BONE MARROW TRANSPLANT

Poster 100

Ex Vivo Fucosylation of Cord Blood Cd34+ Stem Cells Improves the Rate and Magnitude of Engraftment

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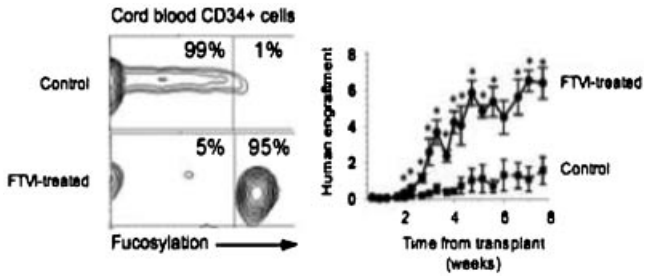
Background: The use of cord blood (CB) as a stem cell source for transplant is limited by cell dose and delayed engraftment. Beyond cell dose, it has been shown that on a cell-for-cell level, cord blood-derived hematopoietic stem cells (HSCs) are deficit in homing compared to adult bone marrow-derived HSCs. Although cord blood HSCs express P-selectin glycoprotein ligand-1 (PSGL1), they lack the terminal fucosylation which is necessary for P and E-selectin binding. These selectins are expressed by the microvasculature of the hematopoietic microenvironment, and appear to play a role in HSC homing.

Objective: To determine if increasing the level of fucosylation of surface ligands on CB HSCs will improve the rate and magnitude of engraftment.

Design/Methods: Freshly collected human cord blood cells were CD34-selected, then exposed to recombinant fucosyltransferase VI (FTVI, kindly provided by OMRF and ASC) in the presence of the substrate GDP-fucose for 30 minutes prior to injection into sublethally-irradiated NOD/SCID/IL-2Rgamma mice. Levels of fucosylation pre- and post-treatment were determined by flow cytometry using the anti-sialyl Lewis X antibody. Each mouse received $\sim 4.5 \times 10^4$ CD34+ cells by tail vein injection. The rate of human engraftment was measured by the appearance of human CD45+ cells in peripheral blood (PB) samples. Multi-lineage reconstitution was measured with human-specific lineage markers.

Results: Following fucosylation, 95% of CD34+ cells had high Lewis X antigen levels, compared to 1% in non-fucosylated samples, confirming significant fucosyltransferase activity (Figure). In mice, peripheral human engraftment could be detected in FTVI-treated HSC mice within 2 weeks post-transplant, compared to >3 weeks in control HSC mice (Figure). In addition, engraftment percentages after 3 weeks remained greater than 4-fold higher in FTVI-treated HSC mice. Importantly, no loss of engraftment was seen and no significant differences were observed in multi-lineage engraftment.

Conclusions: These data demonstrate that ex vivo fucosylation of CB HSCs dramatically improves the rate of engraftment, provides long-term multi-lineage reconstitution and is clinically feasible. These preliminary data support ex-vivo fucosylation as a novel method of improving cord blood transplantation.



Poster 101/PLATFORM SESSION 304

Ex Vivo Expansion of Cord Blood Nk Cells With in Vivo Efficacy Against Acute Myeloblastic and Lymphoblastic Leukemias

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Background: Natural killer (NK) cells have been shown to comprise a significant component of graft-vs.-leukemia effects and have been reported to enhance engraftment and reduce graft-vs.-host disease. Cord blood (CB) is a potentially useful source of NK cells as CB-derived NK cells appear to have enhanced cytokine response, increased antigen-mediated proliferation and increasing availability.

Objective: To develop robust ex-vivo expansion conditions for CB NK cells and evaluate this product in vitro and in vivo against human acute myeloblastic and lymphoblastic leukemias (AML and ALL).

Design/Methods: Either CD56-selected or CD3-depleted CB mononuclear cells were cultured with the CD56- fraction accessory cells with 100 ng/mL IL-2 for 21 days. In vivo NK cytolytic activity was measured via chromium release assays on leukemia cell lines and fresh AML patient samples. Functionality was confirmed in vivo as well through xenografts of K562 (AML), HL-60 (AML) or Nalm6 (precursor B-ALL) cells in NOD-SCID/IL2Rgamma mice. $2–5 \times 10^6$ CB-expanded NK cells were administered on days +1 and +7. NK killing of leukemic targets was demonstrated by flow cytometry.

Results: The irradiated accessory cells enhanced IL-2 mediated expansion of CB CD56-selected NK cells, generating 30-fold expansion, compared to 15-fold expansion in the absence of these accessory cells ($p<0.01$). These NK cells also killed 40–90% of the three cell lines, while killing 60–80% of cells in five AML patient samples in chromium release assays. In CD3-depleted cultures, similar fold expansions were observed, however only 55% of these cells were CD3-CD56+ with 35% CD3+ T lymphocytes and 10% CD3+CD56+ NK/T cells. These CB NK cells demonstrated the ability to significantly reduce leukemia burden in all three xenograft models, decreasing peripheral blasts by 50–70% after one infusion and 60–90% after two.

Conclusions: We have demonstrated the feasibility of a simple enhanced NK expansion approach from cord blood using irradiated accessory cells. These NK cells demonstrate significant cytotoxic activity against human AML and ALL cell lines and patient leukemia blasts. Most importantly, these NK cells demonstrated significant in vivo activity against human AML and ALL. These results support evaluation of CB-derived NK cells as a promising immuno-therapeutic approach in acute leukemias.

Poster 102

Allogeneic Hematopoietic Cell Transplantation (Allo-Hct) for Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia: Impact of Imatinib on Risk of Relapse and Survival

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Background: The utility of Imatinib in either the pre/post-transplant period for Philadelphia positive (Ph+) Acute Lymphoblastic Leukemia (ALL) is unknown. Additionally, there have been concerns regarding Imatinib and cardiac toxicity in both preclinical studies and some clinical series.

Objective: We investigated the outcome of transplantation in pediatric patients with Ph+ ALL who were transplanted since Imatinib became commercially available.

Design/Methods: Sixteen pediatric patients with Ph+ ALL received an allo-HCT at the University of Minnesota between 1999 and 2006. The median age at HCT was 7.4 (range; 2.8–20.8) years. Thirteen (81.3%) patients were male. Seven were in first remission (CR1) prior to HCT and 9-patients were in second remission (CR2). Donor source was HLA matched and mismatched unrelated umbilical cord blood ($n = 11$) or HLA matched sibling bone marrow ($n = 5$). All patients were conditioned with cyclophosphamide and total body irradiation with cyclosporine-based GVHD prophylaxis. Of the 16 patients with Ph+ ALL, nine received

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Imatinib therapy pre/post-HCT (5-pre-HCT and 4-post-HCT) comprising the Imatinib group and the remaining 7-patients, who either never received Imatinib (n = 6) or received it only at time of relapse (n = 1), were the non-Imatinib group.
Results: The median follow-up for the entire cohort was 1.2 (range; 0.7–4.8) years. Overall survival and relapse-free survival at 2 years was 67% and 67% for the Imatinib group compared to 29% and 29% for the non-Imatinib group (p = 0.41 and 0.32, respectively). While the incidence of non-relapse transplant related mortality at 1-year was similar between groups (33% and 14%; p = 0.35), the incidence of relapse was 0% in the Imatinib group versus 57% (95% CI: 0.16, 0.98) in the non-Imatinib group (p = 0.01). Of note, the 1-year cumulative incidence of cardiac toxicity [defined as a reduction in left ventricular ejection fraction of >20% below baseline, cardiac hypertrophy or EKG abnormalities (ST changes and/or T-wave abnormalities)] was similar in both groups, 30% versus 29% (p = 0.77).
Conclusions: The use of Imatinib therapy in pediatric patients with Ph+ ALL in either the pre-or-post-HCT setting decreases the risk of relapse without a concomitant increase in cardiac toxicity or TRM. These data support the routine use of Imatinib in patients with Ph+ ALL undergoing allo-HCT.

Poster 103/PLATFORM SESSION 301

Chemosensitization of Pediatric Cancers to Nk Cell Lysis by Upregulation of Nkg2d and its Ligands Using Hdac and Proteasome Inhibitors

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Background: Relapsed and refractory presents a major therapeutic hurdle because of the resistant nature of these malignancies to conventional chemotherapeutic agents. Several lines of evidence support an evolving role for immunotherapy in the treatment of cancer, particularly in chemotherapy-resistant relapsed disease. As a key component of the innate immune system, natural killer (NK) cells play a crucial role in anti-tumor immune responses. Several reports support the relationship between NK cell recovery or activity and relapse-free survival in patients with AML. Adoptive therapy with NK cells is an attractive approach because haploidentical KIR-mismatched NK cells from related donors are readily available and lack any apparent risk for GVHD. NKG2D, a major activating receptor of NK cells, induces NK-mediated anti-tumor activity when activated by ligands MICA, MICB, and the ULBP family. NK cell killing correlates with the expression of activating and inhibitory ligands on tumor target cells, and expression of these ligands have been shown to be modulated by HDAC inhibitors and proteasome inhibitors in several adult carcinomas and multiple myeloma.
Objective: Determine if HDAC or proteasome inhibitors effect NK cell-mediated killing in pediatric malignancies.
Design/Methods: We investigated the role of HDAC inhibitors MS275 and VPA, and proteasome inhibitors PS341 and NPI0052, in enhancing cell lysis by primary NK cells of human osteosarcoma, AML, and neuroblastoma.
Results: We found that NKG2D ligands MICA/B were upregulated by MS-275, VPA, and PS341 in a majority of pediatric cell lines and primary tumor samples tested, but the degree and the pattern of response to these agents varied greatly. Moreover, PS341 was also able to enhance expression of NKG2D in NK cells, whereas NPI0052 was toxic to NK cells. Overall, these changed led to enhanced cytotoxicity of the target tumor cells.
Conclusions: These results suggest that pediatric cancers may be amenable to treatment with NK cells, and may be greatly enhanced if adoptive therapy of NK cells is combined with upregulation of NKG2D and its ligands by HDAC or proteasome inhibitors. We will present in vivo data testing this chemosensitization approach and our NK cell clinical trials currently open or in development.

Poster 104/PLATFORM SESSION 304

Inhibitory RNA Mediated-Purine Analog Resistance Allows for In Vitro and In Vivo Cell Selection with 6TG

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Background: A major hurdle in gene therapy efforts is the transduction of sufficient numbers of cells to ameliorate disease manifestations. Therefore, the ability to expand a desired population of genetically modified cells would be of great benefit. We have data to support a novel method of in vitro and in vivo cell selection using inhibitory RNA mediated-purine analog resistance (iPAR) and 6-thioguanine (6TG).
Objective: 1) To determine if iPAR and low dose 6TG can be used to select for hematopoietic progenitor cells in murine recipients of transduced BM and 2) To determine if human cells can be similarly provided with iPAR for cell selection.
Design/Methods: Lentiviral constructs are designed to code for short hairpin RNA against murine or human hypoxanthine phosphoribosyl transferase (HPRT). Murine BM was transduced and transplanted into sub-lethally irradiated recipients, which were then treated with low-dose 6TG or PBS. Human leukemia cell lines were similarly transduced and subject to treatment with 6TG in vitro.
Results: Analysis of peripheral blood indicates that treatment with 6TG selects for cells with iPAR, enriching their contribution to myelopoiesis an average of 60-fold (range 10–200) and to B-lymphopoiesis an average of 4 fold (range 2–6) after a single 3 week cycle of treatment (Table). PBS treated recipients had no consistent changes in the percentage of transduced cells contributing to hematopoiesis. 6TG caused moderate neutropenia in one recipient, but was otherwise apparently well tolerated. Human cells provided with iPAR survive and proliferate in the presence of 6TG at near untreated rates, whereas live, untransduced cells are reduced in number by at least 50%.
Conclusions: The provision of iPAR to hematopoietic cells and treatment with 6TG is an effective means of in vivo cell selection in mice and in human cells in vitro. These data suggest that iPAR may be a relatively non-toxic strategy to enhance gene therapy efforts for diseases of the hematopoietic system.

Poster 105

Chronic Graft-Versus-Host Disease (Cgvhd) in Unrelated Cord Blood Transplantation (Ucbt): Single Institution Experience, July 1996–June 2007

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Background: The incidence of CGVHD is lower in UCBT compared to unrelated transplant using adult donors. Details of CGVHD incidence, risk factors, organs involved, severity, and long-term outcome still need to be explored further. Recently, a new NIH guideline for diagnosis of CGVHD has been published to standardize criteria for CGVHD diagnosis.
Objective: To review our experience with CGVHD in UCBT and re-classify CGVHD based on NIH classification and compare it with Seattle classification.
Design/Methods: We retrospectively reviewed CGVHD in 81 consecutive UCBT treated on a single therapeutic trial with consistent GVH prophylaxis. 23 patients were unevaluable for CGVHD based on death prior to UCB infusion (2), death prior to engraftment (7), graft rejection (4), or death before day 100 (10). Thus, 58 patients were evaluable for CGVHD (37 male, 21 female; median age 7.2 yr (range, 0.32–20.36 yr); median weight 24.8 Kg (range, 5.35–82.9 Kg)). Diagnoses included leukemia in 73% (67% ALL, 19% AML, 14% CML or JMML), MDS 3.3%, lymphoma 1.7%, metabolic disorders 7%, immune/genetic disorders 10%, and aplastic anemia 5%. Acute leukemia disease status at UCBT was CR1 19%, CR2 53%, CR3 or greater 22%, and not in remission in 6%. HLA matching was 6/6 (21), 5/6 (24), and 4/6 (13). Conditioning was TBI-based in 64% and non-TBI in 36%. GVHD prophylaxis was cyclosporine and steroids in 93%, cyclosporine and Methotrexate +/− steroids in 7%.

Results: CGVHD was seen in 21 patients (36%) (Limited (10%) and extensive (90%)). There is history of previous Acute GVHD in 81% of patients with CGVHD vs. 38% in patients without CGVHD. NIH reclassification is shown on the table below. Event free survival at 1 and 3 yr were 86% and 63% for patients with CGVHD, and 67% and 67% for patients without CGVHD.
Conclusions: The classic chronic appearance of CGVH is uncommon following UCB transplant. Instead, the acute and overlap variants of CGVH predominate.

NIH Classification	Seattle Classification	
	Limited	Extensive
Acute (persistent, recurrent, late-onset)	2	13
Classic chronic	0	1
Overlap syndrome	0	5

Poster 106

Identification of Genes Associated with All Leukemia Cell Survival in the Alloreactive Post-transplant Immune Milieu

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Background: Allogeneic hematopoietic stem cell transplant is performed for ALL for patients at high risk for relapse. The alloreactivity associated with transplant is thought to produce a clinically significant antileukemia effect. However, relapse remains the most common reason for treatment failure.
Objective: We wished to test the hypothesis that the gene expression profile of ALL cells surviving in the post-transplant environment in which there is significant alloreactivity would differ from the gene expression profile of leukemia cells surviving in a setting of reduced alloreactivity.
Design/Methods: 10e4 murine pre-B ALL cells carrying the human bcr/abl and INK4A/ARF mutations were mixed with C3.SW hematopoietic cell grafts into normal C57BL/6 mice. The strains are MHC I and II matched, but differ at multiple minor histocompatibility antigens. The experimental group (n = 4) received cells from donors primed against recipients to produce a high GVHD environment while control mice (n = 3) received naïve donor marrow. At 14–21 days post transplant leukemia cells were purified from marrow using immunomagnetic selection. Affymetrix expression profiles were generated and analyzed using dChip software.
Results: A small number of genes were observed to be expressed at higher levels in ALL surviving the high GVHD environment.
Conclusions: Analysis of these may provide insight into the mechanisms of relapse and possibly prospectively identify patients who are likely to relapse after transplant.

Gene Name	Fold-Increase	P value	Gene Ontology Function	Gene Ontology Process	Functional significance
Serine proteinase inhibitor	3.37	0.001	Endopeptidase inhibitor Cysteine protease inhibitor	Apoptosis	Upregulation allows evasion of caspase-independent programmed apoptosis.
Chemokine ligand 3	2.65	0.005	Chemokine receptor	GPCR activity Signal transduction	Eosinophil beta-chemokine receptor that signals the IL-13 mediated allergy response.
Macrophage expression factor 1	2.46	0.002	Tyrosine kinase receptor- Cytokine activity Growth factor receptor	Hematopoiesis	Hematopoetic growth factor required for macrophage differentiation.
CCR4-NOT transcription complex, subunit 4	1.44	0.009	Chemokine nuclear transcription complex- Protein and RNA binding	DNA-dependent transcription regulation	Chemokine receptor correlated with poor clinical outcome in T-cell lymphomas.
Diazepam binding inhibitor	1.34	0.004	Acetyl CoA binding Lipid binding	Nuclear transport	Autoantigen that mediates autoimmune destruction of hematopoetic stem cells in aplastic anemia.

Poster 107

Routine Blood Cultures In Pediatric Oncology: Are They Justified?

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Background: The practice of performing routine or surveillance blood cultures is relatively common in pediatric oncology, but has not been critically evaluated. We selected two such practices for study: Blood cultures (BC) taken from hematopoietic stem cell transplant (HSCT) pts on the day of admission for transplant and BCs obtained after 72 hours of intravenous antibiotics (IV abx) for non- HSCT pts admitted for febrile neutropenia (FN).
Objective: To evaluate the yield of performing surveillance BCs in asymptomatic pediatric HSCT pts, and of obtaining late BCs (LBC) in persistently febrile, but stable, oncology patients after 72 hours of IV abx.
Design/Methods: We conducted a retrospective analysis of all BCs taken from HSCT pts, consecutively admitted to our institution between 2000 and 2007 and all BCs obtained from non- HSCT pts, admitted to hospital between 1999 and 2007 for FN.
Results: Surveillance BCs were analyzed in 108 HSCT pts, who underwent 118 transplants. The incidence of positive BCs on admission was 5.9% (7/118) for transplants and 3.4% (7/206) for total BCs taken. Five of these 7 BCs grew Staphylococcus coagulase negative. Repeat BCs in all 7 pts were negative and only 1 pt was treated with an antibiotic appropriate for the isolated organism. None of the untreated pts had any adverse sequelae.
Of the LBCs evaluated in 84 pts (114 episodes) with FN, there were 3 positive BCs (3.5%) after 72 hours of IV abx. The observed frequency of LBCs was 2.6% (3/114) for episodes of FN and 0.6% (3/473) for total blood cultures obtained. Only 1 pt (1.2%) had a positive LBC in the absence of new symptoms.
Conclusions: Our data suggest there is a very low yield to obtaining surveillance BCs on asymptomatic HSCT pts upon admission for transplant, and for routine BCs in stable FN pts after 3 days of IV abx. This implies that more efficient, risk based, strategies for culturing of blood in pediatric oncology pts might be possible. These observations need to be confirmed with larger studies from other centers.

Poster 108

Treatment of CD40 Ligand Deficiency with Reduced Intensity Transplantation Using Highly Purified Cd34 Cells from a Partially-Mismatched Unrelated Hematopoietic Stem Cell Donor

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Background: CD40 ligand (CD154) deficiency (Hyper IgM syndrome) is a severe X-linked recessive immune deficiency. Activated T cells do not

express CD154 and cannot interact appropriately with CD40 on B cells, resulting in poor immunoglobulin class switching. Allogeneic hematopoietic stem cell transplantation can be curative. Our patient presented at age 5 months with severe Pneumocystis carinii pneumonia, normal IgM and undetectable IgG and IgA. CD154 was not detected on activated patient T cells. Sequencing of the CD40LG gene demonstrated a G>C mutation at position 157. The patient had no HLA matched siblings. A 9/10 unrelated donor with a DRB1 mismatch was available.

Objective: We wished to determine if this immune deficiency could be treated with a mismatched unrelated donor transplant using a reduced intensity preparative regimen and highly purified CD34 cells.

Design/Methods: At 10 months the patient underwent a preparative regimen consisting of busulfan, fludarabine, and cyclophosphamide. Immune suppression consisted of rabbit ATG (days −3 to −1), methylprednisolone (days +1-+18) and OKT3 (days +1-+18). No other immunosuppressant drugs were administered. CD34 cells were purified using a Miltenyi Clinimacs device. The final product had no detectable CD8 or CD4 cells and a total of 7 × 10e6 CD34 cells/kg were infused.

Results: The patient did not experience significant toxicity or serious infection. He did not receive filgrastim and ANC exceeded 500 on day 16. Platelets exceeded 20,000 on day 20. He did not experience acute GVHD. At three months molecular chimerism studies showed 99% donor cells. CD3 counts were 301/ul and CD19 were 328/ul. 36% of the CD3 cells were CD45RA+ CD45RO−, demonstrating generation of naïve T cells. PMA/ionomycin treated T cells expressed CD154.

Conclusions: CD40 ligand deficiency can be treated with transplant using a partially mismatched unrelated donor without eliciting GVHD using a reduced intensity preparative regimen, highly purified CD34 cells, and brief but intense peritransplant immune suppression. For patients lacking an HLA matched sibling this approach has the advantage of reduced risk of severe GVHD compared with conventional MUD transplant and possibly earlier and more reliable engraftment than that associated with unrelated umbilical cord blood transplants.

Poster 109

FK506 in Combination with Methotrexate As Prophylaxis for Graft-Versus-Host Disease in Pediatric Patients After Allogeneic Blood/Marrow Transplantation: Mayo Clinic Experience

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Background: Tacrolimus in combination with methotrexate has proven efficacy in the prevention of graft-versus-host disease (GVHD) in allogeneic blood and bone marrow transplantation. Pediatric experience with the use of both tacrolimus and methotrexate for the prevention of GVHD is quite limited.

Objective: To review the records of all our patients who underwent either peripheral blood stem cell or bone marrow transplant using tacrolimus and methotrexate as their primary GVHD prophylaxis in order to describe the frequency and severity of adverse regimen effects and the incidence and severity of acute and chronic GVHD with this regimen.

