cine and ultrasound has led to the possibility of in utero diagnosis and treatment with intrauterine transfusions. Rare cases of premature infants surviving and undergoing chronic transfusion therapy have been reported. Hematopoietic stem cell transplant (HSCT) has become increasingly used to cure beta thalassemia, and provides the potential to cure α^0thalassemia. To date, 3 reported cases of α^0thalassemia have been treated with HSCT. Two children underwent matched sibling HSCT at 20 and 21 months of age and one a 5/6 HLA matched sibling umbilical cord blood transplant (UCBT). One child had received intrauterine transfusions. We report a case of an infant with α^0thalassemia successfully treated with intrauterine transfusions followed by unrelated donor UCBT. The mother, of Cambodian descent, noticed decreased fetal movement at 23 weeks gestation and ultrasound revealed hydrops fetalis. Based on nationality and previous fetal loss, the suspicion of α^0thalassemia was raised. UCB sampling revealed a hypochromic, microcytic anemia with target cells. The Hgb electrophoresis demonstrated Hgb Barts and Portland. Subsequent genotyping confirmed deletion of all 4 α-globin genes. The patient received 3 intrauterine transfusions prior to delivery at 32 weeks. The hospital course was relatively uneventful and included PRBC transfusions. After discharge, he received 3 transfusions. Following informed consent, at 6 months of age, he was conditioned for transplant with busulfan, cytoan and ATG. Lacking an HLA matched sibling, he received a 5/6 HLA matched unrelated donor UCB unit delivering 11.8×10^7 nucleated cells/kg. Neutrophil engraftment (ANC>500) was achieved on day + 15. FK506 and methotrexate 5mg/m2 on days 1, 3 and 6 was utilized for GVHD prophylaxis. His course was complicated by moderate venoocclusive disease, small subdural hemorrhage, and viremia. He has not had any evidence of graft versus host disease. Initial chimerism (STRs) showed approximately 63% donor derived cells, since increasing with 100% T-cell engraftment. He clinically is doing well post transplant. This case demonstrates that intrauterine transfusion followed by unrelated donor UCBT is feasible for the treatment of α^0thalassemia.

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CHRONIC GRAFT-VERSUS HOST DISEASE (GVHD) IN CHILDREN WITH MUCOPOLYSACHARIDOSES (MPS) TWO YEARS AFTER UNRELATED DONOR UMBILICAL CORD BLOOD TRANSPLANTATION (UCBT)

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MPS are a group of metabolic storage diseases caused by lysosomal enzyme deficiencies resulting in the accumulation of glycosaminoglycans in multiple organs. Unrelated Cord Blood Transplantion (UCBT) can prevent progression of organ dysfunction in MPS. UCBT can slow or halt cognitive deterioration in early cases of MPS II and III and improves cognitive outcome for MPS I. However, even after full donor engraftment quality of life and neurocognitive outcome can be adversely affected by post-transplant complications such as chronic GVHD and its treatments. In this retrospective analysis, we sought to determine how many children receive systemic immunosuppressive (IS) drugs at the 2 year time point at the single largest center performing UCBT for MPS. Over 90% of Duke patients with MPS are out of state and are followed locally after 6-9 months. However, even these patients return to Duke for yearly visits. Receiving systemic IS drugs was chosen as a surrogate marker of active chrGvHD. Patients between ages 1 and 50 months (median 18 months) underwent UCBT for treatment of MPS I (Hurler), MPS II (Hunter), and MPSIII (Sanfilippo Syndrome). Preparative regimen consisted of busulfan + cyclophosphamide with equine ATG as previously described (COBLT study). Cyclosporine A and SoluMedrol were used as GvHD prophylaxis. Systemic IS is expected to be tapered starting between 6 and 12 months after UCBT. In the absence of active GVHD, patients are weaned off all IS between 12-16 months post-UCBT. Fourty seven (47) MPS patients were transplanted between 1995 and September 2004, (MPSI n=33, MPSII n=2, MPS IIIA n=8 , MPS IIIB n=4) Thirty three (33) out of the 47 were alive 2 years post-UCBT (~70%). Ten out of the thirty three survivors (30%) were receiving systemic IS drug at the 2 year visit with the recommendation to continue. Five of these had limited skin GvHD while five had skin plus other organ involvement (cytopenias in 2 and GI tract in another 3). One received CellCept only and two were on FK506 alone. Three children received 2 agents (FK506 and Prednisone). Four children received three drug combination (FK506, low dose Prednisone, and Daclizumab). Mean CD4+ T cells were $1400/mm^3 \pm 950$. One patient died before the 3 year f/up with compliactions related to GVHD, while all other patients are alive. Taken together, a subset of MPS patients experience prolonged exposure to IS drugs that may hinder neurocognitive outcome after UCBT. Risk factors are currently analyzed.

