380

ALEFACEPT TREATMENT FOR CHRONIC EXTENSIVE GRAFT VERSUS HOST DISEASE
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Alefacept (Amevive®) is an immunosuppressive dimeric fusion protein that is used for psoriasis control. We have recently shown its effect in acute, steroid resistant/dependent GVHD. In this study, we describe the effect of alefacept treatment on chronic extensive graft vs host disease (cGVHD). A total of 12 patients (13 cGVHD episodes) were included in this study. Alefacept dose regimens for children and adults was 15 mg and 30 mg once weekly. A median of 9 (range 1–25) injections were given per patient. Eight out of 12 patients (9/13 episodes) showed response. The median time to initial response was 2.25 weeks (range 1–8). The response was either partial (n = 3), moderate (n = 2) or minimal (n = 4). In 2 responding patients, the response was only temporary. Responding organs included: skin, oral mucosa, eyes and lungs. Complications that appeared during treatment included infection, pericarditis and squamous cell carcinoma of the lip. All these events may be related to other drugs given simultaneously. Seven of the twelve patients are alive all with stable or improved cGVHD. Five patients died due to GVHD progression while none of the patients experienced relapse of the basic disease. As previously reported in psoriatic patients treated with alefacept, we found a consistent increase in the percentage of naive T cells as a consequence of treatment. In conclusion, alefacept is effective for the treatment of cGVHD, dose and treatment’s time intervals should be further explored.

381

SILDENAFIL (REVATIO) THERAPY IN CHRONIC GRAFT VERSUS HOST DISEASE (cGVHD): IMPROVEMENT IN PULMONARY cGVHD SYMPTOMS

Sildenafil (Revatio) has been used in patients with primary pulmonary hypertension but its impact on the post allogeneic transplant recipients with chronic lung graft vs host disease (cGVHD) has not been well characterized. We have noted significant improvement in an index case with peripheral acrocyanosis and dyspnea. Since then we have treated 3 more patients with Revatio as detailed in this report.

Patient 1: 55 yr with Aplastic Anemia 2 years s/p allogeneic peripheral blood stem cell transplant (PBSC) using single dose TBI 550 cGy/Cy 60 mg/kg. Treatment began on 05/15/06 owing to acrocyanosis of fingertips and shortness of breath. She improved dramatically with resolution of cyanotic fingertips and amelioration of her dyspnea within 6 month of therapy. Patient 2: 55 yr with AML, 5 years s/p allogeneic PBSC transplant with single dose TBI 550 cGy/Cy 60 mg/kg × 2. She had aGVHD of skin in the first 100 days and developed symptoms of shortness of breath and cough two years post transplant. Revatio mg tid was begun on 11/06 with marked improvement of her dyspnea. Patient 3: 54 yr with CML. 20 months s/p allogeneic marrow stem cell transplant using fractionated TBI 1200 cGy/Cy 60 mg/kg × 2. She had mild GI aGVHD and developed cough and shortness of breath 1 year post transplant. Revatio mg tid was started in January of 2007 with improvement of shortness of breath. Patient 4: 44 yr, 6.5 years s/p allogeneic PBSC transplant for CML, using single dose TBI 550 cGy/Cy 60 mg/kg × 2. He had stage III aGVHD of the skin and later cGVHD of lungs with cough and dyspnea late in 2006 and noted improvement in pulmonary symptoms. No flushing, diarrehea or visual changes were seen in these 4 patients and the drug has been continued without dose reduction or interruption in all patients at 20 mg TID. No overt changes in pulmonary and/or cardiac function were demonstrated in this cohort of patients, indicating that Sildenafil actions may involve mild peripheral arteriolar and venous vasodilatation in vivo and inhibition of platelets aggregation leading to improved symptomatology. Further clinical trials carefully monitoring quality of life instruments as well as cardiovascular and pulmonary reserve before and after treatment are needed to determine the role of Sildenafil in patients with cGVHD. Individual cardiac and pulmonary function tests are shown below:

Cardiopulmonary Studies before and after Sildenafil

<table>
<thead>
<tr>
<th>Patient #</th>
<th>PFT: Pre-Revatio</th>
<th>PFT: Post-Revatio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.78 L</td>
<td>2.23 L</td>
</tr>
<tr>
<td>2</td>
<td>5.52 L</td>
<td>1.16 L</td>
</tr>
<tr>
<td>3</td>
<td>2.24 L</td>
<td>2.89 L</td>
</tr>
<tr>
<td>4</td>
<td>1.66 L</td>
<td>1.55 L</td>
</tr>
</tbody>
</table>

ECHO-Pre-Revatio: Normal, ECHO-Post-Revatio: NA

Pulmonary artery pressure was 27, <30 and 50 mm Hg in patients 1, 2, 3.