Design/Methods: We retrospectively reviewed our experience with the use of tacrolimus and mini methotrexate in 25 pediatric patients (22 hematological malignancies, 2 severe aplastic anemia, and 1 immunodeficiency, age range 9 months −18 years). Of the 25 patients, 15 received HLA-matched sibling blood/marrow transplant, 9 matched unrelated donor blood/marrow transplant and 1 haploidentical stem cell transplant. Tacrolimus was infused at a dose of 0.03 mg/kg/day as a continuous infusion intravenously and changed to the oral route once mucositis resolved in order to keep a trough level of 5–15 ng/ml. Methotrexate was administered intravenously 5 mg/m2/dose on days +1, +3, +6, +11.

Results: Acute GVHD occurred in 7 patients (n = 4 unrelated and n = 3 matched-sibs). Five developed grade I-II acute GVHD. One developed grade III acute GVHD and none of the patients developed grade IV acute GVHD. One patient with acute GVHD of skin, gut and liver was not classifiable due to insufficient data. Only two patients had extensive chronic GVHD (HLA-matched sibling). Nephrotoxicity with a creatinine of >2 mg/dl was observed in only 3 patients (2 had veno-occlusive disease, VOD). Grade 1 hypomagnesaemia (per NCI toxicity criteria) was observed in 11 patients and grade 2 in 3. 3 patients had mild tremors (neurotoxicity grade 1). 6 patients went on to die of disease recurrence and 2 of multiorgan failure due to VOD.

Conclusions: In our experience, tacrolimus with mini methotrexate is well tolerated with minimal toxicity. Further studies in pediatric patients are needed in order to assess its efficacy for the prevention of GVHD.

HEMATOLOGY – GENERAL

Poster 110

Young Investigator Presentation Genetic Analysis Reveals Multiple Potential Modifiers of Red Cell and Platelet Indices Among Inbred Mouse Strains

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Background: Hematologic disorders, such as sickle cell disease, the thalassemias, and thrombocytopenia syndromes display a wide range of variability, even among patients with identical mutations. These observations suggest that genetic factors, otherwise known as modifier genes, play a role in modulating disease severity. However, such factors have proven difficult to identify in humans.

Objective: Perform genetic analysis in mice to identify genes which modify hematologic phenotypes.

Design/Methods: Using a Bayer Advia blood analyzer we measured 33 hematologic parameters in a population of 207 mice generated from a cross between two inbred mouse strains, C57BL/6J and WSB/EiJ. We performed a genome-wide linkage scan with a total of 200 markers spaced at approximately 10 centimorgan intervals. We analyzed the data by nonparametric interval mapping and applied a correction for multiple observations.

Results: We identified loci with significant linkage for MCV, RDW, hemoglobin distribution width, reticulocyte percentage and mean platelet mass with LOD scores ranging from 4 to 8.1, as well as multiple loci with suggestive linkage to MCV and RDW. We also identified highly significant linkage to chromosome 7 for the CHCM (alternative measure of the MCHC), with a LOD score of 15.6. We combined these data with information from crosses of C57BL/6J to four additional strains (average of 327 progeny per cross) and employed combined cross analysis and haplotype association mapping to narrow our candidate region for the chromosome 7 CHCM locus to 60 genes, including the beta-globin cluster (*Hbb*). Genotyping of this region revealed two known ancestral haplotype blocks, *Hbb(d) and Hbb(s)* which predicted the high and low CHCM strains, respectively, for each cross.

Conclusions: These data indicate that a common founder allele is responsible for a hemoglobin variant in mice. The high prevalence of this allele suggests this locus may be under positive or balancing selection. Similar variants in humans could modify the clinical severity of the hemoglobinopathies, potentially by influencing regulation of cellular hydration through hemoglobin concentration. We have also identified several potential modifier loci for platelet and other erythroid phenotypes outside of the *Hbb* cluster, which could have important implications for the therapy of human erythrocyte and platelet disorders.

Poster 111/PLATFORM SESSION 302

Hematological and Functional Correction of Sickle Cell Anemia with A Gamma-Globin Lentiviral Vector in the Berkeley Transgenic Sickle Mouse

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Background: It is well known that individuals with hereditary persistent fetal hemoglobin (HPFH), along with SCA have a remarkably mild disease phenotype.

Objective: We aimed at converting the SCA phenotype to that of SCA with HPFH via genetic correction of the hematopoietic stem cells to produce fetal hemoglobin (HbF) post-natally.

Design/Methods: We designed a lentiviral vector, sG^bG, encoding human gamma-globin under the control of the beta-globin promoter and elements of the locus control region. Murine hematopoietic stem cell enriched population, comprising of lineage-, Sca+, cKit+ cells from Berkeley sickle mice were transduced with the sG^bG lentiviral vector and subsequently transplanted into lethally irradiated C57/BL6 mice. The Berkeley sickle mice are knockouts for murine alpha and beta-globin and knockins for human alpha and beta-sickle-globin.

Results: At 4.5 months following transplant, HbF accounted for an average of 30%±5% of the total hemoglobin protein in the C57 recipient mice that were 100% donor/sickle RBC chimeras. The hemoglobin increased from 5.7±0.4 g/dl to 9.8±0.7 g/dl ($n = 12$, $P<0.001$); RBC count increased from $5.3\pm0.4 \times 10^6$/mL to $9.1\pm0.73 \times 10^6$/mL ($n = 12$, $P<0.001$), and reticulocyte count decreased four-fold ($n = 12$; $P<.001$).

Functional correction of RBC sickling was also seen: irreversibly sickled cells decreased from 3.1±0.5% to 0.3±0.2%, $P<0.005$; RBC deformability improved shown by improved elongation index of the sickle RBCs; HbF containing sickle RBCs showed improved survival by doubling from 40% to 80% of the total biotinylated RBCs 1 week post biotin injection in a transplanted mouse. The sG^bG group of mice demonstrated smaller spleen sizes than Berkeley sickle mice and a mock transplant control (0.24±0.04 g versus 0.93±0.06 g, and 0.62 g respectively, $P<0.01$). The gene transfer efficiency, as determined by secondary transplants and CFU-S assay was 52%±11%.

Conclusions: In conclusion, we observe a functional correction of the SCA phenotype with a corresponding improvement of hematological indices with expression of gamma-globin.

**Ajay Perumbeti and Tomoyasu Higashimoto contributed equally to this work.*

Poster 112

Microarray Analysis of Liver Gene Expression in Iron-Overloaded Patients with Sickle Cell Anemia and Beta-Thalassemia

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Background: Blood transfusion therapy is frequently used and may be life-saving for patients with beta-thalassemia or sickle cell anemia (SCA), but often results in severe iron accumulation, especially in the heart and liver. Individual differences in handling iron burden during transfusion therapy appear to exist among patients. This pilot study examined whether liver-expressed genes are differentially expressed in transfused patients with SCA or beta-thalassemia, which could affect the degree and rate of iron accumulation.

Objective: To perform microarray analysis on liver RNA obtained from SCA and beta-thalassemia patients with varying LIC.

Design/Methods: As part of a prospective IRB-approved study of non-invasive measurement of iron stores, a 1.0 cm percutaneous liver biopsy sample was collected for total RNA extraction. Microarray experiments were performed using Affymetrix technology and data analyzed using Spotfire software. Candidate genes showing differential expression were validated using quantitative PCR determined in duplicate on an Applied Biosystems 7700HT instrument, using cyclophillin A expression as an internal control to normalize all transcript values. Liver iron content (LIC, in mg Fe per g dry weight liver) was quantified using inductively coupled plasma-mass spectrometry at Mayo Laboratories.

Results: Thirty-two liver samples were divided into three groups based on LIC and diagnosis: Low SCA (LIC = 8,846±3,225, $n = 18$), High SCA (LIC = 21, 487±2, 477, $n = 7$) and beta-thalassemia ($n = 7$). Microarray analysis identified 18 genes showing differential expression among the three LIC groups. Subsequent quantitative PCR experiments validated six of these genes: GSTM1, EIF5a, CHI3L1, CCL18, ACE2 and GNMT, as being differentially expressed. Three genes (GSTM1, EIF5a and CHI3L1) were significantly dowrnregulated, while three others (CCL18, ACE2 and GNMT) were significantly upregulated, in SCA patients with high LIC. EIF5a was also downregulated in beta-thalassemia samples while CCL18 expression was significantly upregulated in beta-thalassemia compared to all SCA patients.

Conclusions: In patients with SCD or beta-thalassemia with transfusional iron overload, several genes demonstrate differential expression in the liver, based on the liver iron content. These preliminary data will be used to investigate liver-expressed genes that affect the host response to severe iron overload.

Poster 113/PLENARY SESSION 300

Testing Patients with Evans Syndrome for the Autoimmune Lymphoproliferative Syndrome (Alps): Results of a Large Multi-Institutional Clinical Trial

Alix E. Seif, MD, MPH[1], Catherine S. Manno, MD[2], Stephan A. Grupp, MD, PhD[3] and David T. Teachey, MD[1]. [1]Divisions of Hematology and Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA; [3]Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA

Background: ALPS is a recently described disorder of disrupted lymphocyte homeostasis, resulting from mutations in the Fas apoptotic pathway. Clinical manifestations in ALPS patients typically include autoimmune cytopenias, organomegaly, lymphadenopathy, and increased risk of development of malignancies. A similar constellation of findings can be seen in Evans Syndrome (ES), is a hematologic disorder defined by idiopathic autoimmune destruction of at least two hematologic cell types. We have previously published the results of a small single institutional trial, showing a significant percentage of patients diagnosed with ES in our initial cohort actually had ALPS.

Objective: We hypothesized a subset of patients diagnosed with ES may have ALPS and tested children with ES for ALPS.

Design/Methods: To confirm our initial findings, we tested children diagnosed with ES in a larger multi-institutional study. Patients were tested for ALPS by: (1) flow cytometric analysis for peripheral blood double negative T cells (DNTs) (CD3+, CD4−, CD8−, TCR●●+) which are elevated in patients with ALPS (normal value at our institution <2.6%), and (2) by the gold standard diagnostic test, defective in-vitro Fas-mediated apoptosis.

Results: We tested a total of 53 patients over a four year period from 22 different institutions. 36 of 53 (67%) had elevated DNTs and 25 of those 36 patients (69%) had defective fas mediated apoptosis. Thus, 25 of 53 (47%) children with a presumptive diagnosis of Evans syndrome in fact have a forme fruste of ALPS. No child with normal DNTs had defective apoptosis. Preliminary analysis from this cohort suggests a strong correlation between having markedly elevated DNTs (>5%) and having ALPS and having severe autoimmune disease (defined as needing immunosuppresive treatment on average at least 2 times/year) and having ALPS.

Conclusions: In summary, in the group of 53 ES patients screened to date, 47% had ALPS. Our data suggest that DNTs are a sensitive first line-screening test and serve as a marker that identifies patients who require definitive testing. We have documented a high prevalence of ALPS among ES patients, a finding of high clinical significance, and we would strongly recommend all patients with ES should be tested for ALPS.

Poster 114

Elevated Levels of Interleukin-5 and Monocyte Chemotactic Protein-1 are Predictors of Bacteremia in Pediatric Oncology Patients Presenting with Febrile Neutropenia

Victor M. Aquino, MD[1], Ana Gomez, MD, PhD[2], Daniel C. Bowers, MD[1], Octavio Ramilo, MD[3], Tamra Slone, MD[1], Tanja Hoffman, RN[4], Naveed Ahmad, MD[5], Naomi Winick, MD[1] and Patrick J. Leavey, MD[1]. [1]Pediatrics

and Pediatric Hematology Oncology, UT Southwestern Medical Center at Dallas, Dallas, TX; [2]Pathology/Laboratory Medicine, Children's Medical Center Dallas, Dallas, TX; [3]Pediatrics and Pediatric Infectious Disease, UT Southwestern Medical Center at Dallas, Dallas, TX; [4]Center for Cancer and Blood Disorders, Children's Medical Center Dallas, Dallas, TX; [5]Clinical Research, Children's Medical Center Dallas, Dallas, TX

Background: Febrile neutropenia is a common reason for hospitalization of children with cancer, however only approximately 15% have bacteremia. Development of laboratory parameters which could identify patients at lower risk of bacteremia could be used to shorten the length of or avoid hospitalization altogether.

Objective: To determine if a cytokine profile could be generated to identify patients at lower risk of bacteremia at the time of presentation with febrile neutropenia.

Design/Methods: We prospectively evaluated children with cancer who presented with an episode of febrile neutropenia. Patients had blood obtained for a complete blood count, blood culture, sedimentation rate, C-reactive protein, protein C level, and cytokine/chemokine profile. We utilized a multiplexed flow cytometric assay which measures 10 cytokines (IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-gamma, TNF-alpha, and GM-CSF) and 5 chemokines (macrophage inflammatory protein 1 alpha (MIP-1 alpha), monocyte chemotactic protein-1 (MCP-1), Eotaxin, monocyte chemotactic protein-1-beta (MIP1-b), and Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES).

Results: 58 patients were enrolled on the study. Four (7%) had bacteremia (2 Pseudomonas aeruginosa, 1 alpha-hemolytic streptococcus and 1 enterococcus). Patients with bacteremia had a higher temperature ($p = 0.02$) and a lower white blood cell count ($p = 0.04$) at presentation. Statistically higher levels of IL-5 ($p = 0.028$) or MCP-1 ($p = 0.019$) were seen in patients with bacteremia. An IL-5 level of greater than 8 picograms/deciliter was associated with a sensitivity of 67% and a specificity of 96% to predict bacteremia. An MCP-1 level of greater than 1650 picograms/deciliter was associated with a sensitivity of 80% and a specificity of 82% to predict bacteremia. Combining the two tests resulted in a sensitivity of 95.6% and a specificity of 66.7%. CBC, sedimentation rate, C-reactive protein, protein C levels and other cytokines/chemokines did not predict bacteremia.

Conclusions: Elevations of IL-5 and MCP-1 are predictive of bacteremia in patients with febrile neutropenia. Prospective studies should be undertaken to determine if these parameters can be used to select children for hospital discharge prior to complete recovery from neutropenia or outpatient management.

Poster 115

Sirolimus is a Safe and Effective Treatment for Patients with Autoimmune Lymphoproliferative Syndrome (ALPS)

David T. Teachey, MD[1], Dirk Schwabe, MD[2], Jack J. Bleesing, MD, PhD[3], Catherine S. Manno, MD[4], Kathleen Sullivan, MD, PhD[5] and Stephan A. Grupp, MD, PhD[4]. [1]Pediatric Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Pediatric Hematology and Oncology, University clinics Frankfurt, Frankfurt, Germany; [3]Hematology and Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [4]Pediatric Hematology and Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [5]Pediatric Immunology, Children's Hospital of Philadelphia, Philadelphia, PA

Background: ALPS is a disorder of abnormal lymphocyte survival caused by defective Fas-mediated apoptosis. Patients with ALPS develop lymphadenopathy, hepatosplenomegaly, and an increased number of an unusual T cell population, characterized as T cells that are CD3+ CD4− CD8− and referred to as double negative T cells (DNTs). Treatment options for patients are limited.

Objective: Sirolimus (rapamycin), an mTOR inhibitor, has been shown to induce apoptosis in B and T lymphocytes. Because ALPS is caused by defective lymphocyte apoptosis, we hypothesized that sirolimus would be effective by inducing apoptosis in these abnormal cells, controlling the lymphoproliferation that is the hallmark of the disease. We previously established this hypothesis using murine models of ALPS, and have opened a phase I/II clinical trial testing sirolimus in patients with ALPS.

Design/Methods: 3 children with ALPS (one type IA; two type III) were started on treatment with sirolimus, dosing agent for a target serum trough level of 5–15 ng/ml. Patients were treated for clinically significant autoimmune cytopenias that either failed standard therapies or in whom these therapies, including high dose corticosteroids, were not tolerated.

Results: All patients had complete resolution of autoimmune cytopenias and normalization of blood counts within 2 weeks of initiating therapy with sirolimus. Two patients had resolution of lymphadenopathy and splenomegaly (one complete; one partial with a greater than 90% reduction). Patients have been treated for 4, 18, and 24 months, respectively. One patient had co-morbid autoimmune arthritis and colitis which also responded to sirolimus. Two of the patients had previously failed treatment with mycophenolate mofetil and all three could not tolerate corticosteroids. One patient had failed treatment with rituximab, methotrexate, cyclosporine, tacrolimus, anti-TNFalpha agents, and cyclophosphamide. All three patients had a greater than 50% reduction in DNTs. Serial PET/CTs were performed on one patient that demonstrated a complete resolution of diffuse PET-avid disease after 3 months. No patient developed any significant toxicity.

Conclusions: We found sirolimus was capable of significantly reducing the lymphoproliferative state and improving autoimmunity in patients with ALPS who had failed other therapies. We will continue to test sirolimus in a larger clinical trial.

Poster 116

Admission Outcome of Pediatric Sickle Cell Disease Patients Based on Hospital Ward Type: The Children's Hospital at Montefiore Experience

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Background: Increased patient volume and disease-specific training have a direct correlation with improved patient outcome for certain illnesses. Data regarding outcomes for pediatric sickle cell disease (SCD) patients treated on a general pediatrics ward versus a dedicated pediatric hematology-oncology ward are lacking.

Objective: To compare the outcome of pediatric SCD patients admitted to a general pediatrics ward or a subspecialty ward within the same children's hospital.

Design/Methods: A retrospective review of all SCD admissions, length of stay (LOS), and transfer to the intensive care unit (ICU) between January 2005 and September 2007 was performed. Patients 1–20 years with a primary or secondary diagnosis of SCD were included. Care was supervised by the Pediatric Hematology-Oncology service. Housestaff for patients were from the same pediatric training program. Each inpatient ward was staffed with a distinct nursing core. Nurses on the subspecialty ward received disease-specific training. Comparison was made to evaluate a difference in patient outcome as determined by LOS and transfer rate to the ICU.

Results: There were 997 SCD admissions to the pediatric hematology-oncology ward and 135 to the general pediatrics ward. Patients admitted to the general ward were older than those on the subspecialty floor (mean age 16 years vs. 11 years; $p<.001$). There were 5 transfers to the ICU from the general floor versus 18 from the subspecialty floor. The rate of ICU transfer from the general ward was 3 times higher than the transfer rate from the subspecialty ward (age adjusted Relative Risk = 3.07). Average LOS for ICU transfers was 11.4 days on the general ward compared to 6.8 days on the subspecialty ward ($p<0.05$). When adjusted for age, there was a trend toward longer overall LOS for patients on the pediatric ward compared with the subspecialty ward (5.3 vs. 4.9; $p = .1$).

Conclusions: Patients with a primary or secondary diagnosis of SCD admitted to a general pediatrics ward compared to a hematology-oncology ward had a higher risk of ICU transfer and a trend toward increased LOS. The medical and nursing expertise of a hematology-oncology ward may improve outcomes of patients admitted with SCD.

Poster 117

Novel Long-term Stem Cell Culture (LTSCC) To Study Mesenchymal Stromal Support Of Hematopoiesis

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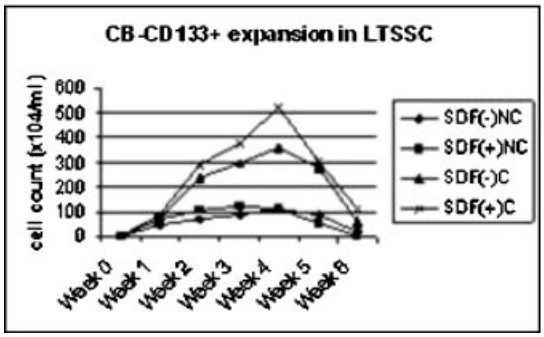
Background: Current long-term hematopoietic assays lack a uniform, clonal feeder layer. In Dexter Long Term Culture (DLTC), hematopoietic cell growth occurs in close association with an adherent bone marrow (BM) stromal microenvironment. The disadvantage of DLTC in analyzing stroma is the difficulty to determine which of the heterogeneous stromal cells actually sustains hematopoiesis.

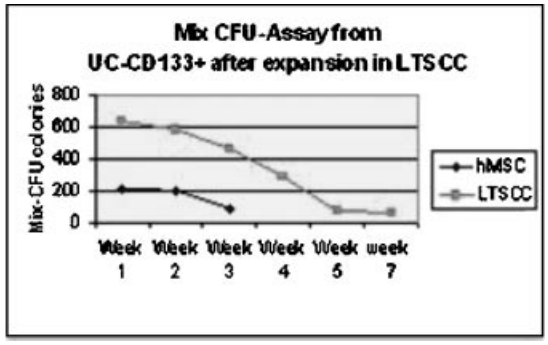
Objective: (1) Determine if a human mesenchymal stem cell line (hMSC) could be induced to undergo BM stroma-like changes that enables growth of cord blood CD133+ cells (CB-CD133+). (2) Analyze the effect of stromal derived factor (SDF-1) on survival and colony-forming capacity of immature CD133+ cells in LTSCC.

Design/Methods: A hMSC line (Cambrex) was induced to form stroma in the presence of SDF-1. CB-CD133+ cells were seeded on this feeder layer.

Results: LTSCC induced BM stroma-like changes in the hMSC feeder layer, with adipocyte formation, thought to be needed for formation of stem cell niches, and supported long-term (>9 weeks) survival of CB-CD133+ cells. Cobblestone areas of active CD133-derived hematopoiesis were seen in LTSCC for up to 9 weeks of culture, comparable to CD34-hematopoiesis in DLTC. SDF-1 acted as a survival factor for immature CD133+ cells and induced a significant ex vivo expansion, while maintaining the capacity for CFU-Mix and BFU-e colony formation up to 7 weeks. Spontaneous and SDF-1-induced adhesion and migration of CB-CD133+ cells were maintained and even increased during the first two weeks of LTSCC.

Conclusions: hMSC can be maintained in LTSCC, and this simplified feeder layer is able to provide niches for cobblestone-area-forming cells derived from CB-CD133+ cells. SDF-1 enhances survival and expansion of CB-CD133+ cells. LTSCC may represent a valuable new model to study stromal-hematopoietic cell interaction, as it should be possible to analyze the contribution of differentiated stromal elements from a clonally derived hMSC line to stem cell niche homing.





