T_{CM} $CD8^+$ TC.

TC from 6 HLA-$A0201^+$ seropositive donors were sensitized in-vitro using HLA-$A0201^+$, $CMVpp65^+$ artificial antigen presenting cells (Hasan et al. JI, 2009) (A2-AAPC). To assess if trans-presentation of IL-15 on AAPCs leads to enhanced IL-15 activity, we sequentially transduced A2-AAPCs with the IL-15 and IL-15 Rα cDNA (A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$). TC were cultured using A2-AAPC with (i) IL-2 (20U/ml) (ii) IL-15 (10ng/ml) (iii) IL-2 plus IL-15 or (iv) A2-$AAPC^{IL\text{-}15R\alpha}$ + IL-2 (20U/ml) (v) A2-$AAPC^{IL\text{-}15R\alpha}$ + IL-15 (10ng/ml) or (vi) A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$

Starting from a precursor frequency of 0.5-2 x 10^4/5 million TC, cultures supplemented with IL-15 demonstrated upto 2000 fold expansion of CMVpp65 tetramer (+)TC and generated 25-70 x10^6 A2-NLV tetramer (+) $CD8^+$ TC, compared to a 300-600 fold expansion and 6-12 x10^6 tetramer (+) $CD8^+$ TC with IL-2 after 21-28 days. Sensitization with A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$ exhibited rapid and significant enrichment of CMVpp65 A2-NLV tetramer (+) $CD8^+$ TC between day 14-28. There were no T_{CM} detected by 21 days among tetramer (+) TC in cultures (i) to (v). However, TC sensitized with A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$ contained 12-15% ($\sim 7 \times 10^6$) tetramer (+) $CD8^+$ T_{CM} which further increased through day 35. In functional assays, sensitization with A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$ generated 35-53% $CD8^+$ CMVpp65 epitope-specific $IFN\gamma^+$ TC compared to 11-26% in cultures with exogenous IL-15 and 2-10% in cultures with IL-2. Cytotoxic activity was also higher for TC sensitized with A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$ (80-90%) compared to cultures with IL-2 (60%).

These data demonstrate that any condition using IL-15 generates higher yields of antigen-specific TC. However, TC cultures supplemented with IL-15 +/- IL-2 only supported sustained expansion of T_{EM} and T_E. In contrast, TC sensitized with A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$ also supported sustained expansion of T_{CM} with augmented functional activity. Therefore $AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$ presents a novel practical approach for expansion of antigen-specific T_{CM} for adoptive immunotherapy.

Differential Expansion and Function of CMVpp65-specific Memory T-cells

Culture Condition	Fold Expansion Tet [+] CD8 TC	Fold Expansion Tet [+] CD62L [+]CD8 TC	% CD8 IFN γ [+] TC	% Cytotoxicity
A2-AAPC + IL-2	200-600	60-90	2-10	28-60
A2-AAPC + IL-15	300-1300	11-82	10-18	60-75
A2-AAPC + IL-2 + IL-15	250-700	25-150	8-20	40-72
A2-$AAPC^{IL\text{-}15R\alpha}$ + IL-2	25-100	4-10	2-7	30-45
A2-$AAPC^{IL\text{-}15R\alpha}$ + IL-15	100-300	20-50	11-20	60-75
A2-$AAPC^{IL\text{-}15/IL\text{-}15R\alpha}$	1200-2300	400-1007	35-53	80-95

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IN VITRO INDUCTION OF ADENOVIRUS SPECIFIC T-CELLS IN RESPONSE TO 15-MER PEPTIDES IN UMBILICAL CORD BLOOD

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Human adenovirus (HAdV) infections are a serious life-threat in hematopoietic stem cell transplantation patients, particularly in children. In recent years, umbilical cord blood (UCB) transplants emerged as an alternative stem cell source. However, the immune reconstitution is severely hampered in CBT recipients leading to higher risk of viral infections. Because no effective antiviral medication exist for severe HAdV infection, replenishing HAdV-specific immunity through adoptive transfer of HAdV-specific T-cells could be a promising treatment.

Aim of this study was to induce HAdV-specific T-cells with low contamination of residual potentially alloreactive T-cells from UCB. Therefore, cord blood mononuclear cells (CBMCs) were cultured with 5 previously described pan-DR binding 15-mer HAdV peptides derived from HAdV serotype 5.[1] To examine if the mainly antigen naive UCB T-cells were able to recognize the different HAdV-peptides, CBMCs of 34 deliveries were cultured with the HAdV peptides for 5 days without adding supplemental cytokines. CBMCs of 17 cord blood samples (= 50%) showed a proliferative response to 43 of the various HAdV-peptides or to the pool of the peptides, with a stimulation index ranging from 1.7 to 19.1 as determined by 3H-thymidine proliferation assay. To induce HAdV specific T-cells, CBMCs derived from 19 deliveries were subsequently cultured for 7 days with the HAdV peptides or a pool of these peptides after adding of 40 IU/ml IL2. Compared with medium stimulated cells HAdV-specific T-cells expressed significantly ($p < 0.05$) more of the activation markers CD25 and CD69 and harbor a memory