IL-2/GM-CSF except for mild flu-like symptoms. The table below summarizes quantitative analysis of immune activation. Values represent means +/- standard error at day 0 (first day of GM-CSF), day 8 (prior to start of IL-2) and day 14 (last day of IL-2 and GM-CSF). P-values are based on paired t-test analysis of day 8 versus day 0 and day 14 versus day 0, respectively. Flow cytometric analysis showed an increase in the numbers of T-lymphocytes (CD3) and T-cell subsets (CD3/CD8 and CD3/CD4) as well as an increase in natural killer cells (CD16/56). Although no differences were seen in the number of dendritic cell subsets, DC1/DC2 ratios decreased with the administration of GM-CSF/IL-2. Limited (n = 4) CD4/FoxP3 analysis did not show change in absolute numbers with administration of GM-CSF/IL-2 (data not shown). In conclusion, cytokine therapy with IL-2/GM-CSF is well tolerated and is an alternative to DLI for relapse after ASCT. Flow cytometry analysis demonstrated a quantitative increase in immune effector cells and polarization to DC2.

*Flow Cytometry of Immune Effector Cells*

| | D0 (Mean ± SE) | D8 (Mean ± SE) | D14 (Mean ± SE) | P-Value (Day 8–0) | P-Value (Day 14–0) |
|---|---|---|---|---|---|
| CD3 (K/uL) | 309 ± 117 | 535 ± 103 | 1306 ± 403 | 0.034 | 0.027 |
| CD3/CD8 (K/uL) | 94 ± 42 | 174 ± 33 | 325 ± 90 | 0.021 | 0.029 |
| CD3/CD4 (K/uL) | 309 ± 117 | 404 ± 102 | 977 ± 310 | 0.249 | 0.045 |
| CD16/CD56 (K/uL) | 124 ± 60 | 404 ± 110 | 496 ± 162 | 0.029 | 0.044 |
| CD19 (K/uL) | 68 ± 39 | 89 ± 36 | 116 ± 31 | 0.183 | 0.044 |
| Tot. Lymphs (K/uL) | 488 ± 167 | 942 ± 160 | 2353 ± 532 | 0.016 | 0.013 |
| CD11(DC1) (K/uL) | 97 ± 60 | 46 ± 32 | 33 ± 27 | 0.108 | 0.101 |
| CD123(DC2) (K/uL) | 32 ± 7 | 40 ± 16 | 45 ± 36 | 0.694 | 0.628 |
| DC1/DC2 | 2.77 ± 1.26 | 0.68 ± 0.35 | 0.61 ± 0.07 | | |

SE = Standard Error; DC= dendritic cells.

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### WHERE TO BIOPSY IN THE GASTROINTESTINAL (GI) TRACT TO DIAGNOSE ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS (AlloSCT)

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**Background & Aims:** Despite therapeutic advances, GI AGVHD continues to be a serious threat after alloSCT contributing to significant morbidity and mortality (Yacoub-Agha, et al, Transplantation 2004). GI endoscopy, although critical to establishing early diagnosis and grading of AGVHD, poses additional risks such as bleeding and perforation to already immunocompromised patients. The objective of this study was to determine if upper GI endoscopy (ED) with duodenum biopsy (Bx) adds diagnostic accuracy to lower GI ED and colon biopsy in pediatric alloSCT recipients. **Methods:** We performed a retrospective review of pediatric alloSCT recipients who underwent upper (UE) and lower (LE) GI ED at Morgan Stanley Children's Hospital-New York Presbyterian between 2001 and 2005. Biopsies were evaluated by a gastrointestinal pathologist blinded to clinical data to correlate biopsy sites with histological findings of GI AGVHD. To examine which sites were more likely to be diagnostic of AGVHD, six pairwise comparisons were made between different sites, using the McNemar Test with Bonferroni correction so that $p < 0.008$ (=0.05/6) represents statistical significance. **Results:** We evaluated 50 patients (age range 0.8 to 17.6 yr, mean 7.6 yr; M 27, F 23) 24 to 100 days post alloSCT suspected of AGVHD who underwent both upper and lower GI ED. The number of Bx sites per patient ranged from 4 to 10, median 7. For upper endoscopy, 48 patients had both duodenal and stomach biopsies, the duodenum was more likely to be positive than stomach, OR 3.7 ($p = 0.005$). For lower endoscopy cases, there were no significant differences in proportion of positive biopsies between ascending and sigmoid, sigmoid and transverse, or left versus right colon. However, comparing LE to UE, left colon was significantly more likely to be positive than duodenum, OR 12.0 ($p = 0.006$). There were 12 cases where the left colon was positive and duodenum negative and only 1 case where the reverse was true. **Conclusions:** Overall, duodenum and colon are most likely sites to be positive for GI AGVHD. Left-sided colonoscopy and left colon biopsy are more sensitive to detect AGVHD compared to upper endoscopy and gastric and duodenal biopsies. Eliminating concomitant upper endoscopy and biopsies if a lower endoscopy is performed could potentially reduce morbidity in pediatric allograft recipients. However, if upper endoscopy is indicated, duodenal biopsies yield a more accurate diagnosis than gastric biopsies.