Poster 118

Pegfilgrastim-Supported Vadriac/ie Chemotherapy in Pediatric Sarcoma Patients: a Phase 2, Randomized, Open-Label Study

Sheri Spunt, MD[1], Helen Irving, MD[2], Jami Frost, MD[3], Leonard Sender, MD[4], Jeannette Green[5], Matthew Guo[5], Dennis Kim[5] and Victor Santana[1]. [1]Oncology, St Jude's Children's Research Hospital, Memphis, TN; [2]Banksia Unit, Royal Children's Hospital, Herston Road, Herston, Queensland, Australia; [3]Pediatric Oncology Program, University of New Mexico Health Science Center, Albuquerque, NM; [4]Children's Hospital of Orange County, Orange, CA; [5]Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA

Background: Children with sarcoma undergoing dose-intensive vincristine/doxorubicin/cyclophosphamide (VAdriaC)/ifosfamide/etoposide (IE) require G-CSF (granulocyte-colony-stimulating factor). Once-per-cycle pegfilgrastim in this setting is desirable.

Objective: To evaluate the absolute neutrophil (ANC) profile, pharmacokinetics, and safety of pegfilgrastim across age groups.

Design/Methods: Patients with sarcoma scheduled to receive VAdriaC (cycles 1&3) and IE (cycles 2&4) that were ≤21 years with ANC≥1×10^9/L were randomized 6:1 to receive single-dose pegfilgrastim 100 mcg/kg or filgrastim (5 mcg/kg/day until ANC≥10×10^9/L) 24 hours after completing chemotherapy. The primary objective was to characterize the ANC profile in cycle 1 (eg, days to post-nadir ANC recovery, incidence of ANC recovery by day 21). Other objectives included pharmacokinetics and safety evaluation.

Results: 43 patients received study drug (pegfilgrastim = 37, filgrastim = 6), 63% males, 13(30%) 0–5 years, 12(28%) 6–11 years, and 18(42%) 12–21 years. Cycle-1 endpoints are presented (table), though caution should be exercised regarding comparisons within age groups due to small sample sizes. The maximum pegfilgrastim concentration was achieved approximately 24-hours postdose and was sustained until ANC nadir occurred. As ANC recovered, pegfilgrastim concentration declined rapidly, consistent with neutrophil-mediated clearance. The pharmacokinetic profile was similar in the 2 older age groups (mean [SD] $AUC_{0-\infty}$ 24.4[11.3] mcg·hr/mL and 29.3[23.2] mcg·hr/mL, respectively); the youngest age group appeared to have higher exposure ($AUC_{0-\infty}$ 47.9[22.5] mcg·hr/mL), possibly because these patients experienced neutropenia that was more severe and of longer duration. Treatment-related adverse events, generally mild to moderate in severity, were reported in 22% of pegfilgrastim and 33% of filgrastim patients; the only treatment-related event reported by >1 patient was bone pain (filgrastim = 17%, pegfilgrastim = 11%).

Conclusions: The ANC profiles and pharmacokinetics observed in this study supported pegfilgrastim 100 mcg/kg use across pediatric age groups and were comparable with the experience of adults. Both pegfilgrastim and filgrastim were well tolerated, with a consistent safety profile observed across treatments and age groups.

	Filgrastim	Pegfilgrastim			
	All (n = 6)	All (n = 37)	0–5 (n = 12)	6–11 (n = 10)	12–21 (n = 15)
Median ANC recovery, days (range)	14 (12,16)	14 (10,31)	15 (12,31)	14 (12,16)	13 (10,15)
Patients without ANC recovery by day 21, n (%)	0	1 (3%)	1 (8%)	0	0
Median duration of severe neutropenia, days (range)	6.0 (0,9)	5.0 (0,24)	8.0 (4,24)	6.0 (4,8)	4.0 (0,7)

Poster 119

Shwachman-Diamond Syndrome Among Other Inherited Marrow Failure Syndromes: A Report from the Canadian Inherited Marrow Failure Registry

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Background: Shwachman-Diamond syndrome (SDS) is an inherited bone marrow failure disorder with a high risk of leukemia.

Objective: To better define the SDS clinical phenotype, we analyzed 29 cases of SDS registered on the Canadian Inherited Marrow Failure Registry (CIMFR) as of September 2007, and compared them to patients with the 4 other commonest IMFSs on the CIMFR: Diamond Blackfan anemia (DBA, 28 patients), Fanconi anemia (FA, 23 patients), Kostmann neutropenia (KN, 11 patients) and dyskeratosis congenita (DKC, 7 patients).

Design/Methods: The CIMFR is a prospective multicenter study to register all patients with IMFSs in Canada. We extracted data on SDS patients from the CIMFR database and compared it to other IMFSs.

Results: SDS was the most prevalent disease on the CIMFR with a male to female ratio of 12 to 15. The median age at diagnosis was 1.2 years compared to 4.6 years, 6.4 years, 1 year and 5 months in FA, DKC, DBA and KN, respectively. At diagnosis, 71% patients had neutropenia, 31% thrombocytopenia and 44% had anemia. Sixty percent had high MCV for age and 78% had high HbF. Initial bone marrow specimens showed hypocellularity in 62% and granulocytic hypoplasia in 42% patients. Sixty-seven percent patients had pancreatic dysfunction, 19% had metaphyseal dysostosis and 62% had short stature. It is noteworthy that two patients developed insulin-dependent diabetes mellitus and one patient had supernumerary thumb. At a median follow up of 4 years on the registry, 4% of SDS patients developed severe aplastic anemia, 22% clonal marrow cytogenetic abnormalities, 22% MDS and 7% leukemia. Thirty one percent needed treatment for cytopenias, compared to 91%, 100%, 100% and 82% of the patients with FA, DKA, KN and DBA respectively. On genetic analysis of the SBDS gene, compound heterozygosity was found in 76% of the patients. The following mutations were identified: 183-184TA>CT/258+2T>C, 258+2T>C/258+2T>C+183-184TA>CT, 258+2T>C/258+2T>C, 258+2T>C/260T>G.

Conclusions: SDS is one of the commonest IMFSs. Although the risk of MDS/leukemia is high, treatment for other hematological complications is less frequently required compared to FA, DKC, KN and DBA. Additional recruitment of patients and longer follow-ups are necessary to accurately assess the frequency of complications and risk factors.

Poster 120

Which Physician Specialty Provides Care to Children and Adult Sickle Cell Disease Patients Enrolled in Medicaid?

Teresa L. Kauf, PhD[1], Abraham G. Hartzema[1], Thomas D. Coates, MD[2] and Nikita Mody-Patel, PharmD[3]. [1]College of Pharmacy, University of Florida, Gainesville, FL; [2]Division of Hematology/Oncology, Children's Hospital Los Angeles and University of Southern California, Los Angeles, CA; [3]Health Economics and Outcomes Research, Novartis Pharmaceuticals, Inc., Florham Park, NJ

Background: Once considered a disease of childhood, median survival among sickle cell disease (SCD) patients exceeds 40 years (y). Transition from pediatric to adult care poses challenges for both patients and providers. Adult patients may face difficulties finding non-pediatric physicians familiar with management of SCD.

Objective: To characterize specialty of physicians providing care to pediatric and adult patients with SCD enrolled in Florida Medicaid.

Design/Methods: SCD patients were identified from administrative claims data between 1/2001–12/2005 using ICD-9-CM diagnosis codes (282.6, 282.60–.64, 282.68–.69, 282.41–.42). Patients with ≥1 inpatient and/or ≥2 outpatient claims ≥30 days apart were included. Individuals ≥65y, additional health insurance, concurrent diagnosis of acute leukemia or hemophilia, and/or <6 months total eligibility were excluded. Self-reported provider specialty from outpatient medical and pharmacy claims were classified as: family practice/general practice (FPGP), hematology (HEM), oncology (ONC), internal medicine (INT), pediatrics (PED), pediatric hematology/oncology (PHO), and other (OTH). Proportion of paid claims by provider type was calculated for pediatric (0–19y) and adult (20–64y) patients.

Results: 4,295 unique individuals with SCD generated 688,285 medical and 261,252 pharmacy claims. Pediatric patients received care from PED, PHO, and OTH (41.3%, 14.3%, and 35.1%, respectively); few claims were submitted by FPGP (6.8%) and INT (2.1%). Conversely, adult patients tended to receive care from INT (19.1%), FPGP (15.2%), ONC (7.9%), and OTH (53.0%). Patients appear to transition to adult care in their twenties, when 8.0% of claims were submitted by PED or PHO. However, a fraction of adult care is attributable to PED (1.2–2.7%). Pharmacy claims exhibited similar patterns.

Conclusions: Adult SCD patients appear to more frequently receive treatment from family practitioners rather than specialists. For survival and quality of life to improve,SCD patients must have access to providers with special expertise in SCD management. Increasing numbers of SCD patients will require medical care well into their adult years. Understanding the types of physicians providing care to SCD patients offers opportunities for targeted SCD management and education efforts, especially in the SCD adult population.

Supported by a grant from Novartis Pharmaceuticals, Inc.

Poster 121

An Assessment of the Long-Term Efficacy and Safety of Deferasirox (Exjade®, ICL670), A Once-Daily Oral Iron Chelator, in Pediatric Patients

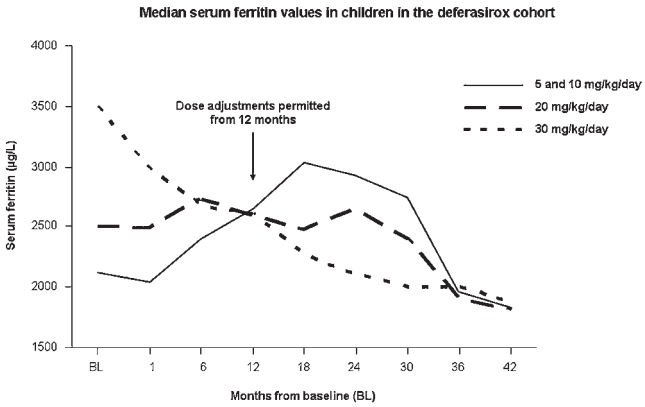
Elliott Vichinsky[1] and Carole Paley[2] on behalf of the 106, 107, 108, 109 Exjade investigators. [1]Children's Hospital and Research Center, Oakland, CA; [2]Novartis Pharmaceuticals Corp, East Hanover, NJ

Background: Children with anemias, such as β-thalassemia, sickle cell disease (SCD) and the myelodysplastic syndromes, often rely on transfusions as supportive care for the treatment of fatigue. Transfusion-dependent patients are usually treated with lifelong iron chelation therapy to prevent the clinical consequences of iron overload.

Objective: To establish the long-term efficacy and safety profile of deferasirox in children.

Design/Methods: A series of four 1-year deferasirox registration studies included pediatric patients (<16 years of age) with β-thalassemia, SCD or other transfusion-dependent anemias treated with deferasirox or deferoxamine (DFO; in two comparative studies). The 4-year extension phases are ongoing, where patients either continue to receive deferasirox (deferasirox cohort) or crossed over from DFO to deferasirox (crossover cohort), with dose adjustments based on efficacy and safety parameters. Efficacy was monitored via serum ferritin, and safety was assessed by the incidence and type of adverse events (AEs). Growth and sexual development were evaluated every 6 months.

Results: A total of 434 patients aged 2–<16 years (n = 289 deferasirox cohort; n = 145 crossover cohort) entered the extensions. Patients in the deferasirox cohort have now received treatment for a median 3.5 years. After dose escalations from month 12, median serum ferritin levels fell below baseline at month 42 in all dose groups (Figure). 390 (90%) children continue to receive deferasirox.



There were 43 discontinuations, 22 due to AEs. Two deaths, both considered unrelated to treatment, occurred in the deferasirox cohort. The most common drug-related AEs, including vomiting (n = 26), nausea (n = 25), abdominal pain (n = 21), diarrhea (n = 19) and mild/moderate skin rash (n = 35), occurred mainly in the first year. There were no significant changes in markers of liver function in the extension phases and no cases of progressive increases in serum creatinine. If intervention was needed, AEs were usually managed with standard treatments. Physical and sexual development proceeded normally in all children.

Conclusions: Over a median period of 3.5 years, treatment with deferasirox provided a dose-dependent overall reduction in serum ferritin in transfusion-dependent children. Deferasirox was generally well tolerated with a manageable safety profile similar to that observed in the 1-year core trials. There was no negative impact on growth and sexual development.

Supported by Novartis.

Poster 122

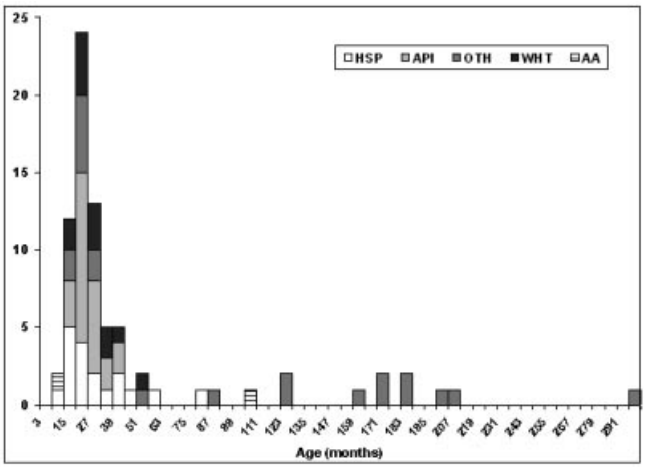
Severe Iron Deficiency Anemia in Early Childhood - Effect of Ethinicy

Lun Li, Jeanette Nichols, Alison Matsunaga and Ashutosh Lal, MD.
Hematology/Oncology, Children's Hospital & Research Center at Oakland, Oakland, CA

Background: Early childhood is a high risk period for anemia from dietary iron deficiency. Infants are screened anemia 9–12 months, but recommendation for follow up between 1–5 years for children at risk is infrequently followed.

Objective: To identify the population at risk of developing severe iron deficiency anemia.

Design/Methods: Retrospective analysis of 106 children with iron deficiency seen at our hospital over the past 10 years.



Results: We identified 78 children with severe anemia (hemoglobin ≤7 g/dL). The age distribution (figure) showed a sharp spike in the second year of life. Only 1% of the subjects presented at <1 year, 17% between 12–18 months, 31% between 18–24 months and 17% between 24–30 months. The number >3 years fell rapidly. The median hemoglobin was 5.0 g/dL with a range of 1.8 to 7.0 g/dL. Among 21 children with hemoglobin <4 g/dL, 20 (95%) were 12–36 months. Transfusions were given to 39/78 (50%) before oral iron therapy. Children of Asian background formed 31% of the group, followed by Hispanic (23%), and White (13%). Only 2/78 (2.6%) children were African-American, although our hospital cares for a large urban African-American population. In children <3 years, the median milk consumption was 32 oz. per day with range from 8–60 oz./day. No other significant contributor to development of iron deficiency was discovered in this population. We observed no trend in a decrease in the number of cases diagnosed per year over the last 10 years.

Conclusions: Our experience indicates that the migrant populations are at greatest risk, while African-American children are protected against severe iron deficiency in the second year. The difference in dietary practices of the different communities may provide clues that can be used for developing preventive strategies.

Poster 123

Oral Iron Chelation Therapy with Once-Daily Deferasirox (Exjade®, ICL670) is Effective and Generally Well Tolerated in Pediatric β-Thalassemia Patients with a High Iron Burden

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Background: Iron-overloaded, transfusion-dependent children with β-thalassemia have an increased incidence of morbidity and mortality if not treated with appropriate iron chelation therapy. Phase II/III studies have shown that once-daily oral deferasirox 20–30 mg/kg/day maintains/reduces iron burden depending on transfusional iron intake.

Objective: A subanalysis of the ESCALATOR trial examined the relationship between dose and efficacy after 1 year of deferasirox treatment in children with β-thalassemia and a high transfusional iron burden.

Design/Methods: The ESCALATOR trial was a prospective, open-label, 1-year study enrolling iron-overloaded patients with β-thalassemia aged ≥2–<16 years previously unsuccessfully treated with deferoxamine (DFO) and/or deferiprone (L1). Patients began treatment with deferasirox 20 mg/kg/day, except for three who started on 10 mg/kg/day, with doses adjusted in response to markers of over- or under-chelation.

Results: Of 167 children enrolled, 150 had received DFO, 1 L1, and 16 DFO+L1 in the year prior to the study. Mean (SD) baseline liver iron concentration (LIC) and serum ferritin levels were 17.1 (8.5) mg/g dw and 3938 (2262) µg/L, respectively. Median (range) deferasirox dose was 22.6 (12–30) mg/kg/day, with 130 children receiving dose adjustments to ≥25 mg/kg/day. The treatment success rate was 56.9% (95% CI 49.5, 64.4). The most common drug-related adverse events were mild/moderate skin rash (n = 16) and vomiting (n = 15). 46 patients with normal baseline creatinine values experienced increases >33% on ≥2 consecutive visits, with values exceeding the ULN in four patients. Baseline ALT values were high in 16 of 21 children who had ALT increases of >5-fold the ULN on ≥2 consecutive visits. Physical and sexual development progressed normally.

	Mean (SD) change from baseline to end of study
LIC, mg/g dw (n = 163)	−3.0 (6.1)
Baseline LIC •7 mg/g dw (n = 143)	−3.5 (6.2)
Baseline LIC <7 mg/g dw (n = 20)	+1.0 (3.9)
Serum ferritin, µg/L (n = 165)	−236 (1237)

Conclusions: These data suggest that with appropriate dosing, deferasirox can effectively control iron burden in heavily iron-overloaded children who were previously unsuccessfully chelated. Dose increases to ≥25 mg/kg/day were required to reach target reductions in iron burden, and were generally well tolerated. These data highlight the importance of timely dose adjustments to achieve therapeutic goals.
Supported by Novartis.

Poster 124

The Effect of L-Arginine Supplementation on Immune Parameters in Sickle Cell Disease in Steady State

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Background: The immune defect of sickle cell (SSD) is primarily due to functional asplenia but various cellular and humoral immune defects also exist. L-arginine (L-Arg) plays an important role in immunity. Depletion of L-Arg in SSD perhaps occurs due to poor nutrition, elevated arginase levels and nitric oxide scavenging and can cause T-cell receptor CD3zeta chain dysregulation.
Objective: Patients with SSD in steady-state (and age-matched controls) were studied after consenting via IRB guidelines for effect of L-Arg supplementation on immune defects. Steady state was defined as no vasoocclusive/febrile episodes, transfusional support or immunosuppression for 5–6 weeks.
Design/Methods: We examined T-cell subsets, CD3zeta chain expression, blastogenesis to PHA and antigen (PPD and candida) with and without L-Arg, as well as cytokine production and plasma L-Arg concentrations.
Results: There were 13 controls (mean, 11 years; 9F, 4M) and 15 patients (mean, 11.5 years; 7F, 8M). Total WBC in patients was $9.5\pm0.9\times10^3$ (vs. $5.6\pm0.5\times10^3$, controls), a significant difference. Absolute lymphocytes were significantly increased in patients. There were no significant differences in T-cell subsets. SSD patients had lower L-Arg levels but the difference approached but did not achieve significance. Blastogenesis to PHA was increased in SSD patients without L-Arg. The response to PHA was enhanced after addition of L-Arg for both groups although patients' response was significantly greater than that of controls (p*0.004). Patients did not respond well to candida and no improvement was seen with L-Arg. Interferon (IFN)gamma was lower in patients and expression insignificantly increased after L-Arg. IL-6 which was seen in higher levels in SSD patients minimally was altered after L-Arg. Increased CD3zeta chain upregulation occurred in the presence of PHA and L-Arg among controls but not for SSD.
Conclusions: L-Arg deficiency in steady state then does not appear to be clinically significant. Cytokine expression and cellular immune response to both antigen and mitogen is defective. L-Arg however did not correct these defects except in the case of blastogenesis to PHA. L-Arg deficiency in SSD is seen in vasoocclusion and studies need to be repeated in crisis states. The deficiency may also be a relative one, given the patient's increased needs.

Poster 125

Parameters of Physical Fitness in School Children with Sickle Cell Anemia

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Background: Poor growth and nutritional status in combination with delayed skeletal maturation are common clinical features of SCD. Metabolic studies have indicated elevated resting energy expenditure and elevated protein turn over associated with SCD. Cardiac performance in children with SCD, it has been reported in several studies that these children exhibit perturbed cardiac functions at rest and during exercise. This fact puts sport experts and physicians in a dilemma of either recommending exercise for these vulnerable patients and make them at risk for developing complications or simply advice them not to exercise.
Objective: The current studies aimed at determining parameters of physical fitness in school children with SCD.
Design/Methods: Male school children (150) comprising 3 Groups participated in the studies. Group 1 has 50 normal healthy controls, while Groups 2 and 3 each has 50 children who were suffering from SCT and SCD, respectively. Anthropometrics measurement and parameters of physical fitness were assessed in all subjects. All children were also subjected to a 5-min running exercise test on a flat motorized treadmill at speed corresponding to 5 km/hr. Throughout the test, heart rate was monitored and recoded during exercise and for 10-min during recovery. Blood lactate was measured before and 5 min following the completion of test.
Results: The mean values of lean body mass and height were lower in the SCD children ($P<0.05$) compared with the healthy subjects and SCT individuals. Children with SCD exhibited a higher mean value ($P<0.05$) for percent body fat and fat mass than the normal healthy subjects and SCT individuals. Although all groups tolerated well the treadmill exercise protocol, the SCD group exhibited higher ($P<0.05$) mean values of heart rate during exercise than those observed in the SCT and normal control children. In addition, SCD children showed higher serum lactate values before and after treadmill exercise compared to the other groups.
Conclusions: Children with SCD exhibit high level of adiposity; low level of fitness and their exercise performance appears to be physiologically more stressful as indicated by heart rate and lactate concentration responses.

