and expanded with different dominant responses detected in different donors. In conclusion, we have developed a method for the in-vitro induction and isolation of functional CMV-specific CD8+ T cells from CMV- donors. This may allow the treatment of serious CMV-related complications in CMV+ patients transplanted with a CMV- donor.

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THE USE OF SIROLIMUS COMBINED WITH TACROLIMUS AND LOW-DOSE METHOTREXATE TO PREVENT GRAFT-VERSUS-HOST DISEASE FOLLOWING UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The use of sirolimus (siro) combined with tacrolimus (tacro) and low-dose methotrexate (MTX) recently showed a promising result in preventing acute GVHD after unrelated donor hematopoietic stem cell transplantation (MUD-HCT) (Antin et al. Blood 2003). We studied this approach in 77 patients (female = 30, male = 47) who received a MUD-HCT after full-intensity conditioning (TBI-VP16: 17, TBI-Cy: 10, BuCy: 3, BEAM: 2) or reduced intensity conditioning (FluMel: 45) from April 2005 to March 2007. Patient age ranged from 19 to 67 (median 46). The cohort consisted of 20 patients with AML, 19 with ALL, 15 with NHL, 8 with MDS, 5 with MPD, 4 with HD, 3 with CML, and 2 with CLL. Twenty-seven of 77 patients had low-risk disease (1st/2nd CR, CML-CP, or MDS-RA). Patients received a bone marrow (n = 15) or peripheral blood stem cell graft (n = 62). GVHD prophylaxis consisted of tacro, siro, and MTX 5 mg/m^2 for 3–4 days. High-resolution (HR) molecular HLA typing was performed for class I and II. Forty-two pairs were in HR molecular match in all 10 antigens (HLA-A, B, C, DR, and DQ). Lack of iKIRL was found in 50 pairs.

After a median follow up of 13 months, 50 are alive. The 1-year probabilities of overall survival (OS), disease-free survival, relapse, and non-relapse mortality were 60.6% (95%CI: 52.6–67.6), 55.9% (95%CI: 48.8–62.5), 15.0% (95%CI: 8.5–25.7), and 25.4% (95%CI: 17.9–35.3), respectively. Severe thrombotic microangiopathy was observed in two patients and was reversible.

Acute GVHD grade II-IV and III-IV occurred in 46 (60%) and 17 (22%), respectively. Of 46 patients evaluable, 26 (56.5%) developed chronic GVHD (22=extensive, 4=limited). Multivariate analysis for acute GVHD (II-IV) demonstrated a significantly increased risk with older age (≥ median) (HR 1.9[1.0–3.5], p = 0.05) and a trend for patients without lack of iKIRL (HR 1.9[0.9–3.8], p = 0.08). Degree of HLA match, conditioning regimen, disease risk, gender mismatch, stem cell source, or CMV serostatus had no significant impact on acute GVHD. Reduced intensity conditioning was associated with better OS (76%) compared with full-intensity conditioning (38%, p = 0.01), which remained significant in multivariate analysis (HR: 0.4[0.2–0.8], p = 0.02).

In summary, our results show the combination of siro, tacro, and low-dose MTX is associated with an acute GVHD rate comparable to our historic data after MUD-HCT, and associated with a promising OS when combined with FluMel reduced-intensity conditioning.

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GRAFT REJECTION AS A TYPE I IMMUNE RESPONSE AMENABLE TO MODULATION BY TYPE II DONOR T CELLS VIA AN "INFECTIOUS" MECHANISM