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### THE PHARMACODYNAMIC ANALYSIS BETWEEN CYCLOSPORINE A (CsA) AND CYTOKINE PROFILES OF CD4+ T LYMPHOCYTES FOR THE DEVELOPMENT OF OPTIMIZED IMMUNOSUPPRESSIVE THERAPY WITH CsA AFTER UNRELATED CORD BLOOD TRANSPLANTATION (CBT)

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Although unrelated cord blood transplantation (CBT) is increasing in number, the optimized immunosuppressive therapy for prevention of graft versus host disease (GVHD) has not been established. Cyclosporine A (CsA) is the most common immunosuppressant used for GVHD prophylaxis and individualization among types of stem cell source by using therapeutic drug monitoring (TDM) is essential to optimize pharmacotherapy. Cord blood lymphocytes are naïve and most of CBT are carried out with a human leukocyte antigen (HLA)-mismatched combination especially in adults. Our retrospective analysis showed CsA can be tapered earlier for recipients of CBT than for those of BMT. To optimize CsA administration in CBT, we analyzed proportion of IL-2 producing cells among CD4+ T lymphocytes using multi-color flow cytometric analysis before and after CsA administration. We analyzed 55 peripheral blood samples from 6 allogeneic BMT recipients as controls and 104 peripheral blood samples from 7 CBT recipients. Cells were cultured in the presence of phorbol 12-myristate 13-acetate, ionomycin, and monensin at 37°C for 4 hrs and then stained for surface markers and intracytoplasmic IL-2. Blood CsA levels were simultaneously measured. We found that the level of IL-2 production in CD4+ T lymphocytes was inversely proportional to blood CsA levels for both BMT and CBT. IL-2 production in CD4+ T lymphocytes of cord blood recipients can be inhibited by relatively lower CsA concentration compared with that of bone marrow recipients. To our most interest, there was dramatic difference in required blood CsA level for inhibition of IL-2 production by days after transplantation (e.g. before and after day45) in recipients of CBT, while there was no difference by days after transplantation in recipients of BMT. This finding can explain why earlier tapering of CsA is possible for cord blood recipients. Further analysis is needed to optimize blood CsA level on different days after CBT for GVHD prophylaxis.

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### INCIDENCE AND RISK FACTORS FOR CHRONIC GRAFT-VERSUS-HOST DISEASE (cGVHD) AFTER CORD BLOOD TRANSPLANTATION (CBT)

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**Objective:** To evaluate the impact of patient and transplant characteristics on the incidence of cGVHD after CBT. **Methods:** Retrospective study of all CBT performed at our institution between 1996 and 2007, excluding primary graft failure cases. 114 patients were analyzed. All had high-risk hematologic malignancies; 59% were in complete remission or chronic phase at transplantation. Median age was 37 years (range 18–67) in the adult group (n = 61) and 7 years (range 0.5–17) in the pediatric group (n = 53).

Conditioning regimen was myeloablative for 92% of patients. It included TBI in 39%, and ATG in 55%. GVHD prophylaxis was tacrolimus-based (except for 2 patients) with methotrexate (75%) or MMF (19%). 44 patients (39%) received double CB units and 70 (61%) single units. Units were ex-vivo expanded for 20/44 and 9/70 patients, respectively. Median number of infused total nucleated cells (TNC) was $3.5 \times 10^7$/kg (1–7) and $4.7 \times 10^7$/kg (0.6–56) in the adult and pediatric groups, respectively. cGVHD was defined according to the recent NIH consensus criteria. Risk factors were evaluated by Cox's regression analysis including age, gender, disease status at transplantation, a prior autologous transplant, conditioning regimen, use of ATG in conditioning, GVHD prophylaxis, number of CB units received, number of infused TNC, HLA-A, B, or DRB1 mismatch, and early withdrawal of immunosuppression. **Results:** With a median follow up of 9 months, 21/114 patients developed cGVHD at a median of 126 days post CBT (range 100–276). 62% of these cases (n = 13) were de Novo. Recipient age was the strongest risk factor with a significantly higher 1-year cumulative incidence in adult patients (31%) compared with pediatric patients (n = 4, 8%), p = 0.002), despite a comparable incidence of grade II-IV (38% and 34%) and III-IV (14% and 10%) acute GVHD; and a superior disease free survival in the pediatric group. In adult patients, a prior autologous transplant (n = 16) was the only significant risk factor, and was associated with a higher incidence (50% versus 23%, p = 0.02). cGVHD was extensive in the majority of adult cases (12/17), yet it was associated with a lower rate of relapse (HR = 0.1, p = 0.07) and mortality (HR = 0.4, p = 0.06) when evaluated as time dependent variable in a landmark analysis starting on post SCT day +100. **Conclusions:** Recipient age is a significant predictor of cGVHD following CBT. In adult patients, the impact of prior autologous transplant deserves further evaluation.

