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.

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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.

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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.

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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

animals with cGVHD, with the CD4+ population having a 13-fold reduction compared to syngeneic controls. By cell surface phenotype, the recent thymic emigrant (RTE) CD4 proportion (CD4+ CD44lo CCR7+ CD69-) was most severely affected, demonstrating a 100-fold reduction. The spleens of affected animals were enriched for memory T cells, particularly T effector memory (CD4+ or CD8+ CD44 high CCR7-). Furthermore, the effector memory T splenocytes of mice with cGHVD were more likely to have an activated phenotype as evidenced by CD69 expression. Congenic AHSCT confirmed that the splenic CD4+ compartment was donor-derived. The total number of splenic T regulatory cells (CD4hi Foxp3+ CD25+) was diminished in animals with cGVHD; however, the proportion of this subset was increased in the cGVHD mice. The T reg compartment was also donor peripherally derived. These data show that cGVHD is associated with impaired thymopoiesis. The relative CD4 lymphopenia and diminished RTE numbers likely stem from impaired thymic output and are associated with donor-derived peripherally expanded CD4+ cell predominance.

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CD28-DIRECTED T CELL COSTIMULATION BLOCKADE WITH ABATACEPT TO PREVENT GVHD DURING HIGH-RISK UNRELATED HSCT: A FIRST-IN-DISEASE TRIAL

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We describe a first-in-disease trial of CD28-directed costimulation blockade to prevent GvHD after URD HSCT in patients >12y with advanced heme malignancies (Clinical Trials.Org #NCT01012492). In this trial, 10 mg/kg abatacept is administered on d -1, +5, +14, and +28 in addition to standard GvHD prophylaxis (cyclosporine + methotrexate). 10 of 11 planned patients have enrolled, of which 8 are evaluable for engraftment and toxicity, and 5 are evaluable for the primary immunologic outcome of the study: the incidence of steroid-requiring GvHD at d+100 post-transplant. The median patient age is 46 y (17-74 y). 5 transplants were 7/8 HLA matched and 3 were 8/8 matched. 7 of the 8 patients are currently alive and in remission. 1 patient relapsed at d+98 (and died on d+121 with refractory AML). The other 7 patients are surviving without relapse with a follow-up of 57-377d.

All 8 patients received the 4 scheduled abatacept doses, without infusional side-effects. All patients achieved neutrophil engraftment (median d+19) and donor engraftment (100% CD33 chimerism at d+30). Of the five post- d+100 patients, all demonstrated rapid lymphocyte engraftment (mean ALC >500 cells/μL by d+21). At d+100, their mean CD3+, CD8+ and CD4+ T cell counts were 673 +/- 251, 384 +/- 148, and 229 +/- 119 cells/μL, respectively. T cell reconstitution was accompanied by a shift toward a CCR7-/CD45RA- effector memory (Tem) phenotype, with CD4+ Tem increasing from 22 +/- 6% pre-transplant to 46 +/- 7%, and CD8+ Tem increasing from 15 +/- 4% pre-transplant to 32 +/- 7%, both with a reciprocal decrease in Tnaive cells.

At the d+100 primary end-point, only 1 of the 5 evaluable patients on the abatacept trial developed steroid-requiring GvHD. 3 additional patients are <100 days post-transplant (between d+57-64), and none has developed GvHD. In striking contrast, in a contemporaneously transplanted cohort of 5 patients enrolled on an immune monitoring protocol (and who had the same disease indications and transplant preparative regimens, but who received standard GvHD prophylaxis), 4 of 5 of these patients developed steroid-requiring GvHD before d+100. 2 patients required salvage therapy and one patient died from GvHD.

These preliminary data suggest that abatacept can be safely added to cyclosporine and methotrexate for GVHD prophylaxis during URD HSCT, with encouraging rates of GVHD at the d+100 primary endpoint. As such, they support the conduct of a larger, randomized phase 2 study.