Poster 126

Bordetella Holmesii Bacteremia in Children with Sickle Cell Disease: An Emerging Pathogen

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Background: Sickle cell disease (SCD) increases risk of invasive bacterial infection. Prophylactic penicillin and vaccination have decreased this risk and may have created selective pressure for other organisms to emerge. *Bordetella holmesii* is a recently described Gram-negative coccobacillus with a predilection for asplenic hosts.
Objective: To report the clinical and microbiologic features of *B. holmesii* bacteremia in two patients with SCD.
Design/Methods: We reviewed patients' medical records. The organism was isolated in the BacT/Alert 3D blood culture system. *B. holmesii* was identified through sequencing of its 16s rRNA gene because standard techniques failed.
Results: A 14 year-old girl with sickle-hemoglobin C disease presented to the emergency department (ED) with fever of 38.9°C and history of chills. She was non-toxic; hematologic parameters were at baseline. Clindamycin was given IV and she was discharged. After 56 hours of incubation, Gram-negative bacilli (GNB) were detected in her blood culture. Ceftriaxone was given IV and she was again discharged. The second blood culture grew GNB at 61 hours of incubation. She was admitted to the hospital with the interval appearance of right ankle cellulitis. She was treated with ceftriaxone IV and then oral cefixime with prompt resolution of symptoms and bacteremia. Two months later, a 10 year-old girl with sickle cell anemia presented to the ED with a temperature of 40.2°C. She was otherwise well; hematologic values were at baseline. She was given IV ceftriaxone, observed for 24 hours, and discharged. A blood culture grew GNB at 60 hours of incubation. She was re-hospitalized for treatment with IV ceftriaxone. No focus of infection was identified. The second blood culture grew GNB at 55 hours. She remained asymptomatic and was discharged after 36 hours on oral levofloxacin. A third blood culture one week later was sterile. All positive cultures were confirmed to be *B. holmesii*.
Conclusions: Prolonged incubation time in culture and an uncomplicated clinical course distinguish *B. holmesii* from other invasive bacterial infections in SCD. Optimal antibacterial therapy is not known; resolution without antibacterial therapy has been reported. Providers for patients with SCD should be aware of *B. holmesii* and ensure their microbiology laboratories can identify this new pathogen.

Poster 127

Comprehensive Profiling of Leukocytes and Circulating Endothelial Cells in Asymptomatic Scd Using Microfluidic Isolation

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Background: Patients with Sickle Cell Disease (SCD) have abnormally high baseline leukocyte counts which several epidemiological studies have implicated as a major risk factor in the development of disease related complications. Hydroxyurea and other anti-sickling agent shown to decrease incidence and severity of pain are commonly administered to patients with SCD.

Objective: The objective of this study was to comprehensively quantify leukocytes and other circulating nucleated cells and their activation status in healthy controls, mild phenotype SCD patients and patients on hydroxyurea to determine if: (a) abnormal leukocyte counts in mild phenotype SCD patients simply reflects an ongoing chronic inflammatory state and (b) the effect of hydroxyurea treatment on circulating leukocytes in minimizing occlusive crisis.

Design/Methods: To accomplish this, whole blood from healthy controls ($n = 6$), mild phenotype SCD ($n = 3$), and SCD patients on hydroxyurea ($n = 3$) were depleted of erythrocytes using a 10 second microfluidic lysis procedure demonstrated to obtain the highest yield of all nucleated cell populations with minimal artifactual activation as compared to conventional techniques. Circulating nucleated cells were phenotyped as follows: Lymphocytes (Th1, Th2, Treg, B and NK cells), Monocytes, Granulocytes and Endothelial cells (mature and progenitor). Expression of activation markers including integrins (CD11b, CD18, Cd49d), CD1a, HLA-DR, ICAM-1, VCAM-1, P-Selectin and CD36 on monocytes, granulocytes and circulating endothelial cells was quantified.

Results: Results indicate that mild phenotype SCD patients have ~ 2 fold increase in the number of leukocytes and other circulating nucleated cells (not phenotype specific). Escalation of circulating leukocytes intensifies the response to a stimulus, contributing to occlusive crisis and pain by obstruction of the blood vessel. Treatment with hydroxyurea reduces the leukocyte count and helps to minimize these episodes although levels still remain higher than baseline. Activation studies show that leukocytes and endothelial cells in both mild phenotype SCD and patients on hydroxyurea are not activated and comparable to the healthy controls.

Conclusions: This study therefore provides evidence that leukocytes in asymptomatic SCD patients are not in a chronic state of inflammation; rather high leukocyte counts represent a state, highly favorable for development of an inflammatory response in reaction to a given stimulus.

Poster 128

Hematological and Clinical Features of Hb Dhofar- A Thalassaemic Variant Unique to the Sultanate of Oman

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Background: Hb Dhofar is a doubly substituted haemoglobin ($b^{58\ ARG\text{-}PROL}$,$b^{29\ C\text{-}T}$) unique to the Sultanate of Oman.

Objective: To study the clinical & haematological data of Hb Dhofar in Oman.

Design/Methods: 54 subjects with Hb Dhofar, [37 heterozygotes, 14 homozygotes and 3 compound heterozygotes along with another b thalassaemia mutation] were studied.

Results: In heterozygotes, the level of Hb Dhofar ranged from 8.8%–21.5% (mean±SD; 13.45%±2.5). All heterozygotes had an Hb A_2 of >3.5% consistent with b thalassaemia trait. Hb Dhofar in homozygotes and compound heterozygotes ranged from 26%–59.7% (mean±SD; 41.79%±11.66) with a peripheral film consistent with homozygous beta thalassaemia. Age at presentation in homozygotes ranged between 6 months to 8 years, with a majority of them presenting before the age of 5 years. All had splenomegaly and 6(43%) had undergone splenectomy. All had some degree of frontal bossing and in particular, 2 patients with very infrequent transfusions had very marked thalassaemic facies and stunting of growth. Hb Dhofar can be mistaken for Hb D as the electrophoretic mobility is similar, but differs from it by the variable, and often reduced, quantity of variant Hb in heterozygotes and homozygotes. HPLC results show an erratic low Hb A_2 level due to overlap with Hb Dhofar.

Conclusions: Clinical and haematological data suggests that this mutation behaves like moderate b^+ thalassaemia allele resulting in a moderate to severe thalassemia intermedia phenotype.

Poster 129

Newborn Hemoglobinopathy Screening in a Region of Western New York State

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Background: The primary intent of newborn hemogobinopathy screening is to identify infants with sickle cell disease (SCD) so that preventive care can be initiated early. Testing methods also inadvertently identify infants who are clinically normal heterozygotes.

Objective: We reviewed a longstanding western New York State newborn hemoglobinopathy screening program. The primary aim was to assess local trends in the incidence of SCD and hemoglobin (Hb) S trait. Hemoglobinopathy clinic follow-up and cohort mortality rates were also evaluated.

Design/Methods: Information was extracted from a confidential database and specialty clinic/hospital data. Local birth statistics were obtained from on-line sources and Finger Lakes Health Services and Finger Lakes Regional Perinatal Center data.

Results: From 1980 through 2006, 213 infants were diagnosed with SCD. The incidence of Hb SS in live black births was 0.177% or 1 in 567. (Adjusted incidence: 0.170% or 1 in 587) For 29 of the 213 infants (13.6%) with SCD, there was at least one other sibling with the disease already in the family. 12% of newborns identified during 1990 through 2006 never enrolled in the specialty clinic or were lost to follow-up. Death before age 18 years was documented for 6 SCD cases (2.8%; mortality rate 0.23 per 100 patient years). Four (1.9%) deaths appeared attributable to SCD (mortality rate 0.16 per 100 patient years). From 1989 through 2006, 3,949 newborns were identified with Hb AS. The incidence in black births was 9.6% or 1 in 10.5. (Adjusted incidence 9.1% or 1 in 11) When early and later identified groups were compared, significant increases in the incidence of Hb AS and the S allele were found. ($p = 0.035$; $p = 0.039$; adjusted $p = 0.042$;0.05).

Conclusions: Local newborn screening activities appear to have had an impact on participation in specialized SCD care as well as the disease associated mortality rate. The incidence of SCD has remained unchanged over 27 years and that of the Hb S trait and the S allele may actually have risen over the last 17 years. Local immigration patterns and the perception of disease burden may have contributed. Trait notification and counseling goals should be reevaluated and current approaches improved.

Poster 130

The Immune Response to Pneumococcal Vaccine Among Patients with Sickle Cell Disease: A Retrospective Analysis

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Background: Infection remains the leading cause of death in children with Sickle Cell hemoglobinopathy (SSH). SSH patients have a pneumococcal sepsis risk of up to 400 times that of normal children despite the current use of anti-pneumococcal vaccine SSH patients however may have a defective response to antigens. We and others have previously described poor antibody response to hepatitis B vaccine in SSH patients, for instance, and anergy has been described in individuals with SSH.

Objective: The object of the study was then to identify the degree to which children with SSH have protective antibody titer levels against pneumococcus. This would allow us to identify those individuals at greater risk for pneumococcal disease who might benefit from re-immunization or alternative preventive strategies.

Design/Methods: Charts of 100 patients with SSH were reviewed to gather information on date of vaccination, age of vaccination, antibody titer, type

of vaccines, and number of infections, as well as genotype. Comparative data was collected for hepatitis and polio titers. Eligible patients included children 2–21 years of age with sickle cell disease. Among these patients, 48% had SS while 31% had SC Disease. The data collected was then analyzed using Microsoft Excel 2007.
Results: Data from 64% SSH patients were evaluable for pneumococcal titers. 76% of the SSH patients had evidence of having been immunized against Hepatitis B and of these, 64% had titers in the protective range. 57% of the SSH patients were also evaluated for anti-polio titers; of these, 94% had protective titers. On the other hand, all SSH patients had received the pneumococcal vaccine. 100% of patients with SC Disease but only 80% of patients with homozygous SSD had pneumococcal titers that were protective.
Conclusions: While all patients with Sickle Cell hemoglobinopathy had received pneumococcal vaccine, the degree of protection afforded by the vaccine was influenced by genotype. Patients with SC Disease all achieved immunity against pneumococcus but 20% of patients with homozygous disease lacked protection, probably reflecting their more profound immune defect. Careful monitoring of immune response to the vaccine is advisable since these individuals may have increased susceptibility to pneumococcus, while being presumed to be protected.

Poster 131
Outcome of Pediatric Aplastic Anemia in Honduras 1995–2006

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Background: In Honduras, one of the poorest countries in Central America, bone marrow transplantation is unavailable for treatment of pediatric aplastic anemia (AA). There is limited availability of immunosuppressive medications and blood products. Anti-thymocyte globulin (ATG) and cyclosporine are only recently provided by the Secretary of Health.
Objective: To evaluate characteristics, treatment, and outcome of childhood AA in Honduras.
Design/Methods: Data were retrospectively collected on patients aged 0–18 years with acquired AA at the main public hospital in Honduras, Hospital Escuela, Tegucigalpa, from January 1995–November 2006. Survival analyses were done using the Kaplan-Meier method and cumulative incidence for competing risks using the method of Gray.
Results: Eighty-six children (median age 10.4 years, range 0.2–17.6) were identified. Seventy-one patients (83%) had severe, while 14 (16%) had mild/moderate AA at diagnosis. Forty-nine children (57%) were male. A majority (57 patients, 66%) resided in rural areas. Eleven patients (13%) were exposed to pesticides or herbicides. Of 85 evaluable patients, 65 received immunosuppressive treatment, while 20 patients received supportive therapy only. ATG/cyclosporine was used in 13 patients (15%). Other immunosuppressive drugs included steroids, anabolic steroids, and azathioprine. Forty-one patients expired due to infection in 12 (29%), intra-cranial bleeding in 16 (39%), or other bleeding in 2 (5%) patients; the latter often due to shortage of platelet products. Twenty-six patients (31%) abandoned treatment (lost-to-follow up while on treatment and transfusion-dependent) so likely did not survive. Treatment failure defined as death or abandonment was 85% (standard error 4%). Treatment failure was associated with younger age ($p<0.004$), lower income ($p<0.02$), residence away from the two major cities ($p<0.04$), but less likely when receiving immunosuppressive drugs including ATG/cyclosporine ($p<0.001$). Abandonment, analyzed considering the competing risk of death, was associated with younger age ($p<0.003$), longer travel time to the hospital ($p<0.04$), but also less likely when receiving immunosuppressive drugs including ATG/cyclosporine ($p<0.001$).
Conclusions: Poor outcome, a treatment failure of 85%, for children with AA in Honduras is due to the lack of medications and blood products but also abandonment. Interventions to improve survival for this disorder must address both problems.

Poster 132
Pain Management for Patients with Sickle Cell Disease

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Background: In large urban areas, patients with Sickle Cell Disease (SCD) often use the emergency room as their first point of entry into the health care system. Boston Medical Center (BMC) is a large, tertiary care academic center where the Pediatric Emergency Department (PediED) has 30,000 visits/year. Approximately 200 of these are for SCD vaso-occlusive pain.
Objective: The purpose of this project was to examine the time to analgesia for children presenting to the PediED with SCD pain.
Design/Methods: A retrospective review of electronic medical records of children <22 years of age, presenting to the PediED with SCD related pain from January 1st to December 31st 2006 was performed. The variables analysed for this study were: 1) time from triage to narcotic order, 2) time from narcotic order to delivery of medicine, 3) time to subsequent pain assessments and analgesic dosing. Note was also made of the number of attempts to successful IV access.
Results: 185 encounters were identified. Of these 151 were selected for review based on chart availability and documentation. The mean time from triage to drug administration was 60 minutes (range: 17–139): from triage to physician order was 30 minutes (range: 4–110) and order to RN administration was 25 minutes (range: 2–89). 45% of patient charts did not record pain scores in the initial documentation. The average interval between pain assessments was 1 hour (1 minute to 3 hours.) 70% of charts provided documentation of IV starting. >50% were successful in the first attempt. <9% required more than three attempts.
Conclusions: A child presenting to BMC PediED with SCD related pain waited from 17 minutes to over two hours to receive a first analgesic dose. The wait time was evenly divided between the time for the order to be written and the time for the RN to administer the drug. IV access was not a significant reason for the delay. Charting was incomplete on pain assessment. As a consequence of these findings we are implementing a time-based algorithm for pain management, up-front oral analgesia for selected patients and PCA therapy in the ER.

Poster 133
Fanconi Anemia in a Pediatric Institution in Colombia

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Background: Fanconi Anemia (FA) is a rare autosomal recessive bone marrow failure syndrome with an increased predisposition to malignancy. Most affected children have abnormalities including skin, skeletal, cardiac, genitourinary, gastrointestinal and endocrine, with about 30% having none. FA is confirmed by increased frequency of chromosomal breakage to diepoxybutane and mitomycin C or by complementation studies.
Most individuals often die from hematological complications. The only curative treatment is Hematopoietic Stem Cell Transplant (HSCT).
Objective: To review clinical data of FA patients in a single institution in Colombia.
Design/Methods: A retrospective review was carried out of patients diagnosed with FA at one institution over 10 years. Clinical data, transfusions and treatment are described.
Results: This series included 39 children, including two pair of siblings, 15 (38%) female patients and 24 (62%) male patients with a median age of 9,4 years (range: 4–20) and a mean age at diagnosis of 6,7 years (range: 2–15).
Thirty-three (84,6%) patients had the characteristic FA phenotype. The abnormalities more frequently found were: short stature in 41%; café- au-lait spots in 41,2%; upper limbs abnormalities in 53,3%; patent ductus arteriosus, bivalve aorta in 10,2%, congenital hip dislocation in 10%, kidney abnormalities in 18%; imperforate anus in 2%; and hypospadias in 2%.

Hematological parameters were: mean hemoglobin: 6,3 mg/dl (range: 3,4–14,0), mean corpuscular volume: 94,4 fl (range:68–113), mean neutrophil count: 1293 (range: 23–4100) and mean platelet count: 77905 (range: 3000–441000). The mean bone marrow cellularity was 25% (range: 5–95%). Fetal hemoglobin, alfafetoprotein and TSH were elevated in 44%, 45% and 35% of the patients respectively.
Twenty-nine individuals (74%) had been transfused, 16 (41%) have iron overload and 10 (62%) are currently on chelation therapy.
Seven patients (18%) underwent SCT, one from a cord blood.
Thirty-four (87%) children are alive and 5 patients (12,7%) had died, 3 of them were transplanted.
One patient recently developed hepatocellular carcinoma.
Conclusions: FA is a phenotypically heterogeneous disease that have no been well defined in the latin population. It would be advantageous to do the complementation studies to our children in order to find the prevalent genetic mutations in Latin-America.

HEMOSTASIS (COAGULATION & PLATELETS)

Poster 134/PLATFORM SESSION 302

Phenotypic Correction and Long-Term Expression of Factor Viii in Hemophilia Mice by Nonviral Gene Transfer Using the Piggybac Transposon System

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Background: Gene therapy holds the promise for curing hemophilia. The clinical manifestations for hemophilia A are attributed to defects in the factor VIII (FVIII) gene, which in healthy individuals, small amounts of these proteins are sufficient to establish normal hemostasis. One approach to increase the integration frequency of nonviral vectors in animals and to prolong therapeutic gene expression is transposons. Transposons are mobile genetic elements that can be used to integrate transgenes into host cell genomes. Transposition occurs by a 'cut-and-paste' mechanism that requires a transposase that acts on short inverted repeat sequences flanking the transposable element. The piggyBac transposon, derived from insects, was recently observed to deliver large transposable elements efficiently.
Objective: We hypothesize that the piggyBac transposon system can be used to deliver human FVIII gene to a mouse model of hemophilia.
Design/Methods: We generated a human FVIII expression construct consisting of liver-specific promoter driving expression of a human FVIII cDNA that includes a partial B domain with 6 N-linked glycosylation sites described by Pipe et al (226/N6). The FVIII expression cassette is flanked by the piggyBac transposon 5′ and 3′ terminal repeats (PB-FVIII). We evaluated FVIII activity following gene transfer in a mouse model of hemophilia following systemic delivery.
Results: PB-FVIII was tested in vivo. Factor VIII null mice on the C57Bl/6 genetic background were administered 50 micrograms of PB-FVIII by hydrodynamic tail vein injection. FVIII activity was measured by Coamatic assay two weeks following plasmid administration. The results showed that the PB-FVIII construct achieved FVIII activity nearing 1.2 IU/ml, similar to that of wild type mice, verifying that the expression construct is functional.
Conclusions: This represents the first use of the piggBac transposon system to deliver a therapeutic transgene in an animal model. Ongoing experiments will contrast the levels and persistence of expression in FVIII null mice following co-delivery of the PB-FVIII with a wild type insect transposase, or a transposase codon-optimized for expression in mammalian cells. Experimental endpoints will include human FVIII levels, activity, and functional correction.

Poster 135

Evaluation of Thrombosis Risk and Prophylactic Anticoagulation-Related Bleeding in Pediatric Icu and Post-Surgical Patients with Central Venous Catheters (CVCs)

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Background: Thromboembolic events (TE) in healthy children are rare. Evidence-based practice guidelines for pediatric TE prophylaxis do not exist. Therefore, better understanding of clot prevalence and prophylactic anticoagulation practices for at-risk pediatric patients is required.
Objective: We compared the relative risk of clots in patients without prophylaxis, to the risk of significant bleeding in patients receiving prophylaxis, because this balance is (a) unknown in pediatrics, and (b) is perceived by many providers to be a key determinant in use of prophylaxis for clots.
Design/Methods: We evaluated 1637 consecutive admissions to two units of a tertiary care Children's Hospital: the multidisciplinary ICU, and a surgical floor. Eligibility criteria aimed to identify patients without known clot risk a priori: Inclusion criteria included patients ages 1 month-21 years, admitted >48h, immobile for >24h, with CVCs in place. Explicit exclusion criteria included known hypercoagulable states, concurrent anticoagulation/antiplatelet therapy, history of thrombosis or HIT, and patients requiring hemodialysis. Cardiology ICU and routine oncology admissions were excluded by the choice of geographic units. Data collection included a retrospective component (11/04–4/05) and prospective survailance (5/05–10/05). Eligible patients were followed to symptomatic clot diagosis or discharge. Patients receiving prophylaxis were monitored for bleeding until discharge. We used a days-at-risk approach to analyze data for clot events. The denominator for rates included days at risk not on anticoagulation.
Results: 206 of 1637 screened patients (13%) fulfilled the entry criteria. Of these, 174 did not receive prophylaxis (42 surgical unit, 132 ICU). 9/174 (5%) who were not prophylaxed had a TE event (1 surgical unit and 8 ICU), rate = 4.8 clots/1000 person-days (95% CI 2.2, 9). Of the 32 patients on prophylaxis 3 (9%) developed thrombus (rate 4.9/1000 person-days); 1 (3%) had a mild bleeding event and 28 (88%) had neither. Differences between groups did not achieve statistical significance. 11/12 symptomatic thromboses were CVC-related.
Conclusions: As in adults, the clot rate is substantial in sick, immobilized children with central catheters, even after excluding those with known prior risks. The bleeding risk is low with prophylaxis. These data can inform future controlled trials of pediatric anticoagulation strategies.

Poster 136

ITP in Children: What is the Best Treatment?

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Background: Historically, pediatric patients with acute ITP are given one of the following treatments: watchful waiting, anti-D (WinRho), corticosteroids, or IVIG. Utilizing a decision analytical model, O'Brien et al. suggested that corticosteroids has the optimal balance of clinical utility and cost effectiveness (Pediatr Blood Cancer 2007; 48:173–180).
Objective: The goal of our study was to document the initial outcomes of children with ITP treated at Children's Hospital of Pittsburgh to determine if this conclusion is upheld.
Design/Methods: The following data were collected from patients with new onset ITP between 2001 to 2006: 1) platelet levels at diagnosis and after treatment 2) treatment(s) given, if any, and doses, 3) time required for the platelet counts to reach 20,000/uL, 4) the length of stay in the hospital, 5) additional treatments necessary, and 6) complications or adverse effects related to treatment. The estimated costs of the first treatment for a newly diagnosed ITP patient were calculated, as done in O'Brien et al.
Results: Of 106 males and 86 females whose ages ranged from 2 months to 17 years, 102 received IVIG, 29 received corticosteroids (usually 4 mg/kg/d.), 57 received WinRho, and 4 received no treatment. The median time for IVIG or WinRho patients to reach platelets of 20,000/uL was 1 day. The median time for corticosteroid patients to reach platelet levels of 20,000/uL was 3 days. IVIG or WinRho patients spent a median 3 days in the hospital, whereas patients receiving corticosteroids spent a median 2 days. 44% of IVIG, 17% of corticosteroid, and 28% of WinRho patients experienced

side effects. The estimated costs of treatment for a 20 kg patient treated with prednisone, methylprednisolone, IVIG, or WinRho were $972.72, $1087.72, $3573.41, and $3071.81, respectively.
Conclusions: Compared to treatment with IVIG or WinRho, treatment of ITP with corticosteroids is associated with a slightly slower platelet response, a shorter hospital stay, less adverse effects, and a significantly lower cost. The estimated costs of treatment in this study were comparable to the estimates for each treatment option calculated by O'Brien et al. In conclusion, corticosteroids offer an effective, inexpensive treatment of acute ITP and O'Brien's conclusions are affirmed.

