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EBV-ASSOCIATED LEIOMYOMAS FOLLOWING HAPLOIDENTICAL TRANSPLANTATION FOR X-LINKED SEVERE COMBINED IMMUNODEFICIENCY DISEASE

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In the setting of transplantation, Epstein-Barr virus (EBV) is most commmonly associated with posttransplantation lymphoproliferative disease; however, there are reports of EBV associated leiomyomas for which the virus has some tropism. We wish to report the first case of EBV-associated leimyomas following haploidentical BMT for X-linked severe combined immune deficiency (SCID). Twin A is an 8-year-old boy who along with his syngeneic twin (B) received a paternal T-cell–depleted haploidentical BMT for X-linked SCID at 10 days of life. Twin B experienced rapid donor T-cell engraftment with restored cellular function. Twin A's course was complicated by incomplete donor T-cell engraftment, multiple infections, and GVHD. Twin A received a booster twin-to-twin infusion of unfractionated marrow, resulting in an improved lymphocyte counts, mitogen stimulation response, and clinical improvement without worsening of GVHD. The patient (twin A) did well over the next 6 years until he developed fatigue, weight loss, and exercise-induced shortness of breath. CT scans revealed bilateral renal masses (4 on the right and 2 on the left) with a left pleural-based lung mass. Percutaneous renal biopsy demonstrated leiomyoma with a spindle cell histology. The tumor cells were positive for Ki-67 and actin, but negative for S-100 protein and HMB45. EBV PCR on paraffin-embedded tissue was positive. EBV serologies were unhelpful on both twins as they remain on intravenous immunoglobulin supplementation, but EBV PCR was negative from the peripheral blood of twin B and positive for twin A at 2400 copies per mL. T-cell chimerism studies showed a decrease in donor T-cell engraftment in twin A. Twin A continued to show weight loss with persistant symptoms and was subsequently given an infusion of 10^6 peripheral blood lymphocytes per kg body weight from twin B to supplement his graft. Over the next 4 months, the patient (twin A) has shown an increase in T-cell numbers, improvement in clinical symptoms, weight gain, and stabilization of his leiomyomas.

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RESULTS OF THE CORD BLOOD TRANSPLANTATION STUDY (COBLT): CLINICAL OUTCOMES OF UNRELATED DONOR UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS WITH INBORN ERRORS OF METABOLISM

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The Cord Blood Transplantation Study (COBLT), sponsored by the National Heart, Lung, and Blood Institute, evaluated the outcomes of unrelated donor umbilical cord blood transplantation (UCBT) in 32 patients (56% males; 75% Caucasian) with inborn errors of metabolism. A common protocol was used for the preparative regimen (busulfan, cyclophosphamide, ATG) and GvHD prophylaxis (cyclosporine and steroids). Patients with MPS I Hurler's syndrome (n = 13; 12 reported NEJM 2004:350:1960–9), Hurler-Sheie syndrome (n = 2), Sanfilippo's syndrome (n = 2), I-cell disease (n = 1), Krabbe's disease (n = 7), Tay-Sachs disease (n = 2), and adrenoleukodystrophy (n = 5) with a mean age of 1.83 years were transplanted with an HLA 6/6 (n = 3), 5/6 (n = 14), 4/6 (n = 14), or 3/6 (n = 1) matched unit with a median of $8/6 \times 10^7$ nucleated cells/kg selected from COBLT banks (80%) or other banks (20%). CBUs were screened for enzyme activity to prevent use of a carrier donor. The cumulative incidence of neutrophil engraftment and grade III/IV acute GvHD were 84% in a median of 26 days and 19%, respectively. The probability of survival at 180 days and 2 years was 84%. Seven patients died, 1 before and 6 after transplantation (1 of GvHD with infection, 3 of graft failure, 2 of organ failure, and 1 of hemolytic anemia). The surviving patients with MPS syndromes, Tay-Sachs disease, and Krabbe's disease all stabilized and/or gained skills posttransplantation. One of 5 patients with ALD experienced disease progression, whereas all others stabilized and continue to gain developmental skills. Levels of HLA disparity between recipient and donor determined by retrospective high-resolution DNA typing did not influence engraftment, GvHD, or overall survival. The COBLT study represents the first prospective multicenter trial in children with inborn errors of metabolism undergoing UCBT. UCBT provides rapid access to donors and favorably alters the natural history of the disease and should be considered for patients with metabolic diseases who are eligible for transplantation therapy.

