the gastrointestinal tract and severe histopathology. The specific deletion of recipient dendritic cells surprisingly enhanced the expansion of donor alloantigen-specific T cells and accelerated GVHD mortality due to a failure of activation-induced donor T cell death. Consistent with this, the use of bone marrow-chimeric recipients demonstrated that professional, hematopoietic-derived recipient APC in isolation were limited in their capacity to induce GVHD. In contrast, non-hematopoietic recipient APC in isolation induced universal GVHD mortality with high levels of alloreactive donor T cell expansion and inflammatory cytokine generation. Confocal imaging demonstrated that MHC class II is highly expressed in recipient non-hematopoietic tissue within the dermis and intestinal villi. Donor T cell activation (CD69) and memory differentiation ($CD62L^{lo}/CD44^{hi}$) occurred in irradiated recipients in an antigen-independent fashion and resulted in the acquisition of a memory cell phenotype and Th1 differentiation in lymph nodes. Within 3 days after BMT, these donor T cells began entering the GI tract and interacted with MHC class II^{+} non-hematopoietic cells leading to lethal acute GVHD.
Conclusion: These data challenge current paradigms, demonstrating that lethal acute GVHD can be induced by alloantigen presented solely within MHC class II by non-hematopoietic recipient APC.

430

MEASUREMENT OF ORAL CHRONIC GRAFT-VERSUS-HOST DISEASE

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Oral chronic graft-versus-host disease (cGVHD) is a serious complication of allogeneic stem cell transplantation, and may be the primary site of disease activity. Scales and instruments recently introduced to measure severity and response to therapy have not been prospectively validated. The objective of this study was to describe the characteristics of oral cGVHD and determine the measures most sensitive to change.
Methods: Patients enrolled in the cGVHD Consortium with oral involvement were included. Clinicians scored oral cGVHD according to the 2005 NIH criteria for severity scoring (0-3) and response (erythema, lichenoid changes, ulcers, mucoceles), and patients completed measures. Other evaluated measures included the esophageal response measure, Hopkins mouth score, and weight. Clinicians and patients also rated change on an 8-point scale, categorized as improved (1-3), stable (4-6), or worsened (7-8).
Results: Of 458 participants with cGVHD, 72% (n = 331) had oral cGVHD involvement at enrollment and were followed for a median of 13.6 months (2.0-38.5). Lichenoid change was the most common objective finding (n = 293; 89%), and 25% of patients had only lichenoid involvement. Oral cGVHD was not associated with global quality of life as measured by the FACT-BMT or SF-36. At visits where change could be assessed (n = 501, 52% of follow-up visits), 51% of clinicians and 56% patients reported improvement, with worsening reported in 4-5% for both groups; agreement between clinician and patient perceived change was fair (weighted kappa = 0.41), but only 1% of visits had highly discordant changes (improve vs. worse). Multivariable regression modeling suggested that the serial measurement changes most predictive of perceived change by clinicians and patients were the erythema and lichenoid scores, NIH severity score, clinician assessed pain score and patient assessed Lee oral symptom score. Serial change in erythema and lichenoid features showed synergy, with more combined impact than each feature alone. Perceived changes in oral cGVHD were not associated with change in ulcers, mucoceles, esophageal scores or patient weight.
Conclusions: Oral involvement in cGVHD is common and associated with a wide range of signs and symptoms that generally improve with time. Measurement of oral erythema and lichenoid changes, pain and patient symptoms using 6 questions may adequately capture the activity of oral cGVHD in clinical trials.

431

REGULATION OF INTESTINAL INFLAMMATION BY INTESTINAL MICROBIOTA FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION

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Following allogeneic bone marrow transplantation (allo BMT), patients are at high risk for developing intestinal inflammation secondary to graft-versus-host disease (GVHD). While the impact of the microbiota on GVHD is known to be significant, no consensus exists between BMT centers regarding the optimal approach to target the flora.

We first examined in mouse models the effects of GVHD on gut flora. While BMT alone produced surprisingly few changes in the flora of mice, with GVHD we observed loss of overall diversity, and in particular expansion of Lactobacillales and loss of Clostridiales. We studied the effects of eliminating Lactobacillales from the flora of mice prior to BMT using antibiotics and observed aggravation of GVHD, while re-introducing the predominant species of Lactobacillus mediated significant protection resulting in improved survival. These results from murine models suggest that GVHD produces unique changes in the flora, and that changes induced by antibiotics can aggravate GVHD.

