characteristics (CD34, CD3, CD19, NK, DC1and DC2 cell dose). Survival curve analysis by a log rank test revealed that the low DC2 group had a significant risk of developing acute and chronic GVHD (P=0.000 for both). These results suggest that the DC2 count in the peripheral blood on day-28 is a strong predictor for development of GVHD in recipients of PBSC in a matched related allogeneic SCT.

### 313 A CDNA-BASED ASSAY FOR DONOR-CHIMERISM ANALYSIS OF EPIDERMAL LANGERHANS CELLS

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Early acute GVHD of the skin frequently occurs in patients after allogeneic hematopoietic stem cell transplantation. T cell depletion sufficiently reduces incidence and severity, but does not completely prevent skin GVHD which then leads to a prolonged need for immunosuppressive medication. The activation of donor T cells by residing host antigen presenting cells such as epidermal Langerhans cells (LCs) plays a central role in the initiation of acute GVHD. The absence of LCs in mismatched T cells after depletion delays the switch of LCs from host to host donor in origin in mice. We and others have provided evidence for a delayed switch in LC chimerism after T cell depleted reduced intensity stem cell transplantation in humans. However, most assays used so far either depend on the detection of the Y-chromosome in skin sections of sex-mismatched transplants. In an attempt to set up a sensitive assay of general applicability, we combined the detection of donor chimerism and tissue specific markers in a single multiplex PCR. We established RT-PCRs for 10 different constitutively expressed genes containing single nucleotide polymorphisms (SNPs) inside their coding regions. These PCRs were combined in a single multiplex PCR and the SNPs were analyzed by the primer extension method (minisequencing) and separated by capillary electrophoresis. We tested this approach on PBMCs of 10 patients and their HLA-matched sibling donors. The assay distinguished all pairs in 1 to 6 out of 10 systems. In a subsequent step, the 10plex PCR was combined with the tissue specific markers langerin for LCs and cytokeratin 10 to distinguish LCs from keratinocytes. The expression of langerin and cytokeratin 10 was detected using gene-specific probes in the same minisequencing reaction used for the detection of SNPs. The resulting 12plex assay distinguished sibling donors from the patients with the same specificity and, in the same reaction, detected Langerin as well as cytokeratin 10 in purified LCs and keratinocytes, respectively. In summary, we established a sensitive assay allowing simultaneous detection of donor chimerism together with the tissue specificity of isolated LCs, which is independent of sex-mismatched donors. The addition of further tissue specific markers might allow performing chimerism studies on other tissue resident antigen presenting cells.

The use of a cDNA-based assay might also allow combining chimerism analysis with activation- and maturation specific markers in a multiplex PCR. We established RT-PCRs for 10 different constitutively expressed genes containing single nucleotide polymorphisms (SNPs) inside their coding regions. These PCRs were combined in a single multiplex PCR and the SNPs were analyzed by the primer extension method (minisequencing) and separated by capillary electrophoresis. We tested this approach on PBMCs of 10 patients and their HLA-matched sibling donors. The assay distinguished all pairs in 1 to 6 out of 10 systems. In a subsequent step, the 10plex PCR was combined with the tissue specific markers langerin for LCs and cytokeratin 10 to distinguish LCs from keratinocytes. The expression of langerin and cytokeratin 10 was detected using gene-specific probes in the same minisequencing reaction used for the detection of SNPs. The resulting 12plex assay distinguished sibling donors from the patients with the same specificity and, in the same reaction, detected Langerin as well as cytokeratin 10 in purified LCs and keratinocytes, respectively. In summary, we established a sensitive assay allowing simultaneous detection of donor chimerism together with the tissue specificity of isolated LCs, which is independent of sex-mismatched donors. The addition of further tissue specific markers might allow performing chimerism studies on other tissue resident antigen presenting cells. The use of a cDNA-based assay might also allow combining chimerism analysis with activation- and maturation specific markers in a single assay.

