patients undergoing allogeneic hematopoietic stem cell transplantation (alloSCT) is unclear. We performed a retrospective matched-control study of the outcomes of 31 patients with serological evidence of HC at the time they underwent alloSCT for a variety of hematological malignancies between 1998 and 2007 at the UTMDACC. For comparison, we identified 31 control patients with negative serology for HC, matched on age group, diagnosis, disease risk (poor vs. good), intensity of conditioning regimen (reduced vs. myeloablative) and donor type (referred to as *matched controls*). To confirm the validity of the matching procedure we extended the comparison to the 1800 seronegative patients (*all controls*) transplanted for the same diseases during the same period of time. Multivariate analysis took into account all variables used in our matching procedure.

The median age of the HC patients was 49 (range 26–72); 15 had AML/MDS, 6 CML/MPD, 6 non-Hodgkin lymphoma, 2 myeloma, 1 ALL and 1 Hodgkin lymphoma; 61% of had poor risk disease, 68% had related donors, and 68% received reduced intensity conditioning. These characteristics were identical to those of the matched control group. There were also no significant differences in baseline liver function: immediately prior to starting conditioning, only 7 patients in the HC group and 5 patients in the matched control group had an alanine transaminase (ALT) level greater than the upper limit of normal (ULN), but all less than 3×ULN (ranges 69–185 and 69–178 IU/L); only one patient in the HC group (versus none in the matched control) had a total bilirubin level above the ULN (1.2×ULN).

Median follow-up was 34 (range 3–53), 27 (4–74) and 29 months (1–108), respectively for HC, matched and all controls. Overall survival post-alloSCT was significantly inferior in the HC group, with a median OS of 3 versus 18 and 20 months in the control groups. The cumulative rate of disease progression and acute or chronic GVHD was comparable, but NRM was significantly increased in the HC group. Results were similar regardless of the control group used, validating our matching algorithm. In summary, serological evidence of HC virus infection at the time of alloSCT, even with normal or minimally abnormal liver function tests, is associated with worse survival after alloSCT, due to an increased rate of non-relapse deaths.

Patients with hepatitis C have worse overall survival and non-relapse mortality than controls after SCT

	Matched analysis			Multivariate analysis		
Outcome	HC (N = 31) vs. matched controls (N = 31), %	HR (95% CI)	P	HC (N = 31) vs. all controls (N = 1800), %	HR (95% CI)	P
OS:						
3 mo	58 vs. 87	3.6 (1.2–11.0)	0.03	58 vs. 87	3.9 (2.2–6.8)	<0.001
1 yr	29 vs. 56	2.4 (1.2–4.9)	0.01	29 vs. 56	3.1 (1.9–5.6)	<0.001
NRM:						
3 mo	29 vs. 13	2.5 (0.8–8.1)	0.1	29 vs. 10	3.6 (1.8–7.1)	<0.001
1 yr	43 vs. 24	2.9 (1.1–7.7)	0.03	43 vs. 23	3.3 (1.9–5.6)	<0.01

HC: hepatitis C, OS: overall survival, NRM: non-relapse mortality, HR: hazard ratio, CI: confidence interval.