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We investigated the mechanism(s) whereby rapamycin-generated donor Th2 cells (Th2R cells) prevent graft rejection after allogeneic bone marrow transplantation (BMT). Analogous to Th1/Th2 cytokine balance in GVHD, we hypothesized that host-versus-graft reactivity (HVGR) may involve type I cells amenable to inhibition by type II cells. Fully MHC-disparate BMT was performed: [BALB/c(H-2 d) into B6(H-2 b)] or [B6-into-BALB/c]; lethal host irradiation (XRT;1050 cGy); and post-XRT add-back of host T cells (0.1×10^6). Wild-type (WT) or Th1/Tc1 host T cells mediated rejection (5/5 and 10/10 mice, respectively); in contrast, host Th2/Tc2 cells yielded full donor chimerism (10/10 mice). STAT1 signaling, which dictates Th1 differentiation, was required for rejection, as post-XRT add-back of STAT1 knockout (KO) host T cells yielded full donor engraftment (5/5 mice). HVGR was quantified by cytokine-capture flow cytometry: host T cells were harvested post-BMT, stimulated with donor APC, and allospecific host T cells was enumerated. Mice receiving WT or Th1/Tc1 host T cells had increased post-BMT allospecific CD4+ and CD8+ T cells secreting IFN-γ(cohort [C] #3> C#2; $p < 0.05$; C#4>C#2; $p < 0.05$). In contrast, Th2/Tc2 or STAT1 KO host T cell add-back yielded nominally increased alloreactive host T cells (C#3 and C#4>C#5, $p < 0.02$; C#3>C#6, $p < 0.002$). These results suggested that donor Th2R cells may prevent rejection by reducing host Th1-type differentiation and promoting host Th2-type differentiation rather than by mediating host T cell clonal deletion. To evaluate this, allospecific 2C TCR transgenic T cells were utilized as host T cell add-back; BMT was performed ± donor Th2R cells. Low-dose donor Th2R cells relative to host 2C TCR transgenic cells (200:1) resulted in rejection (5/5 mice); 2C cell expansion and alloreactivity were nominally decreased by this Th2R cell dose. However, high-dose Th2R cells (1000:1) promoted full donor engraftment (7/10 mice); rejection prevention was associated with reduced expansion but not total elimination of 2C cells and reduced 2C cell IFN-γ allospecificity (C#11 < C#10, p = .008). Furthermore, in this cohort, cytokine profile of purified post-BMT 2C cells was analyzed: the cells were skewed towards a Th2 phenotype (reduced IL-2, IFN-γ; increased IL-4, IL-10). Taken together, Th2R cells prevent rejection by a mechanism that promotes type II cytokine "infectious transplantation tolerance" without allospecific clonal deletion.

Role of Th1/Tc1, Th2/Tc2, STAT1 KO in Graft Rejection: Abrogation of 2C Cell Rejection by Donor Th2R Cell Infusion

	Model #1 B6→BALB/c (Inocula)	Alloreactive Host CD4 Cells (# CD4+ IFN-γ + T cells; × 10^3/spleen)	Alloreactive Host CD8 Cells (# CD8+ IFN-γ + T cells; × 10^3/spleen)	% Donor Cells
Cohort				
1	BM	1.8 ± 1.8	0 ± 0	88 ± 2
2	Host T	5.1 ± 1.6	3.1 ± 2.2	-
3	BM + Host T	772 ± 256	653 ± 218	0.8 ± 0
4	BM + Host T1	2129 ± 723	1787 ± 574	0.8 ± 0
5	BM + Host T2	91 ± 31	22.2 ± 6.6	10 ± 2
	Model #2 BALB/c→B6 (Inocula)			
1	BM	0.1 ± 0.1	0.5 ± 0.2	54 ± 5
2	Host T	24 ± 12	7 ± 1	-
3	BM + Host T	1249 ± 285	3927 ± 555	1.6 ± 0.3
6	BM + Host T	48 ± 43	68 ± 54	61 ± 6
	STAT1 KO Model #3 BALB/c→B6 (2C TCR Host Add-back)	Absolute # of 2C TCR Host T cells (×10^3/spleen)	Absolute # of 2C TCR IFN-γ alloreactive host T cells (×10^3/spleen)	% Donor Cells
7	BM	-	-	90 ± 2
8	Host T	307 ± 56	77 ± 18	-
9	BM + Host T	3656 ± 1189	817 ± 226	0.4 ± 0.1
10	BM + Host T + Donor Th2R (200:1 ratio)	884 ± 144	525 ± 140	1.7 ± 0.3
11	BM + Host T + Donor Th2R (1000:1 ratio)	152 ± 43	30 ± 17	92.6 ± 1.2