382

β2 INTERGRINS SEPARATES GRAFT-VERSUS-HOST DISEASE AND GRAFT-VERSUS-LEUKEMIA EFFECT
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Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation. Migration of donor-derived T cells into GVHD target organs plays an essential role in the development of GVHD. β2 integrins are critically important for leukocyte extravasation through vascular endothelia and for T cell activation. We asked whether CD18-deficient T cells would induce less GVHD while sparing graft-versus-leukemia (GVL) effect. In murine allogeneic bone marrow transplantation models, we found that recipients of CD18−/− donor T cells had significantly less GVHD morbidity and mortality compared with recipients of WT donor T cells. Analysis of allo-reactivity showed that CD18−/− and WT T cells had comparable activation, expansion and cytokine production in vivo. Reduced GVHD was associated with a significant decrease in donor T cell infiltration of recipient intestine and with an overall decrease in pathologic scores in intestine and liver. Finally, we found that in vivo GVL effect of CD18−/− donor T cells was largely preserved, because mortality of the recipients transplanted with CD18−/− T cells plus tumor cells was greatly delayed or prevented. Our data suggest that strategies to target β2 integrin have clinical potential to alleviate or prevent GVHD while sparing GVL activity.

383

ELUCIDATING THE ROLE OF THE Fas-Fasl PATHWAY DURING CUTANEOUS GVHD ACROSS MINOR HISTOCOMPATIBILITY ANTIGENS IN VITRO
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The role of the Fas-FasL pathway in the pathological development of graft-vs-host disease (GvHD) directed to miHA remains unclear. We have recently established a new in vitro culture system to study cutaneous GVHD in mice and used this system to test the involvement of the Fas-Fasl pathway. Primary epithelial cells (pEC), constitutively expressing Fas, were obtained from ear skin of BALB. B mice. Confluent pEC were co-cultured with unseparated T cells from MHC-matched, miHA-mismatched C57Bl/6 (B6) mice. Two days later, Fas expression was increased on the pEC (58.7 ± 8.8, as compared to 21.9 ± 3.2 in pEC cultured alone, p < 0.03). To test whether pEC death observed by day 4 in co-culture was attributed at least in part to the up-regulated Fas, BALB. B pEC were co-cultured with T cells derived from B6 gld /gld mice (gld). Despite their FasL deficiency, gld T cells killed BALB. B pEC
by day 4 as efficiently as wt B6 T cells, with a similar reduction in pECP viability, from 93 ± 6.7 to 43 ± 6.4 in the presence of gld T cells and 44 ± 8.6 in the presence of wt B6 T cells (p > 0.5). However, upregulation of Fas on pECP co-cultured with gld T cells was delayed and seen only by day 4 rather than day 2. The capacity of gld T cells to modulate the epithelial environment appears intact, since they induced the upregulation of H2Kb by 10-fold and IAβ by 3-fold on the pECP, similar to the effect induced by wt B6 T cells. We also found higher interferon gamma (IFNg) in the supernatants of co-cultures with gld T cells (141.2 ± 17.8), as compared to wt B6 T cells (100 ± 20.1, p < 0.004). However, in contrast to the expression of H2Kb and IAβ which were up-regulated 5.5-fold and 2.8-fold by IFNg added to pECP cultures, Fas expression was not altered by this cytokine even after 7 days and at high doses (50 and 500 ng/ml). In summary, T cells increased the expression of Fas on mHA-mismatched pECP. This event was of no evident consequence, since FasL expression on "donor" T cells was not required to kill "host" pECP in the 4-day period studied. Fas-deficient T cells also modulated the expression of epithelial MHC molecules and might be associated with the high levels of IFNg, a key cytokine during GVHD, detected in supernatants of co-cultures. However, IFNg did not regulate Fas on pECP, indicating that other cytokines/mechanisms are responsible for this effect. Altogether, our results suggest that the Fas-FasL pathway may have a minor role in graft-versus-host disease (GVHD), detected in supernatants of co-cultures. However, IFNg did not regulate Fas on pECP, indicating that other cytokines/mechanisms are responsible for this effect. Altogether, our results suggest that the Fas-FasL pathway may have a minor role in the early pathogenesis of cutaneous GVHD.

### 384 Daclizumab and Infliximab for Treatment of Steroid Resistant Acute Graft Versus Host Disease (GVHD) in Pediatrics

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Patients with steroid resistant aGVHD have a poor prognosis. Daclizumab (anti-IL2 receptor alpha chain), and infliximab (anti-TNF) have shown activity in early clinical trials in adults. We are reporting our experience using daclizumab and infliximab in 3 pediatric patients. All received daclizumab (1 mg/kg/dose IV on days 1, 4, 8, 15 and 22) and infliximab (10 mg/kg/dose IV on days 1, 8, 15 and 22). Steroid tapering was at the discretion of the attending physician based on clinical condition and response to treatment.

UPN 377 was a 19 month old girl with Hemophagocytic Lymphohistiocytosis who received a related bone marrow graft match for 8/8 alleles. She received a preparative therapy consisting of busulfan, VP16 and cyclophosphamide and methotrexate (mtx) and cyclosporine (csa) for GVHD prophylaxis. She developed stage 3 grade 2 aGVHD of the skin on d + 32 post transplant. After 19 days of steroids (2 mg/kg/day), daclizumab and infliximab were started. GVHD improved by d + 36. The patient developed extensive chronic GVHD and is presently 522 days post transplant with a Lansky score of 100%.