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SERIAL VORICONAZOLE THERAPEUTIC DRUG MONITORING IN RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Voriconazole is effective for prophylaxis and therapy of invasive fungal infections (IFI). It is metabolized by the CYP450 system 2C19, 2C9 and 3A4 isosenzymes. We have reported that significant interpatient variability exists in plasma voriconazole concentrations after allogeneic HSCT and that about 15% of patients have no detectable voriconazole in the plasma despite adequate dose (Trifilio et al. Cancer 2007;109:1532–1535). Low plasma levels have also been associated with decreased survival in patients with aspergillosis and increased breaktrough Candida glabrata infections (Trifilio et al. Bone Marrow Transplant 2007;40:451–456). Treatment or prophylaxis for IFIs after HSCT is prolonged, and changing conditions are likely to alter voriconazole pharmacokinetics which may predispose to treatment failure. We studied the relationship between the first and second voriconazole levels in 29 allogeneic HSCT recipients whose first plasma voriconazole level was adequate (>2 μg/mL). The first level was drawn 6 days after starting voriconazole (usually day +6 post-HSCT), and the second level was drawn at a variable time after the first (median 9 days; range 1–252 days). The majority of patients received the drug at the dose of 200 mg twice a day orally. The two levels were strongly correlated to each-other ($r = 0.72$; $p < 0.0001$), but not to the interval between the two ($p < 0.6$) or weight ($p < 0.49$). 10 patients had a level <2 μg/mL on the second measurement. Optimal discriminant analysis revealed that all 11 patients with an initial voriconazole level ≤4.6 μg/mL had a second level that was <2 μg/mL ($p < 0.02$). Neither the interval between the two levels ($p < 0.15$) or weight ($p < 0.75$) predicted whether a patient's second voriconazole level was <2 μg/mL. However, when the analysis was expanded to a total of 43 patients in whom the first level was ≥1 μg/mL, 10 of whom had a second level of <1 μg/mL, no factor could be found that could reliably predict for a second level of <1 μg/mL. These data suggest that serial plasma voriconazole levels can change in an unpredictable fashion over time after HSCT - sometimes decreasing to levels that may place the patient at risk for breakthrough IFIs. Patients who require prolonged voriconazole administration may benefit from ongoing therapeutic drug monitoring and dose adjustment.

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EFFICIENT INDUCTION AND ISOLATION OF A PRIMARY CMV-SPECIFIC CD8+ T CELL RESPONSE FROM CMV SERONEGATIVE DONORS FOR THE TREATMENT OF SERIOUS CMV-RELATED COMPLICATIONS IN CMV SEROPOSITIVE PATIENTS TRANSPLANTED WITH A CMV SERONEGATIVE DONOR

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Cytomegalovirus (CMV) disease is a significant cause of morbidity and mortality after allogeneic stem cell transplantation (allo-SCT). Especially in CMV seropositive (CMV+) patients transplanted with a CMV- donor, a high incidence of CMV related mortality is seen. We recently demonstrated in a phase I/II clinical study the feasibility of selecting CMV-specific CD8+ memory T cells from CMV+ donors using the interferon-gamma (IFNg) capture assay and CliniMACS isolation after peptide stimulation of the CMV-specific donor T cells. We have illustrated the in-vivo potential of these T cells after adoptive transfer in 5 patients with persistent CMV reactivation despite seropositivity of the donor, resulting in clearance of the CMV load. However, no suitable method was available for the induction of primary immune responses against CMV for the treatment of persistent CMV reactivation in the high risk group of patients transplanted with a CMV- donor. In the current study we investigated the possibility to induce and isolate CMV-specific T cells from CMV- healthy donors by in-vitro priming and selection. We used as responder cells CD45RO- PBMC from HLA-A1, A2, A3, B7, or B8 positive CMV- donors (n = 13). By CD45RO depletion we removed the majority of regulatory T cells capable of inhibiting the initiation of the response. Naïve donor T cells were cocultured in the presence of IL-7 and IL-15 with mature monocyte-derived dendritic cells loaded with a cocktail containing 1μg of each relevant CMV pp65, pp50, or IE1 derived 9-mer peptide. At day 10, the responses were specifically restimulated with peptide loaded autologous PBMC. At day 20 CMV-specific CD8+ T cells were detected by specific tetramer or pentamer staining, and isolated by flowcytometric cell sorting or magnetic bead isolation, or further enriched by another restimulation, followed by isolation of CD137 or IFNg expressing T cells at day 21. In 13/13 CMV- donors CMV specific T cells could be detected at day 20 of the immune response in frequencies ranging from 0.01–0.4%. Functional CMV-specific T cells against all 3 major immunogenic CMV proteins pp65, pp50, and IE1 were isolated

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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