Poster 137
Phenotypic and Genetic Characterization of a Large Pedigree with Thrombocytopenia

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Background: Congential thrombocytopenias are a heterogenous group of disorders. Autosomal dominant, recessive and x-linked forms have been described. Autosomal dominant thrombocytopenias are frequently associated with large platelets, varying degrees of nephritis, hearing loss and cataracts (syndromes usually associated with mutations in the *MHY9* gene). Discovery of genes causing thrombocytopenia will advance the understanding of thrombopoiesis and platelet function.
Objective: We have identified a large pedigree of approximately 82 individuals in which several members exhibit autosomal dominant thrombocytopenia with normal platelet size. The bleeding pattern is mild, although affected individuals require platelet transfusion for surgical procedures or more severe bleeds. No other physical or functional abnormalities have been identified in affected individuals. The goal of this project is to phenotypically characterize the pedigree and to identify the gene responsible for this unique disorder.
Design/Methods: We have collected blood and saliva samples from 24 members of the pedigree for phenotypic and genetic analysis. Selected platelet studies have been performed to more closely delineate the platelet phenotype of the affected individuals. Platelet function via flow cytometry, characterization of platelet ultrastructure, and evaluation of thrombopoietin levels were performed. Extensive genotyping with the Affymetrix 1 million DNA Gene-chips has been completed in selected individuals. Linkage analyses are in progress.
Results: Affected individuals have thrombocytopenia since birth (29,000 to 92,000) but normal platelet ultrastructure by optic and electron microscopy. Megakaryocytes in the bone marrow show abnormal hypolobar forms. Thrombopoietin levels are within normal range in affected and unaffected individuals. Preliminary flow cytometry studies suggest normal platelet granule release but decreased $a_{2b}b_3$ activation in two affected members of the family demonstrated by decreased affinity for the monoclonal antibody PAC 1 after platelet activation.
Conclusions: We describe a novel large pedigree with autosomal dominant thrombocytopenia. Preliminary platelet function studies indicate potential abnormalities of activation of the fibrinogen receptor $a_{2b}b_3$. Ongoing linkage studies will hopefully lead to the identification of the gene responsible for this disorder.

Poster 138
Experience with Recombinant Activated Factor VII at a Large Children's Hospital

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Background: Recombinant activated factor VII (rFVIIa) has a limited set of FDA-approved indications for the treatment of bleeding. However, over the past decade, ''off-label'' use became commonplace at many institutions. Randomized-controlled trials of rFVIIa are rare – especially so in pediatrics, where case series predominate. Pediatric case reports highlight the efficacy and low-adverse event rate of rFVIIa, though a significant thrombosis rate has been seen in adult patients.
Objective: This study sought to analyze all rFVIIa use at a large children's hospital for usage patterns, drug efficacy, and adverse events.
Design/Methods: Recipients of rFVIIa were identified retrospectively via the hospital's electronic medical record (EMR), with the EMR start date at the study beginning. Using a standard evaluation form, data on patient diagnosis, lab data, other treatments, and outcomes were compiled.
Results: Over a 33 month period, 66 patient episodes were treated with a total of 616 doses. Of note, the administration of rFVIIa required approval by a hematologist prior to use. The most common indication was GI bleeding (36.4%). Only one episode involved an FDA-approved indication. The median number of doses was 2, with 39.4% of patients receiving only one dose and 66.7% receiving 5 or less. Nine of these episodes involved prophylaxis alone. When used for treatment, 36.8% of episodes were successful in controlling bleeding, 22.8% were partially successful, 33.3% were unsuccessful, and 7% did not have outcome data recorded in the EMR. The overall 7-day mortality for the cohort was 21.2%. Thromboses developed within 7 days in 13.6% of patients, though none appeared to be primarily responsible for a fatality.
Conclusions: rFVIIa has a very prevalent rate of off-label usage. In a majority of cases, it appears to be successful in stopping or slowing catastrophic bleeding. However, the thromboses rate of 13.6% is the highest rate reported in children so far. Further investigation is needed to determine the risk-benefit ratio of rFVIIa usage in these critically ill children.

Poster 139
Is the Initial Lymphocytic Count a Significant Risk Factor for the Development of Chronic Immune Thrombocytopenic Purpura? A Single Institute Report

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Background: Idiopathic thrombocytopenic purpura (ITP) in the acute form is a common, benign, self-limiting disease in children. Chronic ITP is diagnosed when ITP persists >6 months. Older age is associated with increased risk for development of chronic ITP.
Objective: To determine if the total WBC and the differential count are predictive of the course of ITP.
Design/Methods: In accordance with institutional IRB procedures, we conducted a retrospective chart review of patients diagnosed with ITP at Children's Hospital of Michigan. 225 patients were diagnosed from 4/1993 till 07/2007. We obtained data on the age, gender, ethnicity and CBC with differential count. SPSS (V.15.1) was used to analyze the data.
Results: Of the 225 patients diagnosed with ITP, 184 patients were eligible for analysis (acute ITP n = 142, chronic ITP n = 42). Males were 142/184 (77%) and females 42/184 (23%). Both genders were equally distributed between acute and chronic ITP patients.
101/184 (55%) of patients were Caucasian, 46/184 (25%) were African American and 36/184 (20%) belonged to other races.
Patients with chronic ITP had statistically significant lower WBC and lymphocytic count at diagnosis (Table 1).
Conclusions: Viral infection's are considered triggers for acute ITP. The lymphopenia observed in our review may offer clues to mechanism involved in pathogenesis of chronic ITP. Previous studies noted persistence of CD56+ CD3- NK cells in patients with ITP refractory to conventional therapy, and difference in cytotoxic T-cells subsets between normal individuals and chronic ITP patients, but have not evaluated them at presentation. The initial reduced lymphocytic count could be used as a prognostic factor for chronic ITP after further prospective studies are done to identify the underlying immunologic differences between both groups.

Table #1 White blood cell differential count and chronic ITP

	Acute vs Chronic	N	Mean	Std. Deviation	P value
Age (years)	Acute	142	5.2	4.2	<.001
	Chronic	42	8.4	4.6	
WBC at presentation	Acute	142	9.0	4.1	.001
	Chronic	42	6.8	3.0	
Absolute Lymphocyte at Diagnosis	Acute	124	4.5	3.2	.002
	Chronic	37	2.8	1.8	

Poster 140

Universal Thrombophilia Screening with risk Stratification and Anticoagulation Management in Pediatric Renal Transplant Recipients

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Background: According to the North American Pediatric Renal Transplant Cooperative Study data, vascular thrombosis is responsible for 12% of renal transplant failures. Congenital and acquired prothrombotic disorders have recently been found to play a major role in defining outcome of the renal transplants.

Objective: To identify renal transplant patients at a higher risk for allograft thrombosis and develop a risk stratification protocol for post-operative anticoagulation.

Design/Methods: All pediatric renal transplant recipients were screened prior to transplant for acquired and congenital thrombophilic risk factors. Patients were assigned a risk category (Level I–IV) based on the presence of thrombophilic risk factors. Their post-operative anticoagulation plan using unfractionated heparin and low molecular weight heparin (LMWH) was pre-determined based on their risk level (Table).

Risk Categorization and Management Plan

Risk Level	Thrombophilia	Management
Level I	Evidence of Thrombophilia (other than MTHFR) and previous thrombosis	Heparin postoperatively and then LMWH bid for 6 months
Level II	Evidence of Thrombophilia (other than MTHFR), No history of thrombosis	Heparin postoperatively, LMWH bid for 3 months, then qd for 3 months
Level III	MTHFR with normal homocysteine	Heparin postoperatively, then LMWH qd for 3 months
Level IV	No evidence of thrombophilia	No anticoagulation

Low-dose unfractionated heparin drip was initiated a few hours after the transplant and titrated to achieve a 1.5 fold aPTT level. Anticoagulation was changed to LMWH on day 2–4, when the risk of major bleed was over, and serum creatinine had normalized.

Results: Ten patients (age 2–17 years) have been screened so far and 8/10 were found to be at high risk for thrombosis (3 at Level II, 5 at Level III). The prothrombotic risk factors identified were Prothrombin gene mutation (2), MTHFR (8), Lupus anticoagulant (2) and low protein S (1). Six patients have undergone renal transplant and have been treated with LMWH post-operatively as per our plan, with successful outcome. No patient has developed allograft thrombosis. One patient developed a peri-renal hematoma requiring evacuation 7 days after the surgery, but completed prophylactic LMWH therapy.

Conclusions: Renal allograft thrombosis is preventable. Universal screening can stratify risk and allow an effective intervention to reduce that risk. Our Institution has developed a unique risk stratification protocol to identify patients at high risk for allograft thrombosis and prevent this complication by post-operative anticoagulation.

Poster 141

Severe Acquired von Willeb Rand Syndrome Complicating Management of Wilms Tumor

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Background: Current literature suggests that acquired von Willebrand syndrome (AVWS) is associated with Wilms tumor (WT) only infrequently and when it does occur, is of relatively little clinical significance. Treatment recommendations for prevention and management of bleeding symptoms are thus poorly defined, despite the important role that extensive surgery with nephrectomy occupies in current WT protocols.

Objective: We describe two patients with WT, AVWS, coagulopathy and clinically significant bleeding (both pre- and intra-operatively) who recently presented at our institution.

Design/Methods: Case review.

Results: Patient 1 (see table for further details) had epistaxis and prolonged bleeding from venipuncture sites as part of her presentation and experienced profuse bleeding during resection despite apparently adequate correction of her laboratory parameters. Patient 2 presented with hematuria and anemia and exhibited persistent laboratory evidence of AVWS despite vigorous factor replacement. Due to concerns about bleeding risks, plans for resection were deferred and patient 2 was started on neoadjuvant chemotherapy; however, after two cycles, the tumor progressed without improvement of her coagulation abnormalities. Immediately prior to surgery, to minimize bleeding complications, patient 2 received plasmapheresis (to treat elevated serum hyaluronic acid and whole blood viscosity) in addition to aggressive blood product and factor replacement. In spite of these interventions, she experienced moderately diffuse intra-operative bleeding that only improved after the renal vessels were ligated. In both cases, the coagulopathy and all bleeding symptoms resolved promptly after tumor removal.

Laboratory Values at Presentation and Tumor Characteristics

Patient	Age	aPTT	VW Antigen	VW Activity	Factor VIII	Stage and Histology
1	29 mos	49.6	28%	11%	13%	Stage 2, FH
2	7 mos	64.5	28%	12%	14%	Stage 2, FH

Conclusions: Our experience suggests that AVWS in the setting of WT is not always benign, as patients with AVWS and WT can present a surgical challenge in achieving adequate hemostasis. A careful bleeding history should always be undertaken in patients with suspected renal tumors and consideration given to pre-operative screening for AVWS, especially when the standard of care involves surgery as first-line treatment.

Poster 142

Prospective Study of Direct Thrombin Inhibition with Argatroban in Pediatric Patients Requiring Nonheparin Anticoagulation

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This study evaluated the safe, effective dose of intravenous argatroban for prophylaxis or treatment of thrombosis in patients 16 years requiring nonheparin anticoagulation. The argatroban dose for systemic anticoagulation was 1.0 g/kg/min (0.25 g/kg/min for patients with impaired hepatic function; 0.5 g/kg/min for patients <6 months old, reduced to 0.125 g/kg/min with impaired hepatic function), adjusted to achieve aPTTs 1.5–3 times the baseline value, or for procedures, an initial bolus then an infusion with doses adjusted to achieve ACT of 200–300 seconds. Primary efficacy endpoints were occurrence of thrombosis and thromboembolic complications. Primary safety endpoints were major bleeding and death secondary to bleeding complications. The study population comprised 18 patients (1.6 weeks to 16.1 years; 12 males) with confirmed (8), suspected (5), or risk of (2) heparin-induced thrombocytopenia (HIT), or heparin resistance or antithrombin deficiency (5). 17 patients received continuous argatroban, 6 underwent procedures, 5 received a bolus [median (range), 250 (11.2–250) g/kg], and 14 completed the study. The median (range) treatment duration was 2.9 (<0.1–13.8) days. The median (range) infusion dose was 1.5 (0.38–13) g/kg/min in 13 patients with normal hepatic function and 0.8 (0.4–0.9) g/kg/min in 4 patients with serum total bilirubin >2.0 mg/dL. Of 11 patients administered 1.0 g/kg/min initially, target aPTTs were achieved without

dose adjustment in 4 patients by 2 h and with no or little dose adjustment in 8 patients by 9 h and all patients by 26 h. Within the 30-day follow up, 5 patients had 6 thrombotic events (2 patients had an event during therapy). No patient required amputation or died due to thrombosis. In each patient with confirmed HIT, the platelet count recovered during treatment. Two critically ill patients had major bleeding (each fatal): 1 patient died 2 days after argatroban cessation due to cerebral infarction and intracranial hemorrhage, and 1 patient on ECMO for heart failure had fatal subarachnoid bleeding on study day 25. In this study of direct thrombin inhibition in both infants and children requiring nonheparin anticoagulation, argatroban was well tolerated and rapidly provided adequate levels of anticoagulation for noninterventional or interventional needs in seriously ill pediatric patients.

Poster 143

In Vitro Platelet Activation in Sickle Cell Disease Under Pathophysiological Shear Stress

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Background: Stroke is a devastating complication of sickle cell disease (SCD) that is associated with stenosis of the distal internal carotid and cerebral arteries. Such areas of stenosis create regions of high shear stress. Interaction of von Willebrand factor (VWF) with circulating platelets is the initial trigger for thrombosis in arterial stenosis and exposure of blood to high shear stress promotes such interaction.

Objective: To assess and compare *in vitro* platelet activation in patients with SCD to healthy African Americans under pathophysiological high shear stress.

Design/Methods: Blood was obtained in Phe-Pro-Arg-Chloromethylketone (PPACK) from SCD (HbSS/HbSβ^0) patients ages 7 to 17 years and healthy adult African American (AA) volunteers. Platelets were isolated by centrifugation and the δ-granules were loaded with radioactive serotonin. Platelets were then reconstituted with packed red blood cells to a target hematocrit of 40% and platelet count of 300 000/μL. The reconstituted whole blood was perfused at high shear stress through a length of polyetheretherketone (PEEK) tubing that includes an artificial stenosis, comprising a short section of narrow-bore (130 μm) tubing. Platelet activation was evaluated from secretion of [^{14}C]serotonin and erythrocyte lysis from release of hemoglobin by a colorimetric assay.

Results: Healthy AA showed modest platelet activation (average [^{14}C]serotonin release of 6.8%) under high shear stress. SCD patients had appreciably higher platelet activation (average [^{14}C]serotonin release of 13.6%) under the same conditions. There was minimal erythrocyte lysis in both healthy AA and patients with SCD. The signaling mechanism that underlies platelet activation in this model is now under investigation.

Conclusions: The data suggests that platelets in blood from SCD patients are more readily activated under high shear stress than platelets in normal blood. This may contribute to the increased risk of stroke in patients with SCD.

Poster 144

Anatomic Distribution of Childhood Deep Venous Thrombosis: An Interim Analysis of the HTRS TERegistry

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Background: In contrast to adults who predominantly present with lower extremity Deep Venous Thrombosis (DVT), childhood DVT has previously been reported to be more predominant in the upper extremities. This difference has been attributed to the greater utilization of central venous lines (CVL) in the upper extremity venous system of children in comparison to adults. Since 2005 the HTRS has maintained a registry (TERegistry) of patients with thromboembolic disease (TE).

Objective: The main objective of this interim analysis was to assess whether the anatomic distribution of childhood thrombosis in the TERegistry is similar to previously reported observations.

Design/Methods: The TERegistry is a multi-institutional, prospective registry for patients with TE including demographic, clinical, laboratory, treatment, adverse event, and outcome data. The TERegistry was queried for children <20 years old and the anatomic location of their DVT. The DVTs were then classified by anatomic region; some DVT episodes involved multiple regions.

Results: There were 49 objectively confirmed first episodes of DVT in 49 patients with a median age of 7.1 years (range: 0–20); 55% of whom were male. Fifteen (30.6%) of the patients had DVTs involving multiple anatomic regions; one of whom had a simultaneous arterial thrombus. The anatomic distribution of the DVTs were:

	Head/Neck	Thorax	Abdomen	RUE	LUE	RLE	LLE
n (%)	21 (42.8)	7 (14.2)	16 (32.6)	4 (8.1)	1 (2)	11 (22.4)	11 (22.4)

Conclusions: In contrast to previous observational studies our data suggests that lower extremity DVT in children is more common than upper, similar to adults. It is possible that this reflects a selection bias as pediatricians may be developing increased comfort with the management of CVL related thrombosis and therefore are less likely to report them. The predominance of head/neck and intra-abdominal DVT may be reflective of a selection bias to report more severe episodes. This information should be taken into consideration when drawing conclusions from analysis of this dataset.

This project is supported by an HTRS grant.

Poster 145

Management of Hemophilia and Mild Head Trauma: A Survey of Current Clinical Practice Among Aspho Physicians

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Background: Intracranial hemorrhage (ICH) in patients with hemophilia can occur following mild head trauma, yet there is a paucity of evidence regarding optimal management.

Objective: The goal of this survey was to assess self reported practices of pediatric hematologists/oncologists regarding the management of hemophilia and mild head trauma.

Design/Methods: A questionnaire was emailed to 1, 077 ASPHO members with generalized questions and two case scenarios. The case scenarios consisted of well appearing toddlers with severe hemophilia (not on prophylaxis), who had either fallen from a height (case#1), or from standing (case#2). Respondents were asked to select from six management options. The case scenarios were then altered to include bruising, prophylactic factor infusion 24 hours prior, trauma 48 hours prior, wearing a soft helmet, or emesis.

Results: The response rate was 37%. Of the 397 respondents 252 completed the survey. Eight-nine percent reported the routine use of CT to evaluate patients with hemophilia and head trauma, 41% had concerns regarding CT imaging. For the initial case scenarios CT imaging was selected by 69% (case#1) and 56% (case#2) of the respondents. The presence of a bruise or vomiting increased the use of CT imaging in the two case scenarios to 84–83% and 93–91%, respectively. Prophylactic factor 24 hours prior and wearing a soft helmet did not significantly decreased head CT use.

Conclusions: The current self reported care of pediatric patients with hemophilia and mild head trauma is diverse. More than half of the respondents selected CT imaging for mild head trauma without signs or symptoms of ICH. Physicians appear to be using bruising as a surrogate marker for head trauma severity, although our recent retrospective review demonstrated that bruising was neither sensitive nor specific for the presence of ICH[1]. Surprisingly, the use of prophylaxis did not result in a significant decrease in head CT use and was similar to soft helmet usage. This variation in clinical practice demonstrates the lack of evidence

regarding optimal management of mild head trauma in patients with hemophilia.

1. Witmer, CM, Raffini, L, Manno, C. Utility of Computed Tomography of the Head Following Head Trauma in Boys with Haemophilia. Haemophilia 13(5):560–566, 2007.

Poster 146

A Prospective Cohort Study Determining the Incidence of Complimentary Alternative Medicine Therapy in Children Receiving Anticoagulation

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Background: Complementary alternative medicine (CAM) is increasing in popularity in children. CAM consists of neutraceuticals, vitamins, dietary supplements, message therapy etc. There is little evidence regarding the safety and efficacy of CAM. CAM has clinical effect and implications. With each additional agent used interactions between ceutical agents increase exponentially and may result in harm to the patient. To reduce the potential for adverse events it is important that children and their parents report their use of CAM to their health practitioner to facilitate integrative medicine and reduce the potential for adverse effects. Increasing numbers of children with chronic conditions are requiring long term anticoagulation for treatment of thrombosis or for thromboprophylaxis of cardiac valves or shunts. When children receiving anticoagulation use CAMs, this may influence their level of anticoagulation resulting in thrombosis or hemorrhage. However, despite patient education in anticoagulated children, CAM use is highly under reported to the health team.

Objective: A pilot study to determine the incidence of CAM use in anticoagulated children and their families, their reasons for use and what types of CAM are used.

Design/Methods: The pediatric anticoagulation program at Stollery Children's Hospital follows all outpatient children requiring anticoagulation. A questionnaire was developed to empower families to disclose CAM use in a non-threatening, non-judgmental way to determine their use of CAM and their reasons for CAM use. The questionnaire was mailed to anticoagulated children and their families.

Results: Eighty-seven questionnaires were mailed, 67 (77%) responses were received. Twenty-five (37%) of anticoagulated children were taking neutraceuticals while 15 (22%) of their parents were taking neutraceuticals. The general perception of the children and their parents was that neutraceuticals may offer benefit without harm.

Conclusions: 37% of children requiring anticoagulation are taking neutraceuticals in addition to prescribed medication. Neutraceutical use may influence patient's levels of anticoagulation. It is important to question children and their families about CAM use in an open non-judgmental manner to facilitate partnership and education regarding the potential implication of specific CAM use in relation to anticoagulation. This will assist parents and their children to make informed decisions.

Poster 147

Enoxaparin Whole Milligram Dosing in Children

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Background: Enoxaparin is commonly used for thromboprophylaxis in clinically stable children. Enoxaparin dosing is based on patients' weight and results in decimal dosing. Due to the high concentration of Enoxaparin the resultant decimal dose makes precise measurement difficult. Dilution is necessary and often results in ten-fold medication administration errors. Enoxaparin may be administered in whole milligram doses via insulin syringe where one milligram of Enoxaparin equals one unit on the 100 unit graduated insulin syringe.

Objective: To determine if whole milligram Enoxaparin dosing via an insulin syringe could effectively achieve and maintain therapeutic anti factor Xa levels (0.5–1.0 U/ml) in infants and children with decreased dose measurement errors.

Design/Methods: A retrospective chart review of 514 children. Data was collected on underlying diagnosis, reason for anticoagulation, anti Xa levels, hemorrhagic events, and medication errors identified.