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### EARLY DETECTION OF SEVERE ACUTE GRAFT-VERSUS-HOST DISEASE USING PLASMA MARKERS OF T-CELL ACTIVATION

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Severe acute graft-versus-host disease (GVHD) is a significant cause of transplant related mortality in patients that receive allogeneic hematopoietic stem cell transplantation (HSCT). Treatment of grade III and IV GVHD is frequently ineffective. Preemptive treatment based on the early detection of cytokines associated with T-cell activation and subclinical GVHD might be a more effective strategy, similar to the monitoring of CMV infection after HSCT.

Various plasma markers of T cell activation, soluble IL-2 receptor (sIL-2R) in particular, have been shown to correlate with cellular immune responses in several diseases, including GVHD. Building on this work, as the first step toward developing an early treatment approach for severe GVHD, we conducted a prospective, observational study evaluating three plasma markers of T-cell activation. sIL-2R, sCD40L and sCD8 were evaluated as screening tests with the goal of detecting GVHD in its incipient stages prior to clinical manifestations.

We measured plasma levels of these markers on days 5 and 10 following HSCT. Testing was performed in 50 transplants (49 patients). The median age of patients was 14 (range: 0–67); 19 received genotypically matched related transplants, 4 received mismatched related transplants, 20 received unrelated transplants, and 7 received unrelated cord blood transplants. This population included patients with both malignant and non-malignant diseases and who received myeloablative and reduced intensity conditioning regimens. Nine patients developed grade III or IV GVHD. Receiver operating curves were generated for each of the markers at both time points. This analysis showed sIL-2R and sCD8 to be more accurate markers than sCD40L. Day 10 levels correlated more closely with outcome than those measured on day 5. A parallel testing strategy combining sIL-2R and sCD8 yielded results superior to using either marker alone.

An elevation of sIL2r above 20 ng/mL or sCD8 above 4400 units/mL on day 10 was seen in 8 out of 9 patients that developed grade III or IV GVHD yielding a sensitivity of 0.89. For the specificity calculation, patients with grade II GVHD were considered to be cases, since these patients routinely receive systemic treatment. This method produced a specificity of 0.57.

The results of this pilot study demonstrate the feasibility of using biomarkers of T-cell activation for the early detection of severe acute GVHD. Plans are underway for a larger, more definitive observational study.

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### THE RELATIONSHIP BETWEEN TACROLIMUS SERUM CONCENTRATIONS AND ACUTE GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC STEM CELL RECIPIENTS

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Tacrolimus is commonly used as prophylaxis of GVHD following allogeneic HSCT. While there is an association between increasing serum tacrolimus levels and the incidence of renal toxicity, there is no established relationship between tacrolimus levels and the incidence of GVHD. We studied the outcome of 92 consecutive allogeneic blood HSCT recipients receiving tacrolimus-based GVHD prophylaxis. 55 had received conventional-intensity conditioning (usually busulfan-fludarabine; 34 unrelated donors and 21 sibling donors) where tacrolimus was combined with short-course methotrexate, and 36 had received reduced-intensity conditioning (melphalan ± cyclophosphamide; all unrelated donors) where tacrolimus was combined with mycophenolate mofetil. For each patient, a weekly average tacrolimus level was determined for each of the first 7 weeks post-transplant by averaging all the levels available for that week. The relationship between the average weekly tacrolimus levels and the occurrence of any grade of acute GVHD was examined. The ANOVA test was used to compare the weekly average tacrolimus levels between patients who developed GVHD and those who did not. The cumulative incidence of acute GVHD was compared by tacrolimus level categories each week. A total of 1385 tacrolimus levels were available (6–45 per patient; median 14). The cumulative incidence of acute GVHD at 60 days was 37% (95% CI: 29–49%). As the table below shows, the average tacrolimus levels amongst patients going on to develop acute GVHD tended to be lower than those not developing acute GVHD; significantly so for week 4 and showing a trend towards significnace for weeks 5 and 6. The cumulative incidence of acute GVHD amongst patients whose week 3 average tacrolimus was <10 was 51% (95% CI: 39–68%) compared with 24% (95% CI: 14–41%) for those with a level of ≥10 (P = 0.015). Similarly, the cumulative incidence of acute GVHD amongst patients whose week 4 average tacrolimus was <10 was 43% (95% CI: 32–59%) compared with 20% (95% CI: 10–41%) for those with a level of ≥10 (P = 0.046). The relationship between higher acute GVHD incidence and lower tacrolimus levels was observed for other weeks and tacrolimus cut-off levels, but the differences were not statistically significant. Our data suggest that there is a relationship between tacrolimus levels and acute GVHD. How this affects relapse rates, transplant-related mortality, and survival remains to be determined.

*Median average tacrolimus levels in patients with or without acute GVHD*

| Week | Acute GVHD | No acute GVHD | P |
|---|---|---|---|
| 1 | 13.6 | 13.1 | 0.97 |
| 2 | 11.1 | 12.1 | 0.21 |
| 3 | 8.9 | 10.9 | 0.20 |
| 4 | 7.3 | 8.5 | 0.031 |
| 5 | 8.6 | 10.2 | 0.057 |
| 6 | 7.8 | 9.1 | 0.06 |
| 7 | 7.5 | 8.1 | 0.25 |