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KINETICS OF ENGRAFTMENT FOLLOWING DUAL UNRELATED CORD BLOOD TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING

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Reduced intensity conditioning (RIC) is a safer alternative to conventional myeloablative conditioning for patients at high risk of transplant-related morbidity/ mortality. For these patients lacking adult donors, unrelated donor cord blood transplant (CBT) is a feasible alternative. However, CBT with RIC may pose unacceptable risks of graft rejection, hence compromising outcome. To enhance engraftment, dual donors CBTs with RIC have been performed with promising results in pediatric patients in our centre. Little is known on the kinetics of engraftment and the defining factor/s determining the final engrafted unit in this setting. We analyzed these variables in 8 patients (6 with acute lymphoblastic leukemia (ALL), 1 with hemophagolymphohistiocytosis (HLH), 1 with CD40 ligand deficiency) who received mismatched, dual unrelated CBT after RIC with fludarabine, cyclophosphamide, low dose TBI ± low dose ATG between 2004 - 2006. All except the patient with HLH engrafted. In 6 evaluable patients, mixed chimerism was noted in 50% of the patients early after transplant (≤D+14). Analyzed separately, all patients with ALL achieved ≥ 90% donor chimera by D+34 after transplant. All but 1 patient with ALL (83%) are in continuous complete remission to date. The patient with CD 40 ligand achieved complete donor chimera on D+125 after transplant. Contrary to reports of prolong mixed chimera states after RIC, which may predispose to graft failure and relapse, the kinetics of engraftment in our patients with ALL was quick and robust. Early engraftment kinetics also revealed dominance of one of the two units in majority of these patients. Amongst other factors, the final engrafted cord units have higher post-thaw cell doses. These data demonstrated that sustained, full donor chimera can be achieved early after RIC transplants with dual CBT in pediatric patients with ALL without compromising survival and relapse risks.

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MOBILIZATION OF PML-RARA NEGATIVE PERIPHERAL BLOOD STEM CELLS AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT IN PEDIATRIC PATIENTS WITH RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

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Autologous stem cell transplant has failed to demonstrate benefit in most children with relapsed acute myelogenous leukemia. Ten to twenty percent of children with acute promyelocytic leukemia

(APL) treated using all-*trans*-retinoic acid (ATRA) based therapy will relapse. An analysis of salvage therapy in patients < 66 years old from two sequential European multi-center trials demonstrated the efficacy of autologous peripheral blood stem cell transplant (PBSCT) performed during molecular remission.

Since 2004, APL recurred in four patients following ATRA based therapy. Table 1 (below) summarizes their clinical characteristics. After achieving molecular remission, the patients received cyclophosphamide 2 gm/m^2 for two days followed by G-CSF mobilization. All PBSC products were negative for PML-RARA by RT-PCR. To date, three patients received stem cell infusions following cyclophosphamide (60 mg/kg X 2 days) and total body irradiation (200 cGy X 6).

All three transplanted patients remain in molecular remission 27, 13, and 8 months following PBSCT. Engraftment of WBC occurred at 13, 15 and 18 days, with discharge days of 16, 18, and 20 post-PBSCT. Maximum toxicity was Grade III mucositis (NCI CTC *v*.3.0).

PML-RARA negative stem cells can be mobilized in pediatric patients after arsenic trioxide or FLAG therapy using cyclophosphamide and G-CSF. Three patients have durable molecular remission following PBSCT. Autologous PBSCT is an option for pediatric patients with relapsed APL that avoids the complications of graft *vs* host disease, prolonged immunosuppression, and the higher transplant related mortality of allogeneic transplantation.

Table 1. *Clinical characteristcs, salvage therapies and mobilization outcome.*

Age at dx (yrs)/gender	Sanz Risk Group[1]	Length CR 1 (mos)	Relapse Site	Re-induction therapy	# days harvested	Total CD34$^+$/kg X 10^6
14 M	High	11	Marrow + CNS	Arsenic trioxide + Intrathecal Ara-C	3	2.19
15 M	Intermediate	24	Marrow	Arsenic trioxide	3	2.35
2 M	Intermediate	14	CNS	FLAG[2] + Intrathecal Ara-C and Methotrexate	1	207.00
12 M	Low	26	Marrow	Arsenic Trioxide	1	14.53

[1] Sanz, Blood 2000: 96(4), 1247-1253 [2] FLAG (fludarabine 25 mg/m^2 X 5 days; cytarabine 2 gm/m^2 X 5 days; G-CSF)

