mice had significantly poorer cGVHD scores than control mice (WT BM cells into WT recipients), suggesting the role of host parenchymal tissue cell expression of B7H1 in cGVHD. Taken together, the PD-1 axis, especially B7H1 expression on recipients, regulates the frequency of IL-17+ IFNγ+ T cells and contributes to the pathogenesis of cGVHD.

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Post-Transplant Cyclophosphamide (PTC) As Sole Graft Versus Host Disease (GVHD) Prophylaxis in Patients Undergoing HLA Matched Sibling Donor Stem Cell Transplant (SCT) for Severe Aplastic Anemia (SAA)

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Between September 2010 and June 2012, 15 patients with SAA underwent HLA identical sibling donor SCT using Flu-darabine 180 mg/m2 IV over 6 days and Cyclophosphamide 100 mg/kg IV over 2 days. Five patients had in addition a single fraction of Total Body irradiation (TBI) 200 cGy. Cyclophosphamide 50 mg/kg/day IV on day +3 and +4 was the sole GVHD prophylaxis. G-CSF mobilized peripheral blood stem cells (PBSC) was the graft source. Ten males and 5 females with a median age of 25 years (range: 8–42) had SCT. Median PBSC cell dose infused was 9.5 x 10^6 CD34/kg (range: 5.4–17.2). Thirteen engrafted (86.6%) with median neutrophil and platelet engraftment of 15.4 days (range: 15–17) and 16.6 days (range: 12–32) respectively. Grade II–IV GVHD seen in 3 patients (23%) at 42, 49 and 68 days post SCT. Two responded to combination of cyclosporine and prednisolone while one patient with grade IV GVHD expired 64 days post SCT. Of 11 evaluable patients, 4 (36.3%) developed chronic GVHD which was limited in all. Two patients with de novo chronic GVHD were managed with prednisolone alone. Overall 7 patients (46.6%) have not required any immunosuppression after SCT while 3 have required immunosuppression therapy. 11 (73.3%) are alive and well including 7 patients who did not require any immunosuppressive therapy following SCT. The use of post transplant cyclophosphamide as GVHD prophylaxis following sibling donor transplant for SAA is associated with low rates of GVHD. A large number (46%) did not require any immunosuppression post SCT. Larger studies are required to understand the utility of this prophylaxis in sibling donor transplants for aplastic anemia.

Efficacy and Safety of Immunomodulation with Fast Withdrawal of Imunosuppression (FWI) and Donor Lymphocyte Infusions (DLI) for Prevention of Relapse in Children Receiving Allogeneic Hematopoietic Stem Cell Transplant (HCT) for Hematologic Malignancies

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Persistence of mixed chimerism (MC) following myeloablative HCT in pediatric hematologic malignancies is related to a high risk of relapse. We initiated a prospective study of FWI and DLI in patients with MC at 30 days post-transplant. We are reporting preliminary results on 43 enrolled patients with a mean age of 10±6.5(SD) years. Fifty-eight percent of patients had myeloid malignancies, 40% lymphoid malignancies and 2% had biphenotypic leukemia. Based on day +30 bone marrow and peripheral blood chimerism results, 26/43 (60%) patients were found to have MC, and were assigned to the intervention arm of the study consisting of FWI and DLI; 12 patients (28%) had full donor chimerism (FDC) or early graft-versus host disease (GVHD) and were assigned to observation arm, and 5 (12%) could not be assigned to either arm due to early death or relapse. FWI started at a median of day +50 (range 40-85), and ended at a median of day +75.5 (range 49-113). Following FWI, 9 patients (35%) converted to FDC. Of 17 patients who remained MC following FWI, 15 proceeded to DLI, 1 did not receive further intervention due to GVHD and one relapsed prior to DLI. Acute GVHD developed in 3/26 (12%) patients undergoing FWI and in 9/12 (75%) of patients in the observation arm (P < .01). Two patients undergoing intervention developed grade II aGVHD which resolved and 1 developed grade IV aGVHD that progressed to fatal cGVHD of the lungs. In the observation arm, 2 patients developed grade I, 5 developed grade II, 2 developed grade III, and 1 developed grade IV aGVHD. Chronic GVHD developed in 6 patients (2 in the intervention and 4 in the observation arm). One of 6 patients developed de novo cGVHD following DLI. The incidence of acute and/or chronic GVHD was 15% in the intervention arm of the study. Toxic death rate due to GVHD was 4%. There were 11 events (3 treatment-related deaths and 8 relapses). Mean follow-up of living patients was 17.6±10 (SD) months. EFS for the entire cohort was 71±7(SD)% and was not significantly different between the observation arm and the intervention arm. Ten patients (23%) had evidence of disease by flow or cytogenetics, at the time of HCT. Based on chimerism results, 4 were assigned to the intervention arm, 3 to the observation arm, and 3 could not be assigned to any arm of the study due to early relapse or death. EFS was significantly lower in patients with positive disease prior to transplant than in those without evidence of disease (EFS 27±15% vs. 86±7%). Among 26 patients undergoing intervention, relapse was significantly more common (P = .014) in patients with positive disease pre-transplant. Our data indicate that post-transplant immunomodulation is safe and has overall low GVHD risk (15%). Our schedule of FWI was not adequate to prevent relapse in patients coming to transplant with persistent disease. We would recommend an earlier, (day 30) and more aggressive schedule of immunosuppression withdrawal for these patients.

