There is no effective therapy for patients(pts) who develop SR-GVHD following allogeneic hematopoietic cell transplantation(HCT). Cytokines such as IL2 & TNFα have been identified as mediators of acute GVHD. However, single agent antibody therapy against the IL2 receptor(daclizumab) or TNFα (infliximab) has only modest activity in SR-GVHD. We hypothesized that concomitant blockade of both TNFα & IL2 would be more effective in this condition than inhibition of either cytokine alone. Between 2/02 and 10/07, 131 pts with solid tumors(n = 69), hematologic malignancies(n = 15) or nonmalignant hematologic diseases(n = 47) underwent nonmyeloablative HCT from a 6/6(n = 128) or 5/6(n = 3) HLA-matched family donor at our institution. Pre-transplant conditioning consisted of cyclophosphamide & fludarabine given alone(n = 89) or with ATG(n = 42). Cyclosporine, plus either MMF(n = 45) or MTX(n = 86) was used as GVHD prophylaxis. Twenty one pts developed grade 3–4 SR-GVHD(GVHD that did not improve after ≥6 days of ≥1 mg/kg methylprednisolone [MP], including ≥3 days of high dose MP) a median of 28 days following HCT; involved organs included the lower GI tract(n = 21), liver (n = 3) & skin (n = 7). All pts received daclizumab (1 mg/kg, days1,4,8,15,22) & infliximab (10 mg/kg, days1,8,15,22). Measures to minimize the risk of opportunistic infections were rigorously applied & included aspergillus prophylaxis, empiric broad-spectrum antibiotics, surveillance blood cultures & a rapid reduction in the dose of MP (≤1 mg/kg). We observed a remarkably high response rate following therapy, with 19/21(90%) pts experiencing complete resolution(CR) of GVHD in affected organs. Responses were usually delayed(median onset 2 weeks) but durable, with all responders recovering sufficiently for hospital discharge. The most notable complication associated with therapy was the development of invasive fungal infections in three pts; in two of these, prophylactic antifungal therapy had been prematurely discontinued due to drug toxicity. Nine patients are alive at the time of this analysis, with a median survival of 255 days (range 37–1724 d). Four pts died from progression of their underlying solid tumor, two from complications related to CMV, & four from complications related to GVHD (including three from invasive fungal infections). These data suggest combined TNFα/IL2 blockade is an effective therapeutic option for pts with SR-GVHD & highlight the need for aggressive antimicrobial prophylaxis in the management of this condition.

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RESPONSE TO STEROIDS AS PRIMARY THERAPY FOR ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) IN 280 HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) RECIPIENTS: COMPARISON OF UNRELATED DONOR (URD) BONE MARROW (BM) AND UMBILICAL CORD BLOOD (UCB)

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Acute GVHD remains a major cause of morbidity and mortality after allogeneic HSCT. To date, there has been no comparison of URD HSC sources in terms of response to primary therapy for acute GVHD. Therefore, we evaluated the response in 280 patients with grade II-IV acute GVHD, treated with prednisone 60 mg/m^2 as initial therapy between 1995–2006 at the University of Minnesota. One hundred and thirty-one patients received URD donor BM, and 149 received URD UCB. UCB recipients were more likely to be: transplanted more recently, have greater HLA disparate grafts, treated with non-myeloablative conditioning, and receive CSA/MMF as GVHD prophylaxis. The groups had similar recipient age, gender, maximum initial acute GVHD grade, and days from HSCT to acute GVHD. The day 28 response was defined as the maximum acute GVHD grade (and organ stage) at 28 days (±14 days) of prednisone therapy. Complete response (CR) was defined as resolution of acute GVHD in all organs maintained for 28 days (to day 56) without additional treatment. Partial response (PR) was defined as improvement of GVHD in all organs without CR and without worsening in any organ. CR and overall response (OR=CR+PR) occurred in 102 (36%) and 167 (60%), respectively. Adjusting for differences between groups, factors favorably associated with achieving CR included use of non-myeloablative conditioning (RR 2.1 vs 1.0 for myeloablative conditioning [95% CI, 1.2–3.6, p = .03]), onset of acute GVHD later than day +30 (RR 3.4 vs 1.0 for earlier onset [95% CI, 1.9–5.8, p < .01]), grade II-III GVHD (RR 21.4 vs 1.0 for grade IV GVHD [95% CI, 2.8–165.2, p < .01]) and single organ GVHD (RR 2.1 vs 1.0 for multiple organ GVHD [95% CI, 1.2–3.6, p < .01]). These factors were also associated with OR. Probability of survival at 6 months after initiation of steroid therapy was 64% (95% CI 59–69%) for the entire cohort. In Cox regression, factors associated with lower 6 month mortality were use of UCB (RR 0.6 vs 1.0 for BM [95% CI, 0.4–0.9, p = .02]), and grade II-III acute GVHD (RR of death 0.4 vs 1.0 for grade IV [95% CI, 0.3–0.7, p < .01]). While mortality was less in recipients of UCB, more effective GVHD therapy is needed for patients with multiple organ involvement and for those with grade IV acute GVHD regardless of HSC source.