### 314 SELECTIVE DEPLETION OF ALLOREACTING T CELLS BY TH9402-BASED PHOTODEPLETION AS A TRANSITIONAL STRATEGY FOR GVHD CONTROL IN HLA-MISMATCHED AND MATCHED DONOR-RECIPIENT PAIRS

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Selective depletion (SD) is a strategy to eliminate host-reactive donor lymphocytes from blood stem cell allografts to prevent GVHD and maintain GVL-effects. We investigated a photodepletion (PD) process, whereby allo-activated donor cells are labeled with a photosensitizing rhodamine-based dye, 4,5-dibromorhodamine 123 (TH9402), and exposed to visible light, which preferentially eliminates allo-activated dye-retaining cells. Stimulator cells were prepared from recipients treated with rhodamine-labeled mononuclear cells (MNCs) and cultured using anti-CD3 and 100 IU IL-2/ml. Responder cells (leukapheresis MNCs) from 3 random HLA-mismatched volunteers and 3 HLA-matched sibling donors were cocultured 1:1 with irradiated stimulators for 3 days. Cultured cells were incubated with 7.5 μM TH9402, followed by dye-extrusion and exposure to 5 Joule light in the PD light source (Celmed Bioscience Inc., Canada) at ≥510 cells/ml in FEP plastic bags. Depletion efficiency was studied by mixed lymphocyte reactions (MLR) in mismatched pairs and by helper-T-lymphocyte precursor (HTLp) frequency assay in mismatched pairs. All six clinical-scale experiments provided sufficient reduction of allo-reactivity and retention of third party responses as measured against a pool of 5 donors. In mismatched pairs mean reduction of allo-reactivity was 703-fold (≥141) when compared to unmanipulated donors. Third-party responses were maintained, with a mean reduction of only 1.3 ± 0.15-fold. In matched pairs alloreactivity was reduced below the “GVHD-threshold” of 1/100,000 whilst third party responses remained above 1/100,000 precursors. This establishes a clinical scale PD process capable of highly efficient removal of alloreactive lymphocytes from mismatched and matched MLRs while maintaining desirable third party responses. As PD targets activation-based surface marker expression resulting in more consistent and effective depletion. This approach will now be tested in a clinical SD trial.

### 315 PHARMACOKINETICS (PK) OF MYCOPHENOLATE MOFETIL (MMF) IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT (ALLOSCT) RECIPIENTS

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MMF PK in children may impact on the incidence of moderate/severe acute GVHD following AlloSCT (Osunkwo/Cairo et al, BBMT 2004). The objective of this study is to evaluate effects of the PK of MMF on pediatric AlloSCT recipients. From 1/04 – 8/06 we enrolled 31 pediatric AlloSCT with 27 being evaluable: mean age 8 yrs; wt 54.3 kg; M/F=12:15; NBL PR (n=1), AMC (CR1 [n=4], CR2 [n=1], CR3 [n=1], relapsed/induction failure [n=1]), SAA (n=5), CML CP (n=1), ALL (CR1 [n=1], CR2 [n=2], CR3 [n=1]), HD CR2 (n=1), ALL 1, HD CR2 (n=1), HDL refractory (n=1); donor sources: MFD (6/6 PBSC [n=7], 6/6 BM [n=3], 5/6 PBSC [n=5], 6/6 related CB (n=1), UCB (6/6 [n=2], 5/6 [n=3], 6/6 [n=7], and 8/10 MUD PBSC (n=1). Cohort 1 [6 yrs] (n=8), 2 [6-12 yrs of age] (n=9), 3 [12-16 yrs] (n=10). GVHD prophylaxis included tacrolimus (on Day –1 or 1st day of conditioning to maintain concentrations 5-20 ng/mL) and MMF (900 mg/m2 iv Q6H starting on Day +1, then converted to PO [same dose] after Day +14). Serum samples for MPA were drawn on Day +1, +7, +14 and +14 at hour 0, 0.5, 1, 2, 3, 4, and 6 post-dose. MPA plasma concentrations were determined by reverse-phase HPLC. MDF dose was adjusted to maintain AUC0-24h 1.5-20 μg/L. The mean CD34+ cell dose/kg = 27.5x10^6, TNC dose/kg = 50.3x10^6. Time to neutrophil (ANC ≥ 500/mm3 x 2 d) and platelet engraftment (untransfused count ≥ 20K x 7 d) was 22 d and 36 d, respectively. Mean f/u was 448 d. Mean MPA PK on Day +14; Cmax=17.6 mg/L, Tmax=1.82 h, total MPA trough=0.85 mg/L, AUC0-24h=39.4 mg•hr/L, T1/2=1.3 h, Vd=1.5 L/kg, and CL=1.2 L/kg at a mean MMF dose of 1056 mg/m2 iv Q6H. Age cohorts are shown in Table 1. Incidence of GI adverse events attributable to MMF was 59% (nausea/vomiting [n=13], diarrhea [n=8], abdominal pain [n=3], pneumatois intestinalis [n=1], gastrocitis [n=2]. Kaplan-Meier probability of grade II-IV aGVHD following related (n=14) and unrelated (n=13) donors was 60.8% (15/27 evaluable pts). mCGVHD was 25.2% (5/22 evaluable pts) and 1 year OS was 64.4% (CI: 45.6-81.1%). In comparison to MMF PK in adult AlloSCT pts
receiving cyclosporine/MMF (Nash et al, BBMT 2005), children have significantly higher MMF clearance rates (1.2 vs 0.54 L/kg/h). MMF doses ≥ 3-fold higher than those used in pediatric SOT recipients were required to achieve AUCC0-6=30-60 μg·h/L. Short T1/2 and rapid clearance of MMF in pediatric AlloSCT pts may be related to a lack of enterohepatic cycling and enhanced UDP-glucuronosyltransferase activity.