Results: All 514 patients were prescribed whole milligram Enoxaparin dosing and achieved therapeutic anti factor Xa within a mean time of 2 days. No infant or child required decimal doses to achieve therapeutic levels. Five children achieved an initial supra-therapeutic anti factor Xa level ($\geq$1.36 U/ml, requiring a single whole milligram dose decrease. There were no associated hemorrhagic events.

Conclusions: Whole milligram Enoxaparin dosing administered via an insulin syringe safely and effectively achieved therapeutic levels in infants and children in addition reduced incidence of Enoxaparin dosing errors, and injection pain and trauma suggests that whole milligram Enoxaparin dosing via an insulin syringe that we observed suggests that this method should be considered for standard of care.

Poster 148

Increased Prevalence of Thrombotic Events After Pediatric Liver Transplantation

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Background: Thrombotic events (TE) may contribute to morbidity after pediatric liver transplantation (LT).

Objective: To determine the prevalence of TE post-LT, the differences between patients (pts) with and without TE, and the safety and efficacy of anticoagulation (AC) in those pts.

Design/Methods: A retrospective chart review of all pts undergoing primary LT between Jan 02-Oct 07 was performed. The data included pts with TE within the first month post-LT confirmed by Doppler ultrasound, defined as abdominal (aTE) or non-abdominal (non-aTE), with sub-analyses by student T-test comparing differences between pts with and without TE.

Results: A total of 88 pts [median age 4.1 yrs (range 0.06–17.3)] underwent primary LT with deceased (65%) or live donor (LRD) (35%) allografts. A total of 15 (17.1%) pts developed at least one TE post-LT, with aTE occurring in 7 (8%) and non-aTE in 8 (9.1%) pts. Post-LT aTE included involvement of the hepatic artery (HAT) (3), portal vein (2), hepatic vein (3) and inferior vena cava (1). Only patients with HAT developed TE-related complications: death (1), and prolonged transaminitis (2). Nine symptomatic non-aTE occurred in 8 pts, including deep vein thrombosis (DVT) in 7 [with 6/7 (85.7%) central venous line (CVL) related] and superficial vein thrombosis in 2. The median time post-LT for both aTE and non-aTE detection was 5 days (range 1–31). Nine pts received AC with heparin followed by enoxaparin [median AC length: 92 days (range 11–105)], while 6 did not, including: death (2), bleeding (1), thrombocytopenia (1), and superficial TE (2). No major bleeding occurred. Amongst 15 pts with DVT, 11 had Doppler ultrasound for TE follow up within 6 months from its detection, including 54.4% with complete resolution, 18.2% with partial response, and 27.3% with no change in thrombus size. Neither age at LT, cold ischemic time, duration of LT surgery, CMV status nor early acute cellular rejection rate significantly differed among pts with or without TE ($p>0.05$).

Conclusions: The prevalence of early TE in a large population of pediatric LT recipients is 17.1%, with 9.1% prevalence of symptomatic non-aTE due to line-related events. Anticoagulation is safe in post-LT patients without a coagulopathy.

Poster 149

Survey of Physician Management of Children with Severe Chronic Refractory Immune Thrombocytopenic Purpura

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Background: Chronic immune thrombocytopenic purpura (ITP) is defined by a platelet count of $<150 \times 10^9$ beyond 6 months from diagnosis. Most children have a mild course with little bleeding and adequate response to treatment, but some have a severe refractory course with hemorrhage, impaired quality of life, and poor responses to therapy. Physician attitudes regarding management of children with severe chronic refractory ITP and the role of splenectomy have not been characterized.
Objective: We aimed to evaluate physician attitudes towards splenectomy in children with severe chronic refractory ITP.
Design/Methods: A 15-item questionnaire was sent in April 2007 to all 919 members of ASPHO using web-based software, 853 members had valid e-mail addresses and received the survey. The questionnaire presented a 5-year-old female with ITP for 1 year who was unresponsive to steroids, IVIG, and anti-D immune globulin and was having bleeding episodes that interfered with her daily activities.
Results: Two hundred and ninety-seven surveys (35% response rate) were returned and 295 were evaluable. Thirty-three percent of respondents stated that they would recommend splenectomy, while the remainder would try alternative therapy first. Performance of splenectomy was unrelated to years in practice ($p = 0.23$) or number of ITP patients seen per year ($p = 0.48$). Long-term risk of sepsis was the greatest concern reported by respondents. Of those who would not recommend splenectomy, 67% reported that they would treat with rituximab, 11% high dose steroids, 7% vincristine, and 15% other medications such as azathrioprine and cyclosporine. If such initial therapy failed, 47% would proceed with splenectomy. Those who reported a preference for rituximab initially were more likely to recommend splenectomy following failure than those who preferred other drug therapy ($p<0.0001$).
Conclusions: Only one third of respondents considered splenectomy to be first line treatment for this patient with severe refractory ITP. Of those who would not undertake splenectomy, 130 (67%) would recommend rituximab. With the advent of new approaches such as rituximab and oral thrombopoetic agents it is critically important to compare their cost, adverse effects and efficacy with splenectomy in order to optimally guide treatment practices.

Poster 150

The Safety and Efficacy of Anticoagulation Therapy in Children with Cancer: A Systematic Review of the Literature

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Background: Recent advances in anticancer therapy in children have resulted in increased cure rates. However, this cure comes with increased frequency of secondary complications, including thrombosis. Although widely studied in the adult population, evidence for the safety and efficacy of anticoagulation therapy in children with cancer is limited.
Objective: To evaluate the safety and efficacy of anticoagulation therapy in the treatment of thrombosis in children ($\leq$18-years of age) with cancer.
Design/Methods: Trials, cohorts, case reports and case series were identified through OVID Medline, EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews and PubMed. Studies published in the English language between 1976 and 2007 were included to evaluate progression over the past thirty years. Studies were included only if there was information regarding the details of cancer, anticoagulation therapy or outcome of thrombosis (e.g. resolution or recurrence) or complications of therapy (e.g. bleeding). When necessary, primary authors were contacted for additional information.
Results: Of 1073 citations, twenty studies were eligible for review. There were 4 case reports, 4 case series, and 12 cohort studies (11 retrospective and 1 prospective) describing a total of 86 patients. Thrombosis resolution was reported in 74/86 cases (86.0%), bleeding in 5 (5.8%) and recurrence in 3 (3.5%). Of 69 patients treated with mono-anticoagulant, thrombosis resolution was reported in 58 (84.1%) cases, bleeding in 3 cases (4.3%) and recurrence in 2 patients (2.9%). Resolution is reported in 16 of 17 patients treated with combination therapy (94.2%), bleeding in 2 (11.8%) and recurrence in 1 (5.9%).
Conclusions: The data on the safety and efficacy of anticoagulation therapy in pediatric cancer patients is very limited and is based mainly on retrospective observational studies. There is a suggestion that current anticoagulation therapy is safe and effective. However, multicentre collaborative studies are necessary to determine the safety and efficacy of anticoagulation therapy in pediatric oncology.

Poster 151

Age-Related Reference Values of Adamts-13, TFPI, Homocysteine And Natural Anticoagulants

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Background: Thromboembolic events are an increasingly common in children. The laboratory diagnosis of hemostatic disorders in children differs from that in adults.
Objective: To distinguish the differences in ADAMTS-13 (A disintegrin-like and metalloprotease with thrombospondin type 1 repeats), TFPI (Tissue factor pathway inhibitor), von Willebrand factor collagen binding assay (VWF:CBA), homocysteine and various inhibitors of hemostatic parameters between children and adults, and to establish the normal range of these parameters in children of different age groups.
Design/Methods: Plasma from healthy children aged 2 months to 16 years ($n = 127$) and adults ($n = 30$) were assayed for ADAMTS-13, VWF:CBA, TFPI, homocyteine and natural anticoagulants were measured. Children were categorized into 4 age groups: <1 yr, 1–5 yrs, 6–10 yrs, and 11–16 yrs.
Results: There were no significant difference in mean levels of VWF:CBA and free protein S between adults and children of all age groups. However, children <1 yr had significantly lower mean values of protein C activity ($p<0.001$), protein C antigen ($p<0.001$), total protein S ($p = 0.01$), and antithrombin ($p = 0.002$) than adult. Additionally, children $\leq$5 yrs had significantly lower level of ADAMTS-13 compared to adults ($p<0.03$). Children in all age groups had significantly lower level of plasma homocysteine and significantly higher level of TFPI compared to adults.
Conclusions: There are age-related physiologic difference in ADAMTS-13, TFPI, homocysteine and natural anticoagulants between children and adults. Our data serve as a useful reference guide in interpreting test results of inhibitors of hemostatic parameters in children suspected of thrombotic disorders.

Poster 152

Clot Resolution in Children $\leq$3 Months of Age Implies Active Fibrinolysis

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Background: The fibrinolytic system in infants has been described as ''down regulated'' compared to adults based on decreased levels of measured profibrinolytic components (tissue plasminogen activator, tPA, and plasminogen) and inhibitors (plasminogen activator inhibitor, PAI). (Andrew) Additionally, thrombin generation is decreased in infants which may disturb the balance between clot lysis and clot presence. However, the overall balance has not been determined in infants. Ultrasound can be used to monitor clot presence and assess the rate of clot thrombolysis in vivo.
Objective: To assess clot resolution in infants $\leq$3 months of age to assess rates of thrombolysis.
Design/Methods: A retrospective cohort of 58 infants $\leq$3 months of age who were objectively diagnosed with venous ($n = 38$) or arterial ($n = 20$) thrombosis were followed with ultrasound. Ultrasounds were performed at 2, 4, 6, 8, 12 and 16 weeks after the clot was diagnosed to determine the presence/size of the thrombus; 36% were occlusive, 19% were non-occlusive and 55% were confirmed thrombosis but percent occlusion was not described. The study was approved by the Health Research and Ethics Board of University of Alberta.
Results: The cohort consisted of $n = 36$ males, with the underlying illnesses being congenital heart disease (CHD) $n = 33$, general medical illnesses $n = 25$. Thrombus resolution can be divided into 3 general resolution periods for both venous and arterial thrombosis; 6 weeks (early), 12 weeks

(medium) and 16 weeks (late) accounting for approximately 50%, 25% and 25% of infants, respectively. Approximately 50% of the patients' thrombi were resolved by 6 weeks.

Conclusions: Infants ≤3 months of age with either venous or arterial thrombosis appear to have clot resolution more quickly than older children or adults. The balance between clot presence and lysis in infants with thrombosis may play an important role in whether a thrombus is degraded and should be defined in proper studies. The results of these studies may allow designer anticoagulants in this population.

Poster 153

Microparticles as a Predictor of Acute Deep Venous Thrombosis in a Patient with Antiphospholipid Antibody Syndrome

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Background: Microparticles (MP) are prothrombotic cell membrane fragments released during apoptosis and/or cellular activation. Elevated levels of microparticles may circulate in inflammatory and thrombophilic disorders.

Objective: To define the relationship between elevation of plasma microparticles and development of acute thrombosis.

Design/Methods: The study subject was a Hispanic male with Antiphospholipid Antibody Syndrome (APS) (DRVVT ratio 2.6, ACA IgG >120, β_2GPI IgG>100, IgA 74) complicated by several DVTs. He was maintained on fondaparinux due to thrombosis on warfarin and enoxaparin. Microparticle analysis was performed on three occasions. The first was during routine follow-up when the patient was asymptomatic. The second was when the patient presented with left lower extremity tightness. Physical exam was normal, and ultrasound of the left lower extremity was negative. However, two days later, the patient was diagnosed with acute DVT by venous ultrasound and was treated with unfractionated heparin followed by reinstitution of fondaparinux. The third analysis was two weeks after the diagnosis of DVT. Microparticles within platelet free plasma (PFP) were labeled using monoclonal antibodies against CD 144 and CD 105 (endothelial VE cadherin and endoglin) and CD 41 (platelet integrin αIIb) and analyzed by flow cytometry the same day as collection.

Results: Results expressed as number of MP/ml of PFP are listed below.

Antibody	2 months prior to DVT	2 days prior to DVT	2 weeks after DVT	Normal Range
CD 144	3650	97704	16128	14815±17714 n = 13
CD 105	n/a	352656	24624	5890±8754 n = 5
CD 41	2550	42624	18864	1607±2627 n = 20

Conclusions: These results demonstrate normal levels of circulating MPs in this APS patient while asymptomatic but dramatically increased levels of both endothelial cell and platelet derived MPs preceding the clinical diagnosis of DVT. While previous reports have documented elevated levels of MP during established thrombosis, we believe that this is the first serial analysis of microparticles in an APS patient during an asymptomatic period, incipient thrombosis, and after treatment of acute DVT. MPs may prove to be a sensitive marker of incipient DVT in patients with negative vascular ultrasound studies.

Poster 154

Prevalence of Injuries and High Risk Behaviors Among Children and Adolescents with Hemophilia

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Background: Unintentional injuries are the leading cause of death and disability among children and adolescents. They result in a significant number of hospitalizations and emergency room visits annually, and are a bigger concern in children with bleeding disorders.

Objective: This study examined prevalence rates of unintentional injuries and high risk behaviors among children with bleeding disorders.

Design/Methods: Participants included 43 children (ages 6–18 years) seen in the Hemophilia clinic. 93% were males, 86% had been diagnosed with Hemophilia A, 7% with Hemophilia B and 4.5% with von Willebrand's Disease; 54% had moderate or severe hemophilia.

Results: Parents (18%) reported concerns about their child's education and ≥6 days of missed school per year as a result of hemophilia-related complications. Parents reported their children were engaging in high-risk recreational activities on a regular basis such as jumping on a trampoline (32%), riding an all-terrain vehicle (16%; 18% without a helmet), and riding a bicycle or skateboard (36%; 27% without a helmet). 14% admitted to riding in motor vehicles without safety restraints. Regarding injury prevalence, 27% of parents reported that their child had been injured as a result of involvement in sports and other recreational activities (i.e., soccer, baseball, horseback riding). In addition, parents reported that their children had at least monthly injuries requiring application of ice (36%) and administration of factor (41%), while 39% reported daily injuries that resulted in bruising. In the past year, 27% of parents reported that their children sustained injuries requiring visits to their doctor and 32% injuries required emergency medical attention. Lifetime prevalence of injuries was also high, with 41% reporting broken bones, 39% lacerations, 36% sprains/dislocations, and 25% head injuries, concussions, or bleeds.

Conclusions: This study indicate that many children who are already at an increased risk for injuries, regularly engage in high risk behaviors. There is a need to address these high risk behaviors of children and adolescents with bleeding disorders and the need for further research in this area, including the development and implementation of interventions to promote safety in these patients.

Poster 155

Cerebral Venous Thrombosis in a Pediatric Patient with Active Ulcerative Colitis and Factor V Leiden Mutation: Case Report and Review of Literature

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Background: Patients with ulcerative colitis are at increased risk of thromboembolic events. Cerebral sinus thrombosis, however, is rare in ulcerative colitis.

Objective: Here we present a case of cerebral venous thrombosis in a patient with ulcerative colitis and factor V Leiden mutation.

Design/Methods: A 10 year old male with known ulcerative colitis and no known family history of thrombosis presented with persistent headache and emesis for ten days. Physical examination, including fundoscopic and thorough neurologic exam, was unremarkable. Magnetic resonance imaging (MRI) and venography (MRV) of the brain were obtained.

Results: Imaging showed left transverse and sigmoid sinus thrombosis as well as occlusion of the vein of Galen and straight sinus. The patient was found to be heterozygous for the factor V Leiden mutation (R506Q). Additional evaluation, which included complete blood count, liver panel, prothrombin and partial thromboplastin times, antiphospholid and anticardiolipin serology, antithrombin III and protein C and S activity, and MTHFR mutation analysis, was remarkable only for homozygous A1298C MTHFR mutation (a clinically non-significant mutation), and a mild transient dip in Protein S activity. Patient's symptoms resolved after commencement of enoxaparin 1 mg/kg/dose subcutaneously every 12 hours. Enoxaparin was continued for six months, and given thereafter during colitis exacerbations. He remained asymptomatic after one year, without apparent neurologic sequelae. Repeat MRI and MRV showed no progression of cerebral venous thrombosis.

Conclusions: This is the first reported case of cerebral venous thrombosis in a patient with ulcerative colitis and Factor V Leiden heterozygosity. The role of prophylactic anticoagulation in patients with inflammatory bowel disease and factor V Leiden mutation merits further investigation.

LEUKEMIA/LYMPHOMA/HISTIOCYTE DISORDERS

Poster 156/PLENARY SESSION 300

Improvement in the Infection-Related Mortality for Children with Down Syndrome (DS) in Contemporary Children's Oncology Group (COG) Acute Lymphoblastic Leukemia (ALL) Clinical Trials

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Background: Children with DS-ALL have an increased risk of treatment-related mortality. Recently, the COG suspended accrual of DS-ALL children to trials for standard-risk (SR; AALL0331) and high-risk (HR; AALL0232) ALL because of excess mortality. Treatment modifications were made for DS-ALL patients and the trials were re-opened to DS patients in 10/2006.

Objective: To evaluate changes in treatment-related mortality of DS-ALL pts following institution of treatment modifications.

Design/Methods: AALL0232 uses an augmented BFM backbone regimen with randomized comparisons of 28 days of prednisone (PDN) vs. 14 days of dexamethasone (DXM) during induction (IND), and high dose methotrexate (MTX) vs. escalating dose MTX (Capizzi) during the interim maintenance (IM) phase. AALL0331 utilizes a 3-drug DXM-based induction with a 2×2 post-IND randomization between intensified vs. standard consolidation, and Capizzi MTX combined with augmented delayed intensification (DI) vs. standard IM/DI. Therapy changes for DS-ALL pts on AALL0331/AALL0232 included addition of leucovorin rescue 48 hours after intrathecal MTX until the end of DI, use of a single DI phase with discontinuous DXM (days 1–7, 15–21 vs. 1–21), and expanded supportive care guidelines. In addition, DS-ALL pts enrolled in ALL0232 are now nonrandomly assigned to PDN IND.

Results: During the first 22 months of AALL0232, before the amendment, 4/20 DS-ALL pts died, 3/4 deaths occurred in pts receiving DXM IND (IND mortality rate 15% vs. 4.3% in the CCG 1961 HR ALL trial). On ALL0331, 6/26 DS-ALL pts died during the first 10 months of accrual. Deaths occurred in IND (3), intensified consolidation (1) and standard DI (2). Since the re-opening of trials to DS pts in fall 2006, no deaths have occurred among 10 DS-ALL pts enrolled on AALL0232 and 42 DS-ALL pts on AALL0331; (39 have completed induction). The induction death rate combining both studies was 13.04% (4/46) prior to the amendment vs. 0% subsequently (p-value 0.0089).

Conclusions: Additional follow-up is needed to assess the full impact of these treatment modifications on toxic death among pts with DS-ALL. If this dramatic decline in death is maintained, these modifications should be routinely incorporated into the treatment of children with DS-ALL.

Poster 157/PLENARY SESSION 300

Langerhans Cells in LCH Lesions Over-Express Genes that Regulate Lymphocyte Trafficking

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Background: Langerhans Cell Histiocytosis (LCH) is thought to be caused by pathological proliferation of the Langerhans cell (LC), a dendritic cell normally restricted to the skin. LCH lesions can arise in virtually any organ system, and uncontrolled multi-system LCH is usually fatal.

Objective: In this study, we isolated CD207+ LCs and CD3+ T cells from LCH lesions in order to determine cell-specific gene expression profiles which may elucidate the etiology of LCH.

Design/Methods: Biopsy samples from freshly-excised LCH lesions from 14 patients were processed, and CD207+ and CD3+ cells were isolated by FACS. Matched CD3+ cells were also isolated from peripheral blood from 7 of the 14 biopsy donors. RNA was purified from the cells, quality was verified by the Agilent 6000 Pico LabChip, then cDNA was generated, amplified, fragmented and biotinylated. cDNA probe was also generated in parallel from control CD207+ cells isolated from 30 normal human foreskin samples and from control CD3+ cells isolated from 20 normal human tonsil samples. The experimental and control cDNA probes were then hybridized to Affymetrix U113 Plus 2.0 Array chips which contain targets for over 47,000 transcripts. Chips were scanned with the GeneChip Scanner 3000. The tonsil pool CD3+ cDNA as well as peripheral blood cDNA were used as the reference for the LCH CD3+ samples. The skin pool CD207+ cDNA was used as reference for the LCH CD207+ samples. Spot intensity was normalized to housekeeping genes, and data were analyzed with BRB Array Tools 3.5.

Results: Gene expression among the CD207+ cells was much less variable than the tumor CD3+ cells. Functional analysis showed that the CD207+ cells significantly over-expressed genes involved in lymphocyte migration. Functional analysis of the tumor CD3+ cells compared to peripheral CD3+ cells showed upregulation of genes involved in T-cell immune activation.

Conclusions: This is the first comprehensive study of cell-specific gene expression in LCH tumors. These results prompt us to propose a novel hypothesis of LCH pathogenesis where tumors arise from lymphocyte accumulation rather than clonal proliferation.

Poster 158/PLATFORM SESSION 303

Dexrazoxane (DZR, Zinecard™) Can Be Added Safely to Intensive Multi-Agent Chemotherapy in Patients with T-ALL or L-NHL

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Background: Early clinical studies demonstrated the protective effects of DZR against anthracycline-induced cardiomyopathy, but were limited in their applicability to children. While relatively few side effects have been ascribed to DZR, additive effects with the toxicity of other chemotherapeutic agents remain an important safety concern. Two COG Hodgkin disease studies suggested that DZR was associated with an increased incidence of second malignancies.

Objective: A primary goal of POG 9404, was to determine whether DZR prevented cardiotoxicity when added to a multi-agent, anthracycline-based chemotherapy regimen. A secondary aim was to determine if DZR affected event free survival (EFS), or was associated with an increase in serious adverse events.

Design/Methods: Using a 2×2 factorial design, patients were randomized at diagnosis to treatment with or without DZR and with or without high dose methotrexate (HDM). DZR was given by bolus infusion immediately prior to every dose of doxorubicin at a ratio of 10:1. Between June 1996 and September 2001, 264 and 273 eligible patients with newly diagnosed T-ALL or L-NHL were randomized to No-DZR and DZR, respectively.