From Murine Model to Clinical Trial of Graft-Versus-GVHD, a Second Transplantation From Another Donor for the Rescue From Refractory Acute GVHD

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Background: GVHD is still a major obstacle in allogeneic transplantation despite the progress of immunosuppressive drugs and cell therapy such as mesenchymal stem cells. GVHD is caused by donor lymphocytes, mainly T cells,
attacking various host tissues. A concept of Graft-versus-autoimmunity (GVA), on the other hand, is suggested from the fact that autoimmune diseases are ameliorated by allogeneic transplantation for accompanying hematologic disorder. GVHD can be thought as a typical autoimmune disease caused by donor T cells. From an experience of the autologous (recipient cells) transplantation for severe GVHD, which resulted in successful control of GVHD, but disease relapse probably due to cancelling the GVL effect, we have pursued the possibility of rescue transplantation from another donor for refractory acute GVHD in a murine model and in a clinical trial.

**Methods:** In a murine GVHD model of BDF1 (H-2b^d^b) to B6C3F1 (H-2b^k^k), GVHD mice underwent a second BMT from B6B10F1 (H-2b^k^b) following low-dose TBI 2-3 weeks after first BMT. In a clinical trial, 16 patients who developed severe acute GVHD, refractory to three to five lines of GVHD-specific treatments, underwent allogeneic stem cell transplantation using reduced-intensity conditioning regimens with grafts from a second donor.

**Results:** In the murine model, GVHD could be successfully treated by a second BMT. For successful treatment of GVHD, rapid achievement of full second-donor chimerism was required. The mice were relatively resistant to new development of GVHD by second-donor grafts. The timing of the second BMT, the intensity of conditioning, and donor selection could be important. In the clinical trial, among 15 transplantations that could be evaluated, rescue donor grafts were engrafted in 11 cases and rejected in 4 cases. For patients who achieved rescue donor engraftment, the response rate was 90% (CR 8, PR 2, stable 1). 6 of 8 patients with CR survived without GVHD symptoms, with a median follow-up of 5.8 years. No new development of GVHD by the second graft was observed. In contrast, no long-term survivors were observed in patients who rejected rescue donor grafts.

**Conclusions:** GVHD could be treated by transplantation using second donor graft, which eliminates first donor lymphocytes in the murine model and the clinical trial. We would like to propose the concept of Graft-versus-GVHD, on the analogy of GVA.

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**Response Endpoints for Acute Graft-Versus-Host Disease Treatment Trials**

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**Background:** We evaluated short-term response endpoints for clinical trials testing initial treatment of acute graft-versus-host disease (GVHD). We postulated that the definition of response should include a reduced symptom burden and improved failure-free survival (FFS), with failure defined as death, recurrent malignancy or initiation of second-line systemic treatment. We also postulated that the response endpoint should include an upper limit of the steroid dose at the time of assessment.

**Methods:** In a cohort of 227 adult patients who received initial systemic steroid treatment for grades III-IV acute GVHD between 2000 and 2005, treatment response was evaluated at days 28 and 56 after starting treatment. Standard definitions were used to define complete response (CR) and traditional partial response (PR). Very good partial response (VGPR) was defined as a subcategory of traditional PR when patients had minor rash, minor elevations of total serum bilirubin or minor gastrointestinal symptoms but otherwise met criteria for CR (2009 Joint Statement, BBMT). Sensitivity and specificity analyses were used to evaluate each response definition in predicting FFS at 6 months after initial treatment. Averages of sensitivity and specificity were used to evaluate the trade-off between sensitivity and specificity.

**Results:** Response rates were 33% CR, 24% VGPR and 10% other PR at day 28, and 40% CR, 14% VGPR and 7% other PR at day 56. Residual symptom burden at days 28 and 56 after treatment was lower in patients with VGPR than in those with other PR, indicating that VGPR is preferred over traditional PR. In evaluating day 28 and day 56 response endpoints as predictors of FFS at 6 months, loss of sensitivity outweighed the gain of specificity with CR compared to CR+VGPR (Table 1). Sensitivity for CR+VGPR was similar at days 28 and 56, but specificity was higher at day 56 than at day 28. Since CR+VGPR at day 56 still had a 26% false-positive rate (i.e., 74% specificity), we evaluated response definitions that incorporated an upper limit of the steroid dose as an additional criterion of success (Figure 1). As shown by the averages, sensitivity and specificity showed a balanced trade-off at prednisone limits between 2.0 and 0.5 mg/kg/day, but loss of sensitivity outweighed the gain of specificity at limits below 0.5 mg/kg/day. Incorporation of prednisone doses <0.5 mg/kg/day in the CR+VGPR endpoint definition at day 56 decreased the false-positive rate to 15%. When recurrent malignancy was excluded from the definition of failure after day 56, this endpoint had a false-positive rate of only 4%.

**Conclusion:** Our results support the use of CR+VGPR with an upper limit of the steroid dose at day 56 as the short-term response endpoint for acute GVHD treatment trials.

<table>
<thead>
<tr>
<th>Response definition</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Average</th>
</tr>
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<tbody>
<tr>
<td>Day 28 CR</td>
<td>48%</td>
<td>81%</td>
<td>65%</td>
</tr>
<tr>
<td>Day 56 CR+VGPR</td>
<td>82%</td>
<td>66%</td>
<td>74%</td>
</tr>
<tr>
<td>Day 56 CR+VGPR</td>
<td>64%</td>
<td>82%</td>
<td>73%</td>
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**Figure 1.**