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BUSULFAN DOSE ESCALATION TO INCREASE GENE MARKING OF HEMATOPOIETIC STEM CELLS BY LENTIVIRAL VECTORS IN INFANT RHESUS MONKEYS

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Gene transfer to hematopoietic stem cells (HSCs) using lentiviral vectors may be an attractive approach to treat a variety of diseases. This can be accomplished in the context of an autologous bone marrow transplantation (BMT), where HSC are transduced ex vivo. The myeloablative drug busulfan can then be used to "make space" in the bone marrow compartment, to allow efficient reengraftment of the transduced cells. It is important to identify an optimal busulfan dose that will result in efficient long-term gene marking and have the least toxicity. We performed a lentiviral gene-marking study in infant rhesus macaques using escalating doses of busulfan. Bone marrow (10–15 mL/kg) was harvested from each monkey, followed by a single IV infusion of busulfan over 2 hours in groups of 2–3 using busulfan at 0, 40, 80, 120, and 160 mg/m^2 with dilantin seizure prophylaxis. Peripheral blood busulfan levels were then followed over a period of 4 hours, and the AUC was determined. CD34+ cells were isolated from the harvested bone marrow, cultured for 24 hours in serum-free medium with recombinant cytokines and transduced overnight with an SIV-derived lentiviral vector pseudotyped with the VSV-G glycoprotein. The vector contains a neomycin gene with a mutation in the start codon that abolishes its expression and thus can serve as a nonexpressed marker gene. The next morning, transduced cells were washed and reinfused IV, approximately 48 hours after administration of busulfan. Increasing dosages of busulfan resulted in an increased AUC. However, variability in AUC at each dose level ($\times$ 1.5) was observed, suggesting relatively large interindividual variations in busulfan clearance. At doses of 120 and 160 mg/m^2 busulfan, neutrophil counts transiently dropped under 1000 cells/μL, and platelet counts dropped under 10^5/μL, indicating dose-related neutropenia and thrombocytopenia. Blood chemistries and behavior appeared normal in all animals, and no seizures were observed. Gene marking in mononuclear cells and granulocytes will be measured at monthly intervals by determining the number of integrated proviruses per cell with quantitative PCR. Together, our results suggest that busulfan is safe and has no detectable toxicity at these submyeloablative dosages in infant rhesus monkeys, except for the expected myelosuppressive effects.

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ENGRAFTMENT FOLLOWING REDUCED INTENSITY CONDITIONING (RIC) WITH FLU-BU-ATG AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)—PEDIATRIC EXPERIENCE AT CHILDREN'S MEMORIAL HOSPITAL

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We report the experience of 41 patients (21 males, 20 females; mean age, 8.1 years; range, 0.2–22.6 years) treated with RIC and allogeneic HSCT. Diagnoses consisted of malignancies in 24 (20 hematologic and 4 refractory neuroblastoma) and nonmalignant disorders in 17: immunodeficiencies in 6, metabolic disorders in 5, hemoglobinopathies in 4, and aplastic anemia in 2. Twelve patients had prior HSCT (7 autologous and 5 allogeneic). Recipient's racial origins included 22 Caucasian, 10 Hispanic, 7 African American, and 2 Asian. A standardized RIC regimen comprised of fludarabine, 30 mg/m^2 for 6 days (days −10 to −5), followed by intravenous busulfan, 0.8–1 mg/kg for 8 doses or targeted busulfan at AUC 4000 mmol/min for 2 doses (days −5 and −4) and equine ATG, 40 mg/kg or rabbit ATG, 2 mg/kg for 4 days (days −4 through −1) was administered. Graft-versus-host disease (GVHD) prophylaxis was cyclosporin A (CsA) alone in 19 patients and CsA and mycophenolate mofetil in 22 patients. Growth factor support was not used. Stem cell sources included 27 unrelated donors and 14 related donors; 34 of 41 sources were peripheral blood stem cells. We also assessed engraftment by determining the number of days required to achieve ≥ 50% and ≥ 95% donor chimerism (Do-chim) by VNTR in total leukocyte and T-lymphocyte (CD3) populations.

The median cell doses infused were 5.8×10^8 MNC/kg and 2×10^6 CD34+ cells/kg. Seven of 41 patients failed to engraft, and 3 patients were not evaluable due to early deaths from toxicity (in 2) or relapse (in 1). Four of 16 evaluable patients with nonmalignant disorders failed to engraft versus 3 of 22 with malignancies. Patients failing to engraft were younger (median age, 6.3 vs 8.8 years) and weighed less (median weight, 18 vs 27 kg), but received lower cell infusion doses (3.9 vs 5.9×10^8 MNC/kg; $P = .05$). The median time to a postnadir ANC of 500/μL was 16.5 days, and unsupported platelet count > 20,000/μL was 19 days. Data shown in the table indicate trends for more rapid achievement of Do-chim in patients treated for malignancy. Also, within the 14 malignancy patients, those with clinically significant acute (II-IV) and/or extensive chronic GVHD demonstrated faster Do-chim.