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HIGH-DOSE THIOTEPA, BUSULFAN, AND CYCLOPHOSPHAMIDE FOR ALLOGENEIC STEM CELL TRANSPLANTION IN RISK PEDAITRIC PATIENTS WITH HIGH-RISK LEUKEMIAS

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Between 1992 and 2003, 50 children at M D Anderson Cancer Center with high-risk leukemias received thiotepa (250 mg/m^2 x 3), busulfan (1 mg/kg q 6 hrs x 12 orally or 0.8 mg/kg q 6 hrs iv x 12), and cyclophosphamide (60mg/kg x 2) as a preparative regimen for allogeneic stem cells transplantation. High-risk ALL patients (25) had abnormal cytogenetics (Ph+, 11q23), CR2 after early relapse, ≥ CR3, or were induction failures. Patients will AML (25) were included if they had ≥CR 2, MDS, or were induction failures. Sixteen patients were transplanted with active disease (> 5% blasts on bone marrow aspirate). Busulfan was given orally until 1999 when the intravenous preparation was available. Stem cell sources included HLA matched family members (21) and unrelated marrow and cord blood (29). Two patients failed to engraft and went on to receive a second transplant. Neutrophil engraftment (ANC >500) occurred at a median of 17.5 days (range 11-63) and platelet counts >50,000/μL at a median of 44.5 days (range 13-394). Grade 3-4 toxicities occurred in 24 patients and were predominantly esophagitis and mucositis, emesis, and hepatic dysfunction. All three patients who developed VOD had received busulfan orally. One patient did not have pharmacokinetics performed secondary to machine malfunction. One patient had an acceptable AUC. The final patient had an elevated BU dose that was decreased based on initial pharmacokinetics. Grades I -II acute GVHD was common (27 patients). Grades III-IV occurred in 5 patients. Five patients developed limited chronic GVHD, and 9 patients developed extensive chronic GVHD. Nineteen patients are alive and well a median of 7.0 years (range 3.0 - 13.9). Thirty-one patients died of recurrent disease (13), infection (6), GVHD (5), VOD (3), other causes (4). The two patients who failed to engraft and underwent a second transplant died of disease recurrence. Children with high-risk leukemias are difficult to cure even with high-dose chemotherapy and stem cell transplantation. Of the 16 patients transplanted with active disease, only the patients with AML are long term survivors, suggesting that this regimen alone will not result in a cure, but that additional strategies are required for children with active ALL. This chemotherapeutic regimen had some significant toxicities, however, after the development of intravenous busulfan, this regimen had a significant decrease in nausea and there were no incidence of VOD suggesting further evaluation.

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A NOVEL RAG-1 NULL MUTANT DISPLAYS SEGREGATION OF DNA CLEAVAGE INTO TRANSPOSITION ACTIVITY IN THREE CHILDREN WITH T$^-$B$^-$NK$^+$SCID

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RAG (recombination activating gene)-1 and RAG-2 initiate V(D)J recombination by cleaving DNA at recombination signal sequences (RSS) through sequential nicking and transesterification reactions to yield blunt signal and coding ends terminating in DNA hairpin structures. Ubiquitous DNA double strand repair factors in the non-homologous ending joining (NHEJ) pathway then mediate the rejoining of broken DNA. Severe combined immunodeficiency disease (SCID) comprises a heterogeneous group of primary immunodeficiencies, a proportion of which are due to mutations in RAG-1, RAG-2 or in NHEJ factors, respectively. Here, we report a novel RAG-1 null mutation, R776W, identified in three familial related children from the Diné Native American tribe who manifest a classic T$^-$B$^-$NK$^+$SCID phenotype and engraftment of maternal T cells. Unlike Artemis mutations among Athabascan-speaking Native Americans, which trigger accumulation of the hairpin structure, this RAG-1 null mutant evokes impaired DNA cleavage and binding activity in vitro by preferentially diminishing hairpin formation. Moreover, the V(D)J recombination efficiency of this mutant is also reduced as assessed by an enhanced green fluorescent protein (EGFP) based V(D)J recombination assay. However, the catalysis of other DNA strand transfer reaction, such as transposition, is not substantially affected by this RAG-1 mutant. These results indicate that dysfunction of RAG-1 is responsible for the T$^-$B$^-$NK$^+$SCID phenotype. Segregation of RAG-1 mediated DNA cleavage activity into its transposition activity casts a new light on RAG-1's roles in V(D)J recombination and translocation.

SOLID TUMORS

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FEASIBILITY OF AUTOLOGOUS STEM CELL TRANSPLANT FOLLOWED BY REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR HIGH RISK NEUROBLASTOMA: A SINGLE INSTITUTION PILOT STUDY

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Background: Autologous stem cell transplant (AutoSCT) has improved the outcome for children with high risk (HR) neuroblas-