UPN 394 was an 8 yr old male with ALL in CR2 who received a related bone marrow graft match for 7/8 alleles. He received TBI, VP16 and cyclophosphamide and csA for GVHD prophylaxis. He developed stage 3 grade 2 aGVHD of the skin on d + 24 post transplant. After 2 days of steroids (4 mg/kg/day), he was treated with daclizumab and infliximab. GVHD improved by d + 31. The patient has extensive chronic GVHD. He is 210 days post transplant and has a Lansky score of 100%.

UPN 395 was a 13 month old male with ALL in CR1 who received an unrelated bone marrow graft match for 8/8 alleles. He received TBI, VP16 and cyclophosphamide and csA and mtx for GVHD prophylaxis. He developed stage 3 grade 2 aGVHD of the skin on d + 19 post transplant. After 11 days of steroids (2 mg/kg/day), he was treated with daclizumab and infliximab. GVHD improved by d + 33. The patient relapsed at d + 189 post transplant and has no signs of chronic GVHD.

In all patients, there were no instances of opportunistic viral or fungal infections. This small series suggests that the combination of daclizumab and infliximab is well tolerated and an effective therapy for steroid resistant GVHD in pediatrics. Therefore, we have revised our algorithm for the management of aGVHD (to be displayed at the meeting).

### 385 Monocyte Blood Count and Acute GVHD: Flow Cytometry Analysis Recommended in Prospective Studies

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Patients with a high absolute number of peripheral blood CD14+ monocytes prior to starting the conditioning regimen have shown to have a greater risk of developing acute graft-versus-host disease (aGVHD) (Arpádi et al. BBMT 2007) after allogeneic hematopoietic stem cell transplantation (HSCT). In this study, we initially pooled the data of 154 consecutive patients with hematologic malignancies who received an allogeneic peripheral blood stem cell transplant at three different institutions, utilizing myeloablative (n = 36) or reduced intensity conditioning (n = 118) regimens not including ATG. The blood absolute monocyte count (AMC) was assessed before starting the conditioning regimen and it was calculated by dividing the absolute white cell count by the percentage of monocytes obtained with an automated CBC. The median value of AMC in the study was 0.5 × 10⁹/L (range:0.0–11.8). Of 154 patients, 73 had < 0.5 and 81 had ≥0.5 AMC × 10⁹/L prior to starting the conditioning regimen. All the patients received an 8/8 HLA antigen matched graft from related (n = 114) or unrelated (n = 40) donors. In the group of patients with higher AMC median age was 51 (range:19–71) and donors were unrelated in 27% and sex mismatched in 41% of the cases, whereas in the group of patients with higher AMC median age was 50 (range:18–63) and donors were unrelated in 25% and sex mismatched in 36% of the cases. The rate of acute GVHD grade II-IV in patients with low or high AMC was 44% and 38%, respectively (p=ns). To test whether the AMC obtained by means of automated CBC was comparable to the absolute count of CD14+ monocytes detected by flow cytometry, blood samples from 43 patients were analyzed utilizing both techniques. In this series of patients, mean values of AMC and CD14+ blood cells were 11.9 ± 11.9 and 7.3 ± 6.9 × 10⁹/L and median values were 9.6 (range 95% CI:8.2–15.6) and 6.0 (range 95% CI:5.2–9.5) × 10⁹/L, respectively, (p = 0.0004). In this study, the analysis of monocyte absolute count by automated CBC did not confirm the correlation between high levels of blood CD14+ cells analyzed by flow cytometry and development of aGVHD. Since we demonstrate here that the absolute number of monocytes obtained by cyttofluorimetric expression of CD14 is significantly different as compared to that obtained by automated CBC, future prospective studies addressing the prognostic role of monocyte blood count in allogeneic HSCT should be performed utilizing a flow cytometry-based method.

### 386 Sirolimus in Combination with Cyclosporine or Tacrolimus Plus Methotrexate for Graft-Versus-Host Disease (GVHD) Prophylaxis After Hematopoietic Cell Transplantation From Unrelated Donors


Sirolimus (SIR) is reported to be effective in preventing GVHD when combined with tacrolimus (TAC) and methotrexate (MTX) after related and unrelated allogeneic hematopoietic cell transplantation (HCT). In two consecutive clinical trials, we evaluated the efficacy of SIR plus cyclosporine (CSP Group) or TAC (TAC Group) and MTX after unrelated HCT. SIR was administered as a 12 mg PO loading dose on day -1 (CSP Group) or -3 (TAC Group) followed by 4 mg PO QD. SIR levels were targeted at 4–14 ng/mL in the CSP Group and 3–12 ng/mL in the TAC Group. The dose of TAC was 3.5 mg/kg IV on day -3. The dose of CSP was 0.02 mg/kg/day IV starting on day -3. CSP was targeted at 150–450 ng/mL and TAC at 5–10 ng/mL. All patients received MTX 5 mg/m² IV on days 1, 3, 6 and 11. Nine patients were...