These measures of engraftment may provide a reproducible comparison of engraftment in the setting of RIC and resulting mixed chimeric states. These parameters may provide insight to promote engraftment or to balance a desired graft-versus-tumor effect in patients with malignancies versus limitation of GVHD for patients with no benefit from an excessive alloimmune response.

Table 1. *Median Number of Days to Achieve Donor-Chimerism*

	Leukocyte Do-chim 50	Leukocyte Do-chim 95	Lymphocyte Do-chim 50	Lymphocyte Do-chim 95
Evaluable Pts (n = 26)	18	26	25	33
Non-malignant Disorders (n = 11)	19	53	26	82.5
Malignancy Pts (n = 15)	13	25	21	27
Malignancy & GVHD (n = 7)	13	17	20	19
Malignancy without GVHD (n = 8)	16	32.5	26.5	39

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CHANGE OF RISK FACTORS FOR TREATMENT-RELATED MORTALITY IN CHILDREN UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MALIGNANT AND NONMALIGNANT DISEASES

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Allogeneic hematopoietic stem cell transplantation (HSCT) offers a high chance for cure in patients with nonmalignant disorders. However, acute and late complications as consequence of the conditioning regimen and severe immunosuppression are still a matter of concern. Only few studies specifically focused on the risk for treatment-related mortality (TRM) in children. To evaluate factors influencing the outcome after HSCT we retrospectively analyzed 342 children given allogeneic SCT. The median age was 8.7 years; 230 patients suffered from hematologic malignancies, 112 from nonmalignant disorders (SAA, immunodeficiency syndromes, metabolic disorders, thalassemia, sickle cell disease, hemophagocytic syndromes). The 360-day TRM (in nonmalignant diseases, 0.17) was influenced by donor type (HLA-identical sibling donors vs phenotypically matched family or unrelated donors vs mismatched donors), time of SCT (before and after 1997), stage of disease (early vs advanced), conditioning regimen (myeloablative vs reduced), graft manipulation (T-cell depletion vs no TCD) and history of severe organ dysfunction and/or infections. Most frequent causes of death were organ toxicity, acute GvHD, and infections. The following factors were independent risk factors for TRM: graft failure, resistant disease, CMV IgG-negative donors for CMV IgG-positive patients, HLA mismatch > 2 alleles, TCD graft, and history of severe toxicity and/or infection. The pattern of severe adverse events changed over time. In the first years, GvHD was the most frequent life-threatening complication, followed by toxicity and infections. More recently, viral infections are the major reason for TRM, particularly in patients transplanted from HLA-mismatched donors given T-cell–depleted grafts. The cumulative incidence of TRM in patients transplanted after 1997 was 0.06 from matched sibling donors, 0.14 from unrelated donors, and 0.27 and from HLA-mismatched donors. Factors associated with improved survival incuded the quality of HLA typing (high-resolution techniques in HLA class I and II), reduced intensity conditioning, monitoring and preemptive treatment (pharmacologic and virus-specific cell support) of viral infections, and experience (center size > 10 allogeneic SCTs vs < 10 per year). We conclude that today, the risk for TRM especially in children with nonmalignant diseases undergoing HSCT could be reduced by multidisciplinary approaches focusing the enhancement of immunologic reconstitution.

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TREATMENT OF PEDIATRIC PATIENTS WITH SANFILIPPO SYNDROME (MPS IIIA AND IIIB) WITH UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION

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Umbilical cord blood transplantation (UCBT) favorably alters the natural history of young pediatric patients with Hurler syndrome (MPS I). We asked whether it would also be effective for patients with Sanfilippo syndrome (MPS III). Patients with Sanfilippo syndrome have less somatic and more CNS disease than patients with other MPS syndromes. They are typically diagnosed between 3 and 5 years of age. Without treatment, they develop severe neurologic dysfunction, deafness, inability to speak or ambulate, and severely autistic behaviors. Death occurs between 10 and 13 years of age. Currently, allogeneic stem cell transplantation (SCT) is the only way to deliver healthy enzyme to the brain. We hypothesized that children with Sanfilippo syndrome would benefit from UCBT, particularly if it was performed early in the course of the disease. Between February 2001 and April 2004, 12 children (age 10–59 months, 75% male, 100% Caucasian) underwent UCBT. All were prepared for transplantation with myeloablative chemotherapy (busulfan/cyclophosphamide/ATG), and all received prophylaxis against GvHD with cyclosporine and steroids. They were transplanted with unrelated donor UCB grafts matching at 6/6 (n = 1), 5/6 (n = 7), and 4/6 (n = 4) HLA loci, containing a median of 6.5×10^7 nucleated cells/kg and 1.42×10^5 CD34 cells/kg. Donor cell engraftment (ANC 500/μL) occurred in 9 of 12 children in a median of 24 days. Platelet engraftment (platelet count 50K/μL untransfused) occurred in 7 of 12 children