We then characterized gut flora of eight patients undergoing allo BMT during onset of intestinal inflammation due to GVHD, compared to ten patients without GVHD. We again found patterns of loss of diversity, expansion of Lactobacillales, and loss of Clostridiales, mirroring our findings in mice. We also identified increased microbial chaos early following allo BMT as a potential risk factor for subsequent GVHD. Together, these data increase our appreciation for reciprocal regulation of inflammation and flora in the intestine, and suggest that flora manipulation may improve outcomes for allo BMT recipients.

Table. Summary of GVHD-induced changes in the microbiota

	Mice		Humans	
	No GVHD	GVHD	No GVHD	GVHD
Flora diversity	no change	decreased	no change	decreased
Lactobacillales	no change	increased	no change	increased
Clostridiales	no change	decreased	no change	decreased

432

IDENTIFICATION OF A NEW HY MINOR IN THE UTY GENE USING REVERSE IMMUNOLOGY

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Introduction: Minor histocompatibility antigens located on the Y-chromosome (HY minors) are known to play a pivotal role in allogeneic hematopoietic cell transplantation (HCT) with female donor and male recipient. Only a few HY minors are known. We identified a new HY minor using reverse immunology where candidate minors first were predicted using bioinformatics and afterwards confirmed with standard immune laboratory techniques.
Methods: Patient/donor pairs with female donors and male patients were high resolution HLA typed. Candidate HY minor epitopes located in genes only expressed on the Y chromosome were found using the HLA-pan restrictor (http://www.cbs.dtu.dk/services/HLArestrictor). Post nonmyeloablative conditioning HCT PBMCs from the patients were thawed, stimulated with these peptides and tested for cytokine produktion (TNF-α and IFN-γ) after restimulation using flow cytometri. Approximately 140 peptides were synthesized per patient/donor pair and the test for cytokine production was carried out using a matrix system. When positive cytokine responses were found, the optimal peptide and the HLA-restriction were determined by affinity assays and tetramerstaining. Cytotoxity was demonstrated by staining for CD107a.
Results: A strong cytokine response on an 11-mere peptide located in the UTY gene was found in a patient transplanted for follicular

lymphoma. Within the 11-mere, the peptide with the highest affinity to an MCH allele was a 9-mere and tetrameres with the MCH allele A2402 and the 9-mere positively stained expanded T-cells. The tetramere positive T-cells were found to be CD107a-positive after stimulation with dendritic cells pulsed with the peptide. T-cells specific for that HY minor were found after expansion in two of four A2402 positive patient/donor pairs with female donor and male patient.

Conclusion: A new HY minor has been identified using a high-throughput methodology suitable for identifying more HY minors and possibly also autosomally expressed minor histocompatibility antigens.

433

IS LIVER BIOPSY NECESSARY IN THE MANAGEMENT OF ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS WITH GRAFT-VERSUS-HOST DISEASE?

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Background: Liver function abnormalities are common after hematopoietic stem cell transplantation (HSCT) and liver biopsy is often used to direct treatment. We reviewed 117 consecutive liver biopsies during the last 8 years at our institution.

***Materials and Methods*:** From 2003 to 2010, 117 liver biopsies were performed on patients who had undergone HSCT for hematological malignancies and presented with liver dysfunction. All slides were evaluated for features of GVHD by cholestasis, fibrosis, lobular inflammation, iron deposition and were graded from 0 to 3 (0 = none, 1 = mild, 2 = moderate and 3 = severe).

Results: The median age of the patients was 49 (range 14-66). Fifty seven (49%) patients underwent related donor (RD) and 60 (51%) unrelated donor (URD) transplantations. Preparative regimens were applied based on their disease; GVHD prophylaxis was tacrolimus and mycophenolic acid in 105 (88%) patients; tacrolimus, sirolimus and thymoglobulin in 6 (5%), and other regiments in 6 (5%). Fifty nine patients were alive with a median follow up for the surviving patients of 4.2 years (range 0.9-9.3 years). Kaplan-Meier estimate of overall survival at 6 years was 43% +/- 5%. Liver biopsies were preformed at a median of 173 days post-transplant (range 22-1366 days). At the time of the liver biopsy, the median AST was 168 IU/L (range 18-1425 IU/L), ALT 250 IU/L (range 38-1495 IU/L), total bilirubin 1.1 mg/dL (range 0.1-26.8 mg/dL) and alkaline phosphatase 278 IU/L (range 69-1547 IU/L). On biopsy there were 107 (91%) patients with histological features of GVHD: mild 29 (27%), moderate 56 (52%) or severe 22 (21%). Iron deposition studies showed 5 (4%) patients without iron deposition, 23 (20%) patients grade 1, 38 (32%) patients grade 2, and 51 (44%) patients grade 3. In multivariate analysis of relevant prognostic factors including age, donor, total bilirubin, ALT and histological grade of cholestasis, only total bilirubin of $< = 1.5$ IU/L predicted favorable survival (hazard ratio = 0.34; 95% CI 0.19 - 0.63, p = 0.001).