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NILOTINIB (TASIGNA) THERAPY POST ALLOGENEIC STEM CELL TRANSPLANTATION (ALLoSCT) FOR ADVANCED (>CP[1]) CHRONIC MYELOID LEUKEMIA (CML) AND PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Relapse rate post alloSCT in advanced CML (>CP[1]) and in Ph+ ALL is high. We assessed (study CAN107AIL03T) whether Nilotinib administration post alloSCT intensifies remission and reduce relapses. 24 pts (M-13, F-11), age 36 years (range, 18-58) participated in the study. 17 pts were with advanced CML (BC-10, AP-7) and 7 with Ph+ ALL. 22/24 pts underwent alloSCT (2 are pending) from an HLA-matched sibling (n = 11), matched unrelated (n = 8) or alternative donor (CB-2, Haplo-1). All, but one, had myeloablative conditioning (Bu/Cy or Flu/Bu-14, TBI/Cy-7). GVHD prophylaxis included CSA and methotrexate or MMF. 21/22 pts engrafted in median day+13 (range, 9-38) with 100% donor chimerism. TRT included mucositis in all pts, encephalitis-2, pericarditis-1, VOD-1 and infection-3. 3 pts died very early post transplant from sepsis and multi organ failure. Acute GVHD ≥ Gr II was observed in 10 pts (III-IV in 5). cGVHD was observed in 15 pts (extensive – 9). 16/22 transplanted pts received Nilotinib (200mg x 2/d – 10, 300mg x 2/d – 6) starting at median day +38 (range, 30-158) post alloSCT. 6 pts did not receive Nilotinib post alloSCT due to early death -3, progressive disease -1, severe pancytopenia -1, refusal -1. Nilotinib administration was delayed or dose reduced/stopped in 9 and 7 pts, respectively due to toxicities. All, but 2 pts achieved MMR post alloSCT and 6 of them (CML) converted to CMR following Nilotinib therapy. Kinas mutation (G205E and F359V) were detected only in 1 pt with disease progression. With a median follow up of 16.5 mo (range, 2.5-38) 12 pts are alive while 12 died (Infection -5, GVHD -3, TTP-1, disease progression -3). In 2 pts disease progressed but responded to further therapy. All 6 pts that did not receive Nilotinib died (early death post allo-SCT -3, disease progression -1, GVHD Gr IV -2). Immunological evaluation post Nilotinib administration disclosed no significant change in T, B and NK cell numbers and T cell mitogenic response. Thymic output (TREC) and receptor repertoire (Spectrotyping) analysis indicates continuous thymopoiesis in 8/13 evaluated pts. NK cytotoxic activity against K562 increased in 9/15 evaluated pts. In conclusion, post alloSCT Nilotinib maintenance therapy in extremely high risk pts with advanced CML and Ph+ ALL may prevent relapse and disease progressionand should be recommend as 1) pts achieved MMR and 6 converted to CMR with Nilotinib therapy and 2) only 5/16 pts that received Nilotinib post alloSCT progressed.

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ALEMTUZUMAB FOR STEROID REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE IN PEDIATRIC PATIENTS: A RETROSPECTIVE REVIEW OF 12 PATIENTS REVEALS A RESPONSE RATE OF 83%

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High dose steroids are the mainstay of treatment of acute graft versus host disease (GVHD) with a response rate of 30-70%. A clear second line agent for steroid refractory graft versus host disease has not been established and it remains a significant cause of morbidity and mortality in allogeneic hematopoietic cell transplant (HCT) recipients. In order to estimate the effectiveness of alemtuzumab for the treatment of steroid refractory acute GVHD in pediatric patients, we retrospectively reviewed the charts of 12 patients (median age 6.5years, range 1.25-21years) with grades II (n = 1), III (n = 6), or IV (n = 5) steroid refractory acute GVHD who received alemtuzumab for treatment. Steroid refractory acute GVHD was defined as progression of GVHD after 48 hours of >/ = 2 mg/kg steroid treatment, or lack of response within 5 days. Patients received a median dose of 0.73mg/kg alemtuzumab (range 0.3-2mg/kg) divided over 2-5 days. Seventy-five percent of patients received repeated courses. A complete response, defined as GVHD of grade 0 at 4 weeks following the first alemtuzumab course, was observed in 7 patients (58%). A partial response, defined as a grade improvement after 4 weeks, was