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ALLOGENEIC ANTIBODIES SPECIFICALLY TARGET AML ANTIGEN NUSAP1 AFTER BONE MARROW TRANSPLANTATION

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Allo-antibody (allo-ab) responses that develop after an allogeneic hematopoietic cell transplant (HCT) in sex-mismatch transplant patients are associated with cGVHD, persist even after 2 years post-transplant and are quantitative. We hypothesize that novel minor histocompatibility antigens (mHA) can be serologically identified as targets of allo-ab responses that develop after an allogeneic HCT. For this study, plasma was collected from five AML patients one year post-transplant, pre-transplant, and from their donors and detected against 5,000 full-length human proteins with N-terminal GST epitopes presented on V3.0 ProtoArrayTM (Invitrogen). Targets of allo-Ab were identified after subtracting the pre-transplant fluorescent signal intensities from their one-year plasma results and then characterized for donor plasma response. New allo-Ab responses targeted 60–75 antigens with fluorescent differences ranging 0.5 to 3 logs.

Two of the five patients showed Nucleolar and Spindle Associated Protein 1 (NuSAP1) and Chromatin Assembly Factor 1b (CHAF1b) as the two predominant proteins recognized after HCT. Exon DNA sequencing has failed to identify SNPs encoding disparate amino acid polymorphisms. However gene expression profiles showed NuSAP1 was up-regulated in AML as compared to other types of leukemia. Therefore we hypothesize the allo-ab immune response targets NuSAP1 due to increased expression as a tumor associated antigen. We directly confirmed using RT-PCR that NuSAP1 expression was the highest in AML peripheral blood lymphocytes (Table attached) as compared to all other cell populations sorted from GM-CSF mobilized peripheral blood from healthy donors. To identify if antibodies specifically recognized and targeted the potential tumor antigen NuSAP1 post-transplant, 87 patients were screened by ELISA for allo-ab responses against NuSAP1. Allo-ab against NuSAP1 were significantly higher in patients post-transplant with AML (n = 27) as compared to ALL, CLL, CML, MCL, MM and NHL, with a significance of p = 0.0011 for NuSAP1 using Wilcox test.

NuSAP1 and CHAF1b were also highly expressed on CD34+Thy1+ stem cells from healthy donors by RT-PCR. ELISA results demonstrated that patients post-transplant also developed allo-ab responses to CHAF1b though these responses were not significantly associated with disease. Taken together this data suggests that allo-ab target tumor antigens and molecules highly expressed in the hematopoietic stem cells.

	Relative mRNA expression when normalized to Actin	
Cell Populations	**NuSAP1**	**CHAF1b**
Patient AML PBSC (n = 5)	**17.06827**	**0.27725**
Cell Populations from healthy donors		
CD34+/THY1+ (n = 4)	**9.297199**	**0.621888**
CD3 (n = 7)	**2.243828**	**0.201833**
CD19 (n = 5)	**2.030201**	**0.371341**
PBSC (n = 11)	**2.950317**	**0.086726**