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APPLYING THE HEMATOPOIETIC CELL TRANSPLANTATION-COMORBIDITY INDEX (HCT-CI) IN MYELOABLATIVE MUD TRANSPLANTS PREDICTS NRM AND OS USING A MODIFIED 2-GROUP SCORING SYSTEM

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Background: The HCT-CI is a comorbidity score adapted to hematopoietic cell transplantation, which has identified 3 risk groups with increased non-relapse mortality (NRM) and lower overall survival (OS) (Blood 2005;106:2912). We determined the HCT-CI score in a cohort of patients who underwent myeloablative MUD transplantation in an single institution trial. **Methods:** The analysis included all patients undergoing MUD transplant from 1996–2006 who received a 3-drug GVHD prophylaxis regimen. Comorbidities were obtained by retrospective chart review and scored according to the HCT-CI score. **Results:** 150 patients (median age 40) were included, with 38% low-, 34% intermediate- and 28% high-risk disease, per CIBMTR classification. Diagnoses included acute leukemia in 50%, MDS in 12%, CML in 15%, and lymphoma in 18%. Conditioning regimens were Cy-TBI in 64% and Bu-Cy in 21%. HCT-CI scores of 0, 1–2 or ≥3 were found in 17%, 30% and 53% of patients. The majority of comorbidities were pulmonary (72%). Day 100 and 5-year OS were 82.7 and 33%, with a 23% and 50.4% cumulative incidence of NRM. Five year relapse-related mortality was 15.8%. No statistically significant differences in NRM or OS were detected using the HCT-CI grouping of 0, 1–2 and ≥3. Unadjusted hazard ratio (HR) for inferior survival were 0.9 (CI 0.47–1.85, P = .79) and 1.65 (CI 0.885–3.090, P = .11) for scores 1–2 and ≥3, respectively. We determined an alternate prognostic model based on 2 groups. Statistical modeling separated patients with a score of 0–3 (n = 97, 64%) and ≥4 (n = 53, 35.6%), with a 3 month and 5 year OS of 84% and 45% versus 52% and 10%, respectively (P < .0001). Cumulative incidence of day 100 and 5-year NRM was 16% and 38% versus 43% and 73%, respectively. Unadjusted HR for inferior survival was 2.77 (CI 1.816–4.225, P < .0001) for a score of ≥4. By multivariate analysis, only the HCT-CI score (P < .0001) and the disease risk per CIBMTR (P = .0058) were predictive of OS and NRM, but not age, CMV status, sex- or HLA-mismatch, or regimen. **Conclusions:** Although the HCT-CI score was predictive of NRM and OS in this high-risk cohort, we were unable to detect statistically significant differences between the 3 risk groups defined in the original score. A modified 2-group scoring system of 0–3 and ≥4, respectively, identified a low-risk and a high-risk group. This simplified, 2-tiered scoring system will have utility in clinical decision-making and in defining patients eligible for clinical trials.

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LIPOPOLYSACCHARIDE BINDING PROTEIN PROMOTER VARIANTS INFLUENCE THE RISK FOR GRAM-NEGATIVE BACTEREMIA AND MORTALITY AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Lipopolysaccharide binding protein (LBP) function is dependent upon circulating LBP levels. Disturbance of *LBP* transcription regulation may influence the risk for clinical events. We comprehensively assessed the LBP sequence and identified three haplotype tagging single nucleotide polymorphisms (SNPs) for our studies. Using a nested case control design, we assessed whether genetic variation in the *LBP* gene influences the risk for Gram-negative (GN) bacteremia after allogeneic hematopoietic cell transplantation (HCT), then confirmed the associations by prospectively assessing association with an intermediate phenotype (circulating LBP levels) and a secondary clinical phenotype (mortality). Presence of the SNP 6878 C allele among patients was associated with a two-fold higher risk for GN bacteremia (odds ratio 2.22, p = 0.001). SNP 6878 was in strong linkage disequilibrium with three SNPs in the *LBP* promoter, one of which was SNP 6878 ($r^2 = 0.8$), located in a promoter CAAT box. The SNP 1683 C allele was associated with higher median circulating LBP levels (TT 10.86 ± 0.68 mg/ml; TC 13.86 ± 1.51 mg/ml; CC 20.66 ± 5.89 mg/ml; p = 0.002) and a three-fold increase in mortality risk (hazard ratio 3.30, p = 0.001). These data suggest that genetic variation in the *LBP* promoter variation likely influences the risk for developing GN bacteremia and death after HCT.