Results: Three year EFS was similar for the DZR ($78.7 \pm 2.5\%$) vs. the No-DZR group ($77.2 \pm 2.6\%$, $p = 0.90$). No difference in the 3-year EFS was seen between arms when analyzed separately for the T-ALL ($76.3 \pm 3.1\%$ vs. $76.0 \pm 3.3\%$, $p = 0.91$) and L-NHL patients ($83.7 \pm 4.1\%$

vs. 79.6±4.4%, p = 0.63). The frequencies of severe grade 3 or 4 hematologic toxicity, infection, CNS events and mucositis were similar in patients treated with or without DZR (p<0.24). Acute cardiac toxicity occurred in 1 patient (arrhythmia) on DZR and 4 patients with No-DZR (1 arrhythmia, 4 decreased fractional shortening by echocardiogram). Five toxic deaths occurred with DZR/HDM compared to 2 deaths on the standard, and 1 each on the standard+DZR and standard+HDM regimens. There were 9 second malignancies, 6 had received DZR and 3 No-DZR (p<0.33).

Conclusions: Addition of DZR did not interfere with the anti-tumor efficacy of this doxorubicin-containing, multi-agent regimen, and there was no significant increase in toxicities or second malignancies.

Poster 159/PLATFORM SESSION 303

Outcomes with 4 hr vs 24 hr IV Methotrexate (MTX) Infusions During Intensification Therapy for Childhood ALL: POG 9904/9905: A Childrens Oncology Group Study (COG)

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Background: Post-induction intensification with anti-metabolite based blocks of therapy that include IV MTX have enhanced outcomes for children with ALL. However the relative value of higher peak levels of MTX provided by higher dose shorter infusions vs. prolonged exposure with longer infusions has been debated.

Objective: COG 9904 and 9905 tested the importance of MTX schedule and "concentration × time" by randomizing 1861 pts to intermediate dose (ID) IV MTX delivered as 1 gm/m^2 infused over 24 hrs vs 2 gm/m^2 infused over 4 hrs. The trial had 80% power to detect a difference of 87.5% vs. 82.5% 4-year event free survival (EFS).

Design/Methods: Induction was a dexamethasone-based 3-drug (NCI SR) or prednisone-based 4-drug (NCI HR) regimen. Post-induction, NCI SR pts with trisomies of chromosomes 4 and 10 or a TEL/AML1 translocation were classified as lower risk and enrolled on 9904. NCI SR pts without and NCI HR pts with favorable blast cytogenetics, or pts who did not meet refined NCI HR age and WBC criteria were eligible for 9905. Pts with CNS3 disease, t(9; 22) or t(4;11) were excluded from both studies. Intensification on 9904/9905 included 6 courses of ID MTX (randomized as above) with identical leucovorin rescue. A second randomized question examined the impact of a delayed intensification phase at wk 16 after the 3rd course of IV MTX.

Results: There were no significant differences in EFS between the 4hr and 24hr infusion cohorts (4 year EFS 85.1+/−1.5% vs. 85.7%+/−1.4%, p = 0.814). Reported toxicities grade ≥3, including infection and elevation in ALT, were not significantly different between arms. During intensification the incidence of infection on the 4 hr vs 24hr infusion arms was 31% vs 32% and incidence of elevated ALT was 29% vs 28%.

Conclusions: The 4 hr ID MTX infusion schedule did not improve EFS as compared to the 'standard' 24hr infusion schedule and the toxicity profiles of the regimens are nearly identical. Because the 4 hr infusion can be given as an outpatient, consideration should be given to using this administration schedule in future trials that incorporate ID MTX.

Poster 160/PLATFORM SESSION 303

A Study of Rituximab and Ifosfamide, Carboplatin, Etoposide (ICE) Chemotherapy in Children with Recurrent/Refractory B-Cell Non-Hodgkin Lymphoma (NHL) and Mature B-Cell Acute Lymphoblastic Leukemia (B-ALL). A Children's Oncology Group Report

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Background: Patients with relapsed/refractory B-cell NHL and B-ALL represent a significant challenge for retrieval.

Objective: To estimate the response rate and therapy related toxicities of rituximab combined with ICE chemotherapy in patients with relapsed and refractory B-cell NHL and mature B-ALL.

Design/Methods: Patients received rituximab and ICE for 1 to 3 cycles, depending upon response. Rituximab (375 mg/sqm) was given on day 1 and 3 of each cycle (day 1 only for cycle 3), with ifosfamide (3000 mg/sqm) and etoposide (100 mg/sqm) given on days 3, 4, and 5 and carboplatin (635 mg/sqm) given on day 3 only. G-CSF support was given after each cycle.

Results: A total of 41 treatment courses were administered to the 20 eligible patients. Only one patient was removed from study due to prolonged myelosuppression. Grade 2–5 allergic reactions (fever, rash, chills, hypotension) associated with rituximab infusion were reported in 6 of the 41 treatment courses; all continued with therapy. Of the 6 eligible patients with diffuse large B-cell lymphoma, 3 achieved complete remission (CR), 1 had stable disease (SD), and 2 had progressive disease (PD). Of the 14 eligible patients with Burkitt lymphoma and B-ALL, there were 4 complete responses (CR), 5 partial responses (PR), 1 SD and 4 with PD. Thus the CR/PR rate for the entire group was 12/20 (60%, 95% CI 39%–81%). Overall survival was significantly greater in the responding patients (8 of 12, one with disease, f/u 13 = 30 mos,) vs. the non-responders (0 of 8, p = .0001). Six patients were able to proceed to consolidation with high-dose therapy and stem cell rescue (4 auto, 2 allo), with 5 survivors.

Conclusions: The combination of rituximab and ICE chemotherapy was associated with an encouraging objective response rate and an acceptable toxicity profile.

Poster 161/PLATFORM SESSION 304

Incidence and Predictors of Treatment-Related Mortality in Pediatric Acute Leukemia in El Salvador, A Low-Income Country

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Background: The majority of children with leukemia live in low-income countries (LICs) where cure rates are lower than those in high-income countries (HICs). As intensive treatment becomes available, increased treatment-related mortality (TRM) may offset decreases in relapse rates; improvement of outcomes requires the concurrent reduction of both relapse and TRM. Identification of risk factors for TRM in LICs permits the development of interventions aimed at reducing toxicity and may improve survival.

Objective: To describe the cumulative incidence, specific causes and predictors of TRM among children in El Salvador with acute leukemia.

Design/Methods: We examined Salvadoran pediatric patients with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML –

excluding acute promyelocytic leukemia) diagnosed from January 2000 to June 2007 using the Pediatric Oncology Networked Database (POND). Data were collected prospectively by trained data managers. The Kaplan-Meier method was used to estimate cumulative incidence of TRM; biologic, socioeconomic and nutritional predictors were examined using Cox proportional hazards models.
Results: 469 patients with ALL and 78 with AML were included. The two-year cumulative incidence (±standard error) of TRM was significantly higher among children with AML (33.1±1.7%) than ALL (12.5±6.5%; P<.0001). However, the proportion of all deaths attributable to the toxicity of treatment did not differ significantly between AML (23/47, 48.9%) versus ALL (55/107, 51.4%; P = 0.91). Among children with ALL, low monthly income (hazard ratio [HR]/100 dollars = 0.862, 95% confidence interval (CI) 0.777, 0.957 P = 0.04) and low parental education (HR = 0.456, 95% CI 0.234, 0.888 P = 0.02) significantly increased the risk of TRM. Among children with AML, only higher mid-upper arm circumference was associated with TRM (HR = 1.361, 95% CI 1.012, 1.829 P = 0.04). Biologic variables were not significantly associated with TRM in AML or ALL.
Conclusions: In El Salvador, TRM contributes significantly to mortality in both ALL and AML. Unlike in HICs, the proportion of deaths attributed to treatment is similar for both types of acute leukemia. Socioeconomic factors predict the risk of TRM in ALL but not AML. Understanding the pathways through which socioeconomic status affects TRM may allow the design of interventions to reduce TRM in children with acute leukemia in LICs, but multi-center studies will be required.

Poster 162/Young Investigator Presentation

Pi-103, A Dual Inhibitor of Mtor and Pi3 Kinase, Potentiates Rapamycin-Mediated All Cell Death and Inhibition of Proliferation

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Background: mTOR integrates intracellular and extracellular signals, thereby acting as a critical mediator of lymphocyte survival and proliferation. The mTOR inhibitor (MTI) rapamycin suppresses proliferation and induces apoptosis of pre-B ALL *in vitro* and in human ALL xenografts, although lymphoid growth factors such as IL-7 can overcome the effects of MTI. We hypothesized that PI(3) kinase inhibition would potentiate the action of MTI by blocking growth factor signals upstream of mTOR. LY294002 is a PI3K inhibitor (PI3KI), and PI-103 is a dual MTI and class I PI3KI. In contrast to rapamycin, which selectively inhibits mTOR Complex (TORC) 1, PI-103 inhibits both TORC1 and TORC2.
Objective: To evaluate the targeted inhibition of PI3K, TORC1 and TORC2 with combinations of LY294002, PI-103 and rapamycin in pre-B ALL.
Design/Methods: Murine and human pre-B ALL cell lines were treated with combinations of rapamycin, PI-103, LY294002 and IL-7. Growth inhibition and cell death were assessed via MTT proliferation and flow-based apoptosis assays.
Results: LY294002 and rapamycin acted additively to inhibit ALL in culture, and LY294002 attenuated MTI resistance conferred by IL-7. PI-103 alone decreased ALL cell proliferation and increased cell death in a dose-dependent manner, with an IC50 of approximately 500 nM. Treatment of murine pre-B ALL cell lines with 1 μM PI-103 decreased proliferation down to 20–30% of baseline, untreated cells. Combinations of PI-103 and rapamycin had greater inhibitory effects than each drug individually. Co-treatment with 1 μM PI-103 and 10 ng/ml rapamycin fully inhibited cell proliferation, compared to 40% inhibition with rapamycin alone. This same combination resulted in 50% cell death, versus 15% with rapamycin alone. While IL-7 fully reversed rapamycin-mediated inhibition, the addition of 1 μM PI-103 effectively blocked this IL-7-mediated reversal.
Conclusions: Single agent LY294002, PI-103 and rapamycin all inhibited proliferation and induced cell death in pre-B ALL. However, concurrent treatment with PI-103 and rapamycin resulted in greater inhibition, increased cell death, and overcame the pro-survival effects of IL-7, suggesting that there are non-overlapping inhibitory effects of these agents on mTOR signaling. Our data support the notion that treatment with combination therapy targeting multiple nodes of the growth factor-PI3K-mTOR pathway could improve efficacy and reduce MTI resistance in pre-B ALL.

Poster 163/PLATFORM SESSION 303

Hyperglycemia During Induction Therapy is a Predictor of Overall Survival in Children with Acute Lymphocytic Leukemia

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Background: Recent studies in adult cancer patients suggested an association between hyperglycemia and reduced survival. We hypothesized that hyperglycemia during induction therapy influences relapse free (RFS) and overall survival (OS) in children with Acute Lymphocyctic Leukemia (ALL).
Objective: To explore the relationship between hyperglycemia during induction therapy and survival in children with ALL.
Design/Methods: We conducted a retrospective chart review of 167 children diagnosed with ALL between May 1998 to December 2001 at Texas Children's Hospital. Blood glucose concentrations during induction therapy were reviewed. Patients were considered hyperglycemic if they had one or more blood glucose concentrations >200 mg/dL during induction. We compared the RFS and OS between patients with and without hyperglycemia using Kaplan-Meier and Cox-proportional hazard analyses, adjusting for potential confounding variables. The RFS and OS estimates are reported with standard errors below.
Results: The median time of follow up in survivors was 6 years (range 0.1 to 8.5 years). There were 18 deaths and 36 relapses. 56 patients (34%) met the criteria for hyperglycemia during induction. Compared to the euglycemic group, patients with hyperglycemia had significantly poorer RFS (85%±3.6 vs 68%±6.7, p = .025) and OS (96%±1.9 vs 74%±6.1, p<.0001) at 5 years, respectively. In multivariable analysis, patients with hyperglycemia had 5.4 times (95% CI 1.6–17.7) greater risk for death than patients with euglycemia, independent of NCI risk group at diagnosis.
Conclusions: In children with ALL, hyperglycemia may be associated with shorter relapse free and overall survival. Validation of this finding is needed in a larger prospective study.

Poster 164

Immunostimulatory DNA Induces NK Cell-Mediated Anti-Leukemia Activity in a Syngeneic Acute Lymphoblastic Leukemia Model

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. While current therapies are very successful, the 15% of patients who relapse are generally unresponsive to chemotherapy alone. Evidence that natural killer (NK) cells are involved in a post-transplant graft-versus-leukemia effect suggests immune therapies targeting NK cell activation may be effective adjuvants to current chemotherapy. Immunostimulatory DNA oligodeoxynucleotides containing CpG motifs (isDNA) stimulate anti-tumor immune activity via Toll-like receptor 9 and are in clinical trials for several tumors. We previously reported that isDNA stimulation alters antigen presentation by human ALL cells, enhancing allogeneic Th1 responses. We have also shown that isDNA administration

in vivo reduces the leukemic burden of primary human ALL xenografts in Nod-SCID mice, and that this activity is mediated in part by NK cells.
Objective: To further the development of isDNA as a novel therapeutic approach for ALL, we investigated induction of anti-ALL activity by isDNA in a syngeneic setting, both as monotherapy and as a post-chemotherapy adjuvant.
Design/Methods: We adapted a flow cytometric *in vitro* cytotoxicity assay to assess syngeneic anti-leukemia activity in isDNA-stimulated splenocytes. NK cell contribution to cytotoxicity was assessed by performing the assays with CD49b-depleted splenocytes or with addition of an NKG2D blocking antibody. In addition, leukemia expressing green fluorescent protein was injected into syngeneic mice. After engraftment, mice were treated with chemotherapy followed by isDNA or saline. Mice were sacrificed 3 weeks after treatment and evaluated for leukemic burden by flow cytometry.
Results: isDNA-stimulated splenocytes showed significantly increased killing versus control. The difference between isDNA-treated and untreated controls became more pronounced at higher effector: target ratios ($P<0.0001$). Both depletion and blockade of NK cells significantly decreased killing. At sacrifice, isDNA-treated mice had significantly reduced leukemia burden in bone marrow ($P=0.0019$), spleen ($P<0.00001$) and blood ($P=0.00028$) versus control.
Conclusions: To our knowledge, this is the first demonstration of isDNA-induced anti-ALL activity in a syngeneic model, both as a monotherapy and as a post-chemotherapy adjuvant. The syngeneic model eliminates allo- and xeno-reactive influences, focusing the effects on autologous NK-mediated blast recognition. This suggests isDNA has the potential to treat minimal residual disease and to reduce the incidence of relapse.

Poster Board 165/PLATFORM SESSION 304

The Novel Glycolysis Inhibitor, 3-BrOP, Has Broad Activity Against Acute Lymphoblastic Leukemias (ALL) And Synergizes with Rapamycin

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Background: Rapidly proliferating cancer cells are dependent on glycolysis for ATP production (Warburg effect). The novel glycolysis inhibitor, 3-bromo-2-oxopropionate-1-propyl ester (3-BrOP), has efficacy in pre-clinical models of glioblastoma, colon carcinoma, lymphoma, and most recently acute leukemias. Since mTOR inhibition exacerbates cellular starvation, we hypothesized that rapamycin may synergize with ATP depletion caused by glycolysis inhibition.
Objectives: Determine the effect of 3-BrOP in combination with rapamycin on human ALL cell lines.
Design/Methods: Five human precursor-B and five T-ALL cell lines were treated with 3-BrOP, alone and in combination with rapamycin, to determine IC50s via cell counts, cell cycle and MTT analysis. Synergy was evaluated with CalcuSyn software. Caspase-3 levels were evaluated by Western blot. NOD/SCID mice were injected with GFP-labeled Nalm6 human pre-B ALL cells, and treated with 3-BrOP (20 mg/kg) for 10 days. Flow cytometry was used to detect Nalm6 leukemia cells in peripheral blood samples.
Results: All ten ALL cell lines demonstrated growth arrest, decreased MTT metabolism, and significant apoptosis when treated with 3-BrOP (IC50 10–30 mcM). At 40 mcM, 3-BrOP induced >90% cell death in all lines. In a primary patient ALL sample, the IC50 was 11 mcM. Sub-therapeutic doses of 3-BrOP combined with rapamycin demonstrated significant synergy in MTT and cell cycle assays. Importantly, synergy was observed at multiple doses of 3-BrOP (2.5–40 mcM) and rapamycin (6.25–50 nM) (combination index (CI) = 0.002–0.5). For example, pre-B ALL treated with 10 mcM 3-BrOP and 25nM rapamycin revealed a CI of 0.002, indicating very strong synergy between these two agents. Similar effects were seen in T-ALL (CI = 0.003–0.3). Of note, caspase-3 cleavage was detected by 2 hours of exposure, and was nearly complete by 6 hours suggesting an early and potent pro-apoptotic signal. Preliminary results in a human pre-B ALL xenograft model demonstrate anti-leukemia activity by 3-BrOP at non-toxic doses (4-fold reduction of peripheral blasts).
Conclusions: Inhibition of glycolysis via 3-BrOP induces growth arrest and apoptosis across a panel of human pre-B and T-ALL cell lines, as well as primary patient samples. 3-BrOP is highly synergistic when combined with rapamycin. The novel combination of 3-BrOP and rapamycin offers a promising therapeutic approach in ALL and warrants further evaluation.

Poster 166

Osteoporosis Screening in Childhood Acute Lymphoblastic Leukemia: A First Step in Understanding the Natural History of Vertebral Compression

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Background: Bone pain is a common presenting symptom in children with ALL. Symptomatic vertebral compression fractures have been reported in up to 7% of children at diagnosis while asymptomatic fractures have not been studied.
Objective: The aim of this study is to determine the prevalence of vertebral body compression fractures in children with ALL at presentation.
Design/Methods: 116 children with ALL (age 0.11–16.48 y, mean 6.6 y, male n = 62, pre-B n = 102, T-cell n = 14) who were enrolled in the **ST**eroid-induced **O**steoporosis in the **P**ediatric **P**opulation (STOPP) study were assessed for vertebral compression fractures. Lateral thoracolumbar spine radiographs were performed within 30 days of diagnosis with central review by two independent pediatric radiologists who resolved discrepancies through consensus. The radiographs were scored for vertebral deformity according to the Genant semi-quantitative method.
Results: Forty-one (35%) of patients sustained a total of 113 vertebral compression fracture events (90 thoracic, 23 lumbar). The T6 and T7 vertebrae were the most commonly affected and comprised 41 of the 113 fractures (36%). Ninety-two of the fractures (81%) were graded as mild, 17 (15%) as moderate and 4 (4%) as severe. Eighty-six (76%) of the fractures had an anterior wedge configuration. Twenty-eight patients (68%) had one or two fractures, ten patients (25%) had three to seven fractures and 3 patients (7%) had greater than nine fractures. Only 14 of the 41 patients with vertebral compression fractures (34%) reported back pain.
Conclusions: Vertebral compression fractures are common, and potentially severe, in children with ALL at or soon after diagnosis and may be attributed to the leukemic process. The low incidence of back pain may reflect that the majority of fractures were categorized as mild, with the mid-thoracic region representing a particularly vulnerable skeletal site. Our national, longitudinal bone health study (STOPP) will follow the natural history of vertebral compression fractures in children with ALL from diagnosis through to the completion of chemotherapy and one-year following therapy. The relationship between vertebral deformities at ALL diagnosis and various clinical parameters, including bone mineral density, will also be studied through longitudinal follow-up.

Poster 167

Increased Erythroid and Megakaryocytic Potential in Down Syndrome Fetal Hematopoiesis: How Trisomy 21 May Predispose to Leukemia

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Background: Infants and young children with Down syndrome (DS) develop two related disorders of clonal hematopoiesis: transient myeloproliferative disorder (TMD) and acute megakaryoblastic leukemia (AMKL). Somatic mutations in the GATA1 gene, which encodes an essential hematopoietic transcription factor, are associated with TMD and AMKL in DS. Separately, trisomy 21 or GATA1 mutations are not sufficient to cause TMD or AMKL, suggesting that these genetic aberrations synergize in a multistep leukemogenic process that begins in fetal life. DS neonates also exhibit a variety of non-malignant hematopoietic abnormalities.

Objective: Our objective was to study how trisomy 21 in isolation alters fetal hematopoiesis.

Design/Methods: Fetal liver hematopoietic cells were isolated from DS and control abortuses (13–23 weeks gestation). Constitutional trisomy 21 was confirmed by cytogenetic studies. Fetal hematopoiesis from DS and control livers were evaluated by hematopoietic colony assays and flow cytometry.

Results: No GATA1 mutations were detected by direct sequencing ($n = 24$). Hematopoietic colony assays demonstrated that DS fetal liver contains significantly more erythroid and megakaryocytic progenitors compared to controls. Relative to myeloid colony formation DS progenitors formed 2.6-fold and 2.5-fold increased erythroid and megakaryocytic colonies, respectively ($p = 0.015$ and $p = 0.004$), compared to non-DS samples ($n = 8$ DS and 8 controls). In addition, DS erythroid and megakaryocytic colonies were consistently larger reflecting increased proliferative capacity of trisomy 21 progenitors. Flow cytometry revealed a distinct distribution of hematopoietic progenitors in DS. Specifically, compared to controls, DS fetal liver had an increased common myeloid progenitor (CMP) compartment (65.2% vs 43.5%, $p = 0.014$), and decreased granulocyte macrophage progenitor compartment (24.4% vs 48.5%, $p = 0.003$). Surprisingly, the megakaryocytic erythroid progenitor compartment was not expanded in DS fetuses, suggesting that the CMP may be the population susceptible to TMD and AMKL.

Conclusions: Together, these studies show that trisomy 21 itself increases the proliferative capacity of erythroid and megakaryocytic progenitors in a cell intrinsic manner, supporting the hypothesis that increased dosage of a chromosome 21 gene(s) promotes leukemia by expanding erythro-megakaryocytic lineages, possibly at the CMP stage of hematopoietic development.