Table 1. Age-related IV MMF PK on Day +14.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Total MPA</th>
<th>Mean MPA</th>
<th>AUCC0-6</th>
<th>T1/2</th>
<th>Vss</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 y.o.</td>
<td>(n=8)</td>
<td>13.8</td>
<td>1.9</td>
<td>1.2</td>
<td>37.1</td>
<td>1.7</td>
</tr>
<tr>
<td>6-12</td>
<td>(n=8)</td>
<td>14.8</td>
<td>2.0</td>
<td>0.4</td>
<td>35.8</td>
<td>0.9</td>
</tr>
<tr>
<td>12-16</td>
<td>(n=7)</td>
<td>25.2</td>
<td>1.6</td>
<td>1.1</td>
<td>45.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

316 CHARACTERISTICS OF CHRONIC GRAFT-VERSUS-HOST DISEASE IN BRAZILIAN RECIPIENTS OF ALLOGENEIC STEM CELL TRANSPLANTS: A FIVE-YEAR RETROSPECTIVE ANALYSIS

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Chronic graft-versus-host disease (cGVHD) is the leading cause of non-relapse morbidity and mortality in the post-transplantation period. Incidence rates and clinical characteristics have been changing considerably in recent years, following increases in the use of new sources of stem cells and donor/recipient age and the introduction of new modalities of conditioning regimens. Incidence rates reported in the literature have ranged from 20 to 70%, being lower in the pediatric population and in recipients of cord blood grafts. Use of donor lymphocyte infusions as a strategy to control minimal residual disease or treat relapse is also a risk factor for cGVHD. Among 95 patients analyzed, 29 (30.5%) were females. Median age was 35 years (18-64). Eighty seven patients had hematologic malignancies (91.5%). The conditioning regimen was myeloablative in 79 (83%). Bone marrow was used in 74 (78%) and hematologic malignancies (91.5%). The conditioning regimen was myeloablative in 37 (49%). Extensive disease was present in 42 (56%). Esophagitis (>500/μL) was found in 12 (16.5%). Lymphopenia (>500/μL) was present in 19 (25%). Thrombocytopenia (<100,000/μL) was seen in 32 (41.5%). The findings in our series are in accordance with the clinical profile usually reported in the literature. Because of its high morbidity and mortality, the need for early detection of cGVHD can not be overstated. Systematic, prospective evaluation of patients at risk for this complication can contribute to better understanding of the disease, and possibly, better control of the complications and impact of advanced cGVHD in quality of life and survival expectancy.