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HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR CHEMORADIOTHERAPY-RELATED MYELODYSPLASTIC SYNDROME AND ACUTE LEUKEMIA: A SINGLE-CENTER ANALYSIS OF 47 PATIENTS

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Purpose: To examine the value of allogeneic HCT in the treatment of therapy-related myelodysplastic syndrome/acute leukemia (t-MDS/t-AL) which develops after previous exposure to chemotherapy (CT) or radiotherapy. **Patients and Methods:** We retrospectively analyzed the data of 47 patients who were treated at our institution between 1996 and 2007. The primary diseases included malignant lymphoma (n = 17), breast cancer (n = 13) and other solid tumors (n = 17). The median interval between the diagnosis of the primary disease and the onset of t-MDS/t-AL was 3.9 years (range, 0.6–18.7). Twenty-five patients had AML, 5 ALL, 1 biphenotypic acute leukemia, and 16 MDS. **Results:** Thirty-three patients received disease-adapted CT, with a response rate of 73%, while 14 patients were elected to receive no interventions due to an indolent clinical course such as MDS-RA (n = 13). With a median follow-up of surviving patients of 1.9 years (range, 0.05–10.5) after the diagnosis of t-MDS/t-AL, the 3-year OS for all patients was 55%. Twenty-seven patients (median age, 47 years: range, 3–63) underwent allogeneic HCT from related (n = 12) or unrelated (n = 15) donors after myeloablative (n = 18) or reduced-intensity (n = 9) conditioning regimens. The sources of stem cells were BM in 15 patients, PBSC in 10, and CB in 2. At the time of HCT, 18 t-MDS/t-AL were in CR, 1 was in non-CR, and 8 had untreated t-MDS. Twenty patients (43%) did not undergo HCT due to various reasons, including age older than 65 years (n = 11), indolent clinical course (n = 4), progression of the primary disease (n = 4) or t-AL (n = 3), and patient refusal (n = 2). The 3-year OS was significantly better in patients who received HCT than in those who did not (71% *vs* 31%; P = 0.018). TRM after HCT was 15%. The 3-year OS was better in those who achieved CR after initial therapy for t-MDS/t-AL than in those who did not (74% *vs* 30%; P = 0.002). A multivariate analysis revealed that HCT was associated with a significantly better OS, even after adjusting for age and other significant prognostic factors including achievement of CR in t-MDS/AL, cytogenetic risk group, and an interval between the diagnosis of primary disease and t-MDS/t-AL of longer than 3 years. **Conclusion:** Although this study is contaminated by a selection bias, it still appears that HCT was an effective therapeutic option for patients with t-MDS/t-AL, especially when patients were in CR. These results support a prospective study to examine the value of upfront HCT.

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HEALTHY ALLOGENEIC RELATED DONORS OF PERIPHERAL HAEMATOPOIETIC PROGENITORS: A LONG-TERM PROSPECTIVE STUDY

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One hundred and thirty six healthy donors who underwent aphaeresis collections after G-CSF stimulation have been included in a surveillance protocol for a provisional period of ten years. Once a year, they are required to send a WBC, Hb and platelets count report and clinical information to our Service. To date, the mean observation time is 46(4–120) months. Fifty out of the 136 donors show a follow-up longer than 60 months. As previously described (1), at day +14 after G-CSF administration, a moderate neutropenia or lymphocytopenia, have been documented in 32 and 20 subjects, respectively. Moreover, a mild thrombocytopenia has been diagnosed during the G-CSF administration in 52 donors. After a five years period of observation, a persistent, slight reduction of circulating neutrophils or lymphocytes has been detectable in 5/32 or 5/20 donors, without clinical relevance. As regards clinical data, 1 case of MGUS, Insipid Diabetes, Autoimmune Thyroiditis, Multiple Sclerosis, Intestinal Multiple Polyps and Allergy to certain food, were the main diseases reported by six donors. Four donors