***Conclusion*:** Liver biopsy confirmed the presence of GVHD in most patients. The histological features of GVHD and iron deposition did not adversely influence the survival. The most significant predictor of survival was the presence of cholestasis on biopsy and the elevation of total bilirubin of >1.5 mg/dL. Our data indicated limited value of liver biopsy in management of patients with liver dysfunction after allogeneic stem cell transplantation.

434

GENETIC VARIATIONS IN THE ACTIVATION AND EFFECTOR PATHWAYS OF CYTOTOXIC T LYMPHOCYTES MODULATE ALLOIMMUNE REACTIVITIES AND HAVE PROGNOSTIC SIGNIFICANCE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objectives: Donor T lymphocytes play a critical role in alloimmune reactivities after allogeneic hematopoietic stem cell transplantation (allo-HSCT). CD28, inducible co-stimulator (ICOS) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) belong to the same family of T-cell costimulatory molecules. After activation, three effector pathways have been described for T-cell cytotoxicity: granzyme B/perforin, Fas/Fas ligand (FasL) and secreted molecules such as TNF-alpha. Near recently, several important polymorphisms have been identified in those genes and reported to be associated with the risk of autoimmune diseases, malignancies and allograft rejection in solid organ transplantation patients. However, such information is less available in allo-HSCT. In the present study, we first investigated the influence of those genes polymorphic features on the abilities of T-cell alloimmune responses in allo-HSCT setting.

Methods: We analyzed 10 single nucleotide polymorphisms (SNPs) in the CD28, ICOS, CTLA-4, Granzyme B, Fas and FasL genes in 138 pairs of recipients and their unrelated donors (URDs) and 102 pairs of recipients and their HLA-identical sibling donors.

Results: (1) We found two SNPs in donors influenced the risk of aGVHD. The association was particularly strong in the URD transplantation cohort. Unrelated donor with Granzyme B +55 mutated genotype (AA) was an independent risk factor for grades II-IV aGVHD (P = 0.024, RR = 1.811, 95%CI, 1.080-3.038). While unrelated donor with CTLA-4 CT60 mutated genotype (AA) was protective (P = 0.025, RR = 3.806, 95%CI, 1.187-12.204). (2) However, donor with CTLA-4 CT60 AA genotype was a risk of early CMV infection (P < 0.0001, RR = 0.383, 95%CI, 0.243-0.605) and relapse after allo-HSCT in AML patients (P = 0.047, RR = 2.792 95%CI, 1.013-7.696). Furthermore, AML patients with Fas -670 homogeneous mutated allele (TT genotype) also had a higher risk of relapse (P = 0.003, RR = 3.823 95%CI, 1.566-9.337). (3) The presence of those susceptible alleles in donor and/ or recipient (patients receiving CTLA-4 CT60 AA donor, patients receiving GranzymeB +55 AA donor, AML patients with Fas -670 TT genotype or with all) resulted in a reduced overall survival compared with those with wild-type genotypes (54.9% vs 69.5%, P = 0.029).

Conclusions: The results of this study highlight the important effect of genetic variations in T-cell activation and effector pathways from the donors and recipients on the outcomes of allo-HSCT.

435

SEVERE CHRONIC GRAFT-VERSUS-HOST DISEASE IS ASSOCIATED WITH IMPAIRED THYMOPOIESIS AND PERIPHERAL LYMPHOCYTE EXPANSION

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Allogeneic hematopoietic stem cell transplantation (AHSCT) is a curative therapy for hematologic malignancies. Chronic GVHD (cGVHD) is a significant barrier to successful AHSCT. Although T cells have been implicated in cGVHD pathobiology, the role of the thymus in this process has yet to be clearly defined. We characterized thymus and spleen T cell subsets in a murine model of cGVHD (B10.D2→BALB/c) to investigate the role of the thymus in this process. Once cGVHD was well-established, 4-6 weeks post AHSCT, total thymocyte numbers in mice with cGVHD were more than 20-fold depleted compared to syngeneic controls. Furthermore, the double positive thymocyte cell population (CD4+ CD8+) was most markedly reduced, with a 90-fold reduction in numbers compared to syngeneic controls. Despite evidence of thymic atrophy and diminished thymopoiesis, thymic CD4+ single positive cells were decreased less proportionally (13-fold). Congenic AHSCT demonstrated that this CD4+ population was comprised of donor peripherally derived CD44hi memory T cells, representing an allogeneic infiltrate. Further evidence of diminished thymic output was found in the periphery. Live splenocytes were 3-fold depleted in