317 PRE-TRANSPLANT BIOMARKERS PREDICT GVHD AND TREATMENT-RELATED MORTALITY IN PATIENTS TREATED WITH EXTRACORPOREAL PHOTOPHERESIS PRIOR TO ALLOGENEIC BONE MARROW TRANSPLANTATION

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Accurate prediction of the onset and severity of GVHD and treatment-related mortality (TRM) can improve outcomes through optimized donor selection and GVHD prophylaxis. Current assessments are typically made after bone marrow transplantation (BMT), and often require inherently unreliable qualitative measures. The persistence of host antigen presenting cells, particularly dendritic cells (DC) has been implicated in the onset of GVHD, and a recent data suggests that extracorporeal photopheresis (ECP) can prevent GVHD after allogeneic BMT. We examined blood from 32 ECP-treated patients to identify cell surface markers that might predict GVHD and TRM. Samples were drawn immediately prior to ECP (at baseline) and immediately after ECP, but prior to myeloablative conditioning, then assayed by flow cytometry. Cytometry data were grouped and modeled to assess their predictive accuracy alone or in combination with clinical laboratory values. Logistic regression showed that specific DC subsets present prior to myeloablation were the best predictors of outcomes. The likelihood of grade II-IV GVHD (aGVHD) increases when baseline lin HLA-DR+ CD11c+ myeloid cells make up a smaller proportion of circulating mononuclear cells with a predictive accuracy, reflected in the area under the receiver-operating curve (ROC) of 0.83. The best predictive model for TRM was a lower absolute abundance of circulating lin HLA-DR+ CD11c+ plasmacytoid DC at baseline (ROC=0.86). No additional predictive power arose with respect to aGVHD or TRM after including laboratory values. Models that combined certain clinical laboratory results and demographic factors also predicted these clinical outcomes, and offer a possible alternative to complex cytometric assays. The best such model included baseline measurements of BUN/Creatinine ratio, serum albumin, and the match/relatedness of the graft donor and recipient (ROC=0.82, n=59) for TRM, while baseline neutrophil counts were most predictive of aGVHD (ROC=0.69, n=60).

In summary, we have identified biomarkers that, at least in patients receiving ECP, are present before conditioning and can predict outcomes. Although this study does not address the impact of ECP on antigen presenting cells directly, the DC profiles described here highlight the potential role of ECP in modulating DC, and thereby decreasing the risks associated with BMT. These results encourage larger studies with non-ECP control populations to directly address this issue.

318 EXTRACORPOREAL PHOTOPHERESIS (ECP) FOR THE TREATMENT OF STEROID-REFRACTORY OR -INTOLERANT CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD) FOLLOWING ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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We report our long-term results using extracorporeal photopheresis (ECP) for the treatment of chronic graft-versus-host disease (cGVHD). Between 2002 and 2005, 21 patients with steroid-refractory or -intolerant cGVHD received ECP for a median duration of 10.8 (range 1.3-58.8) months (Table). HSCT types were 14 HLA-matched related donor (MRD), 6 HLA-matched unrelated donor (MUD) and 1 HLA-mismatched unrelated cord blood donor. Standard GVHD prophylaxis included a calcineurin inhibitor with methylprednisolone (18), or methylprednisolone (2) or both (1). Two (9.5%), 11 (52.4%) and 8 (38.1%) cases had mild, moderate and severe cGVHD by CIBMTR criteria. Among the 19 patients with adequate follow-up (>6 months post ECP initiation), the sustained overall and complete response (OR and CR) rates were 84.2 % (16/19) and 42.1% (8/19) with the maximum response occurring at the median time of 1.8 (0.6-3.5) months from ECP initiation. OR and CR rates by CIBMTR severity grading were 50% and 50% in the mild group, 45.4% and 36.4% in moderate group, and 62.5% and 37.5% in severe group. Steroid-sparing (steroid discontinuation or requiring adrenal doses only) was