Poster 168

Increased Carotid Artery Intimal Media Thickness, a Surrogate for Subclinical Atherosclerosis, is Present in Patients with Hodgkins Lymphoma

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Background: Carotid and coronary artery disease (CAD) are well-described late effects of treatment for Hodgkin's Lymphoma (HL). We previously demonstrated increased carotid artery intima-media thickness (CIMT), measured by ultrasound, in 20 HL survivors greater than 5 years from diagnosis compared to 8 sibling controls. Increased CIMT is a valid marker of subclinical atherosclerosis and is associated with an increased risk of future vascular disease.

Objective: To measure CIMT in a cross sectional cohort of patients with HL.

Design/Methods: Thirty-seven patients with HL (23 male/14 female), mean age 20 years (range 4–39), 2–241 months from diagnosis, including those from our original cohort, were evaluated. Twenty patients had received radiation therapy (RT); 3 high dose ($\geq$36 Gy); 17 lower dose (21–25 Gy). Thirty-six patients had received an anthracycline. CIMT was assessed using a standardized scanning and reading protocol and was calculated as the mean of the maximum measurement at 12 sites. Peripheral blood was drawn to evaluate a cholesterol panel and inflammatory markers.

Results: The mean CIMT for the total cohort was 0.75 mm$\pm$0.07 mm, a value equivalent to the 50th percentile CIMT for healthy 35–40 year olds[1]. CIMT was significantly associated with increasing time since diagnosis ($p = 0.000$), age ($p = 0.000$), and male gender ($p = 0.014$). BMI, total and LDL cholesterol, radiation and the presence of B symptoms were not associated with CIMT, although the three patients who received $\geq$36 Gy had higher CIMT values and there was a trend toward increased CIMT with increased BMI ($p = 0.08$).

Conclusions: Subclinical atherosclerosis in patients with HL may evolve early in treatment and survivorship and then increase with age and time from diagnosis. Type of treatment did not predict increased CIMT in our cohort suggesting that factors other than RT may play a role in the development of subclinical atherosclerosis. Further studies are needed to better elucidate the etiology of atherosclerotic disease in HL patients and to evaluate CIMT as a screening tool for cardiovascular disease risk in this population.

1. Stein, Stroke, 2004

Poster 169

The Relationship Between Lumbar Spine Bone Mineral Density and Vertebral Compression at Leukemia Diagnosis

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Background: Vertebral fractures are common and potentially severe, among children with ALL at diagnosis.

Objective: The aim of this study was to characterize the clinical indices associated with vertebral compression at ALL diagnosis through the **ST**eroid-induced **O**steoporosis in the **P**ediatric **P**opulation (**STOPP**) study, a Canadian, pediatric bone health research program.

Design/Methods: A total of 116 newly diagnosed children (age, mean 6.6$\pm$4.08 years, 62 boys) who were enrolled in the **STOPP** study were evaluated within 30 days of diagnosis. Baseline assessments included lateral thoracolumbar spine x-ray, anthropometry, spine areal and volumetric bone mineral density (BMD), vitamin D and calcium intake (Frequency Food Questionnaire), and activity levels according to the Habitual Activity Estimation Scale.

Results: Forty-one patients (35%) had a total of 113 vertebral deformities (90 thoracic, 23 lumbar). Fractures were seen in 32.4% (33/102) of B-cell precursor and 57.1% (8/14) of T-cell immunophenotype. The 41 children with vertebral deformity were compared to those without spinal involvement. The mean age- and gender-matched z score$\pm$standard deviation (SD) for spine areal BMD and height, the raw scores for spine vBMD and the median value, IQR (25–75%) for the remaining parameters were as follows:

Clinical parameter	Compression	No compression	p value
	N = 41	N = 75	
Age, years	5.7(3.6,9.2)	5.1(3.2,11.3)	0.749
Male, n(%)	21(51.2)	41(54.7)	0.722
Height z score	0.30(1.19)	0.31(1.25)	0.993
Spine aBMD z score	−1.5(1.38)	−0.67(1.30)	0.003
Spine vBMD g/cm^3	0.10(0.02)	0.11(0.03)	0.003
Vitamin D intake IU/d	210(129–306)	300(156–427)	0.141
Calcium intake mg/d	1594(1120–2349)	1899(1328–2571)	0.554
Activity (% to waking hours)	21(5–45%)	35(13–53%)	0.087

Of the studied clinical parameters, only the spine BMD z score was associated with an increased odds for fracture. For every 1 SD reduction in spine areal BMD z score, the odds for fracture increased by 60%.
Conclusions: Vertebral compression fractures are a potentially serious complication of ALL at diagnosis. Low spine areal BMD was the only clinical parameter measured in this study that was associated with an increased odds for vertebral compression at presentation. The potential for reshaping of the vertebral bodies and restitution of bone mass for this cohort of children will be determined by longitudinal follow-up.

Poster 170

Mechanisms of Leukemogenesis by Calm-Containing Fusion Proteins: Characterization of a Potent 18 Amino Acid Calm Transcriptional Regulatory Domain

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Background: MLL translocations are common in infant leukemias, with more than 50 described translocation partners. We recently identified the CALM (Clathrin Assembly Lymphoid Myeloid leukemia) gene as a novel MLL partner in an infant with aggressive AML. CALM was first discovered as a translocation partner for AF10, which had previously been identified as an MLL fusion partner in aggressive leukemias and lymphomas. The 660 amino acid (aa) native CALM protein exhibits predominantly cytoplasmic localization, and participates in clathrin-dependent endocytosis and intracellular vesicle transport. We have previously demonstrated that CALM possesses a transcriptional activation domain (TAD) that modulates MLL transcriptional activity, potentially contributing to leukemogenesis.
Objective: To delineate specific domains which constitute the CALM TAD.
Design/Methods: We prepared a set of expression vectors in which portions of native CALM (from aa 256–660) are fused to the GAL4 DNA-binding domain. We also introduced three separate point mutations in a specific 18 aa domain in the CALM$^{256–660}$ construct using site-directed mutagenesis. These vectors were individually co-transfected with a GAL4 promoter-firefly luciferase reporter plasmid (and a Renilla luciferase plasmid, for normalization) into COS7 cells, and luciferase activities were measured 48h after transfection. Results were normalized again to an empty vector (lacking CALM fragments) to account for GAL4 DBD self-activation. Protein levels were confirmed by Western blots.
Results: Significant luciferase activity (265±22x empty vector) was observed with the CALM$^{256–660}$ construct. While CALM$^{256–492}$ showed minimal activity, a substantial increase in activity (to 181±16x) was seen with CALM$^{256–510}$, suggesting the presence of a potent TAD within this 18 aa region. Introduction of mutations into aa 496–498 (KST→AAA), 503–504 (DS→AA), and 507–509 (FDE→AAA) resulted in a significant reduction in luciferase activity (4±0.2x, 4±0.6x, and 11±0.7x respectively), indicating that these residues contribute to the CALM TAD.
Conclusions: We have identified a small (18 aa) domain in the CALM C-terminus that contains aa residues critical to transcriptional regulation. Mutation of specific residues in this domain results in dramatically decreased transcriptional activation. The presence of a CALM-derived TAD suggests a role for CALM-dependent altered transcriptional regulation in leukemias in which CALM is aberrantly expressed.

Poster 171

Targeting Eif4e with Ribavirin Demonstrates A Potentially Effective Strategy Against Acute Lymphoblastic Leukemia (ALL)

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Background: mTOR is the central protein in a large signal transduction pathway involved in nutrient metabolism and cell growth, RNA translation and protein synthesis, and progression through the cell cycle. We have previously demonstrated that mTOR inhibitors (MTIs) are active against ALL in preclinical models. MTIs may be active in ALL through inhibition of one or more of these mTOR-mediated functions. eIF4E is a down-stream target of mTOR involved in cap-dependent translation. Ribavirin has been shown to disrupt elf4E subcellular organization and transport, leading to reduced levels of important signaling and cell cycle proteins including cyclin D1. This suggests that ribavirin could target ALL cell growth through this mechanism.
Objective: We hypothesized ribavirin would be effective against ALL.
Design/Methods: To test our hypothesis, we treated multiple ALL cell lines (4 murine and 3 human) with ribavirin at concentrations ranging from 1nM to 100 uM for 48 to 72 hours We assessed efficacy of treatment by measuring proliferation using MTT assay and cell death using FACS analysis for 7-AAD. We determined if we were hitting target by immunoblot for cyclin D1. We also combined ribavirin with the mTOR inhibitor sirolimus to assess the effect.
Results: We found all ALL cell lines were exquisitely sensitive to ribavirin. We found ribavirin inhibited proliferation and induced cell death at nanomolar dosing (IC50 5–50 nM, depending on cell line). These results were statistically significant in all cell lines by both output methods ($p<0.05$). Micromolar dosing is needed for ribavirin to inhibit viral replication. Thus, these results suggest ribavirin can be used in ALL at far lower doses, potentially avoiding some of the hematologic toxicities associated with ribavirin when it is used as an anti-viral agent. We also found ribavirin had an additive and potentially synergistic effect when combined with sirolimus. Finally, we have preliminary data suggesting ribavirin down-regulates cyclin D1 in ALL at nanomolar dosing.
Conclusions: Targeting elf4E with ribavirin appears to be a novel and effective means to treat ALL. Future work will be directed at using ribavirin in our published and established murine xenograft models of ALL.

Poster 172

In Vitro Drug Sensitivity as a Predictive Tool of Early Clinical Response in Childhood Acute Lymphoblastic Leukemia

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Background: Residual disease or rapidity of response to induction therapy is among the most powerful predictors of outcome in pediatric Acute Lymphoblastic Leukemia (ALL). Various methods to determine response during induction have been in use in clinical investigation. Expeditious and reliable methods to predict response to therapy is an ongoing goal.
Objective: To correlate drug sensitivity of leukemic blasts in vitro with the rapidity of response to induction therapy among patients with ALL.
Design/Methods: We developed a high resolution flow cytometry based cytotoxicity assay for in vitro cellular drug response profiling for pediatric ALL. We performed in vitro tests on cryopreserved bone marrow (BM) samples of 23 patients with newly diagnosed ALL. Fourteen patients were rapid early responders (RER) and 9 were slow early responders (SER) by Children's Oncology Group criteria. Drugs were tested at three different concentrations. Leukemic cell survival index (LCSI) was determined at 48 hours after in vitro exposure to individual standard induction agents for pediatric ALL. LCSI differences between RER and SER were compared using Wilcoxon rank sum test for each drug and concentration. The mixed

effects model was used to evaluate the overall difference of LCSI between RER and SER over the three concentrations (referred to as ''averaged concentrations'').
Results: For dexamethasone, a significantly lower LCSI was seen in the RER compared with the SER cohort at individual and averaged concentrations ($p = 0.01$, mixed effects model). A trend toward a lower mean LCSI in the RER compared with the SER group was noted for asparaginase and vincristine ($p<0.1$). Mean LCSI was not different between the RER and SER groups for daunomycin and prednisone.
Conclusions: This in vitro drug sensitivity assay provides a response profile for dexamethasone that correlates with in vivo response to induction therapy. Research is ongoing with more patient samples in order to achieve a greater statistical power to detect a smaller difference for all drugs tested. These results will determine the potential value of this assay for early risk stratification and modification of therapy in de novo or relapsed ALL.

Poster 173

Brief MRI Screen for Osteonecrosis in Pediatric Patients with Lymphoid Malignancies and Hemoglobinopathies

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Background: Osteonecrosis (ON) has recently been recognized as a serious complication for pediatric patients with hemoglobinopathies and lymphoid malignancies. ON typically is diagnosed only after the patient develops mobility-limiting symptoms. Early diagnosis of ON lesions may potentially allow earlier and more effective therapeutic intervention.
Objective: This study tested a rapid screening MRI protocol of the lower extremities for ON. The prevalence of symptomatic and asymptomatic ON was assessed. The prevalence of osteopenia was also assessed.
Design/Methods: Following provision of informed consent, children older than four years with acute lymphoblastic leukemia (ALL) or lymphoma and those older than seven years with sickling conditions or thalassemia completed a brief MRI protocol lasting approximately 30 minutes including T1-weighted and STIR coronal images of bilateral hips and knees, DEXA scan of the lumbar vertebrae, and screening for symptoms of ON.
Results: Thirty-one children (19 with malignancies and 12 with hemoglobinopathies) have completed the initial MRI. All children completed the screening MRI successfully without sedation. One 4-year-old child required two attempts. Seven children (23%) had evidence of ON by MRI. Three children ages four to eight years with standard risk ALL had mild asymptomatic ON (less than 50% joint surface involvement) and one child had severe symptomatic ON. Two children with hemoglobinopathies had symptomatic ON and one had asymptomatic ON. Thirty-three children (18 with malignancies and 15 with hemoglobinopathies) have completed the initial DEXA, all without the use of sedation. Sixteen children (48%) had osteopenia (Z-score less than -1.0) with six being severe. Four children with mild osteopenia and five with severe osteopenia had ALL. Two children with mild osteopenia and ALL had spinal compression fractures.
Conclusions: ON and osteopenia continue to be important concerns for children with hemoglobinopathies and lymphoid malignancies. Children as young as four years are able to successfully complete brief MRI protocols and DEXA scan without sedation allowing safer and faster diagnosis of ON and related pathology. Asymptomatic ON is likely more prevalent than previously recognized, however the clinical significance of this finding is currently unknown. This study continues to evaluate the natural history of ON in this population.

Poster 174

Evolution of Hemophagocytic Lymphohistocytosis: A Texas Experience

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Background: Hemophagocytic Lymphohistiocytosis (HLH) is a rare, complex disease with historically poor survival. Early and aggressive therapy has transformed this uniformly fatal disease to a survivable condition if targeted treatment is given. As part of an overall evaluation of prognostic markers in HLH, we undertook a retrospective review of patients from two large pediatric academic centers in Texas.
Objective: To report current survival and common clinical markers at presentation in HLH.
Design/Methods: Institutional hematology/oncology and pathology databases were searched to identify patients diagnosed with HLH, malignant histocytosis or hemophagocytic syndrome between 1992 and 2007. Patients meeting HLH 1994 or HLH 2004 diagnostic criteria: fever, cytopenias of >2 cell lines, hypofibrinogenemia, splenomegaly, elevated ferritin, triglycerides, abnormal natural killer cell function and/or soluble interleukin (IL) 2 receptor were included. Labs, age, ethnicity, treatment and outcome were recorded.
Results: A total of 66 patients: 55 from Houston (33 female, 22 male) and 11 Dallas (8,3) met inclusion criteria. Median age at diagnosis was 4.4 yrs (range 0.1–17). Twenty-nine patients (44%) were Latino in a state that is 35% Latino. Splenomegaly and hepatomegaly were documented in 70% and 79%, respectively. Hemophagocytosis was seen in any biopsy specimen from 48 (73%). Acute EBV infection was identified in 27 pts (41%) and CMV in 6 (9%). Median nadir platelet count was 43,000/ml and maximal ALT/AST 534/1104 U/L, ferritin 11,063 ng/ml and triglyceride 354 mg/dl. Soluble IL-2 receptor was markedly elevated; median 10,577 U/ml (range 500–86,849). Forty-two percent of patients had CNS disease. Patients were treated per HLH 1994 or 2004, with a combination of steroids, IVIG, cyclosporine and VP16. At median follow-up of 17 mo. [range 1–139] 43 pts (65%) are alive; 13 after bone marrow transplant.
Conclusions: The 65% survival of patients supports the importance of early and aggressive therapy. This series includes more patients of Latino ethnicity than prior reports. Whether this ethnic difference accounts for improved survival will need to be studied in larger population. HLH is no longer a uniformly fatal disease, and should be considered in critically ill children who may present with signs of sepsis, liver or multi-organ failure.
Supported by a grant from Amgen.

Poster 175

The Role of the API2 Moiety in API2-Malt1-Mediated Malt Lymphomagenesis

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Background: B-cell Mucosal Associated Lymphoid Tissue (MALT) lymphoma is the most common form of extranodal lymphoma. Commonly arising in the setting of *H. pylori* gastritis, 70% of cases of stage I disease are cured by eradication of the infection with antibiotics. The t(11;18) chromosomal translocation, present in up to 40% of cases, is associated with treatment resistance and advanced stage. This translocation creates a fusion protein composed of the N-terminus of API2 (Inhibitor of Apoptosis 2), and the C-terminus of MALT1, a signaling protein which mediates antigen-induced proliferation in lymphocytes. API2-MALT1 activates the pro-survival transcription factor NF-kB, but the mechanism by which this occurs and the contribution of NF-kB activation to lymphomagenesis are not yet known.
Objectives: To investigate the mechanism by which the API2 moiety contributes to API2-MALT1-dependent NF-kB signaling and oncogenesis.
Design/Methods: Co-immunoprecipitation analysis was performed to identify and characterize API2 moiety-mediated protein-protein interactions. The ability of API2-MALT1 expression to promote cellular transformation was evaluated using NIH-3T3 cells in a soft agar assay and murine bone marrow cells in a methylcellulose re-plating assay.

Results: The API2 moiety of API2-MALT1 mediates both oligomerization of the fusion protein and interaction with the TRAF2 (*t*umor necrosis factor *r*eceptor *a*ssociated *f*actor 2) adaptor protein. Deletion or mutation of the TRAF2 binding site on the API2 moiety leads to dramatically decreased API2-MALT1-dependent NF-kB activation, and deletion of both the TRAF2-binding and the oligomerization sites results in near complete loss of NF-kB activation. API2-MALT1 expression results in transformation of NIH-3T3 cells as well as immortalization of murine bone marrow cells, and loss of API2 moiety-mediated oligomerization and/or TRAF2 binding is associated with loss of API2-MALT1-dependent cellular transformation.

Conclusions: These studies suggest that (1) API2 moiety-mediated oligomerization and TRAF2 binding both contribute to maximal API2-MALT1-dependent NF-kB stimulation, and (2) API2-MALT1 is a bona fide oncoprotein capable of transforming cells, and API2 moiety-mediated protein-protein interactions are critical to its oncogenic function. These studies provide insight into the pathogenesis of MALT lymphoma and lay the groundwork for developing novel therapies aimed at disrupting API2-MALT1 function as a means of treating refractory disease.

Poster 176

Perturbed Endocytosis as a Mechanism of CALM-Dependent Leukemogenesis: Development of a Non-Radioactive Assay for Receptor Internalization

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Background: Clathrin Assembly Lymphoid Myeloid leukemia (*CALM*) gene rearrangements, in which *CALM* is fused to *MLL* or *AF10* genes, are found in aggressive leukemias and lymphomas. Expression of MLL-CALM or CALM-AF10 fusion proteins immortalizes murine hematopoietic cells *in vitro*, correlating with leukemogenesis *in vivo*. We have hypothesized that perturbation of normal CALM function contributes to transformation. The native CALM protein is primarily cytoplasmic and functions in Clathrin-Dependent Endocytosis (CDE). We have previously shown that overexpression of CALM-containing constructs (CCC) in COS7 cells impairs CDE of Transferrin (TF) and Epidermal Growth Factor (EGF) using a semi-quantitative radioactive assay (with ^{131}I-TF or EGF) that, while sensitive, lacked specificity for transfected cells. Here we describe the development of a flow cytometry-based assay of CDE that permits specific quantitation in CCC-transfected cells and in leukemia cells with endogenous *CALM-AF10*.

Objective: To develop a novel flow cytometry-based CDE assay that accurately measures TF/EGF internalization.

Design/Methods: COS7 cells transfected with GFP-tagged CCC or six leukemia cell lines (some harboring native *CALM-AF10* translocations) were incubated at 4 C for 1h with AlexaFluor (AF) 633-TF or AF647-EGF to allow binding, followed by incubation at 25C (2–30 min) to permit internalization. Following acid buffer wash and fixing, internalized fluorescence was measured by flow cytometry.

Results: Overexpression of native CALM (aa^{1-660}) reduced the rate of TF and EGF internalization by 53±10%. The portion of CALM in MLL-CALM fusions, $CALM^{256-660}$, reduced TF and EGF internalization by 66±11% compared with controls, validating our previous studies. Transfection of a series of CALM deletion constructs ($CALM^{256-492}$, $CALM^{256-502}$, $CALM^{337-531}$, $CALM^{436-583}$, $CALM^{593-660}$, and $CALM^{601-660}$) demonstrated that a 52 aa region (CALM aa 531–583) was required to perturb internalization. TF internalization was impaired in the setting of *CALM-AF10* expression (U937 and P31/Fuji cells) compared with cell lines that do not express *CALM-AF10* (HL60, K562, MonoMac6, THP1), a likely consequence of perturbed CDE.

Conclusions: Using a novel flow cytometry-based assay, we have narrowed the specific region of CALM critical to CDE perturbation to 52 aa. We have also demonstrated impairment of TF internalization in leukemia cell lines expressing the *CALM-AF10* fusion. These observations further support a role for CDE dysregulation in leukemogenic transformation.

Poster 177

Increasing Prevalence of Transient Hyperglycemia During Induction Chemotherapy for Pediatric Acute Lymphoblastic Leukemia

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Background: Transient hyperglycemia (TH) has long been recognized as a side effect of pediatric acute lymphoblastic leukemia (ALL) induction therapy due to the combination of corticosteroids and asparaginase. Previous studies have described TH in 4–15% of pediatric ALL patients, though their criteria for TH have varied. Age ≥10 years and overweight have been identified as risk factors for TH, but data about gender differences in TH is mixed. Also, as dexamethasone use increases, more data is needed about its effect on TH compared to prednisone. Identification of ALL patients at risk for TH may allow early intervention to reduce potential morbidity from this side effect.

Objective: To determine the prevalence of TH in a cohort of pediatric ALL patients and to determine the impact of type of steroid used and of risk factors such as age, gender, and overweight.

Design/Methods: Retrospective review of patients aged 2–18 years diagnosed with ALL in 2002–2006. Data collected included age, height, and weight at diagnosis (used to calculate body mass index [BMI] percentile for age), type of steroid administered, and random glucose levels during induction chemotherapy. TH was defined as ≥2 glucose values ≥200 mg/dl. Overall prevalence of TH was calculated. Risk factors were evaluated using Chi-square analysis and logistic regression.

Results: 98 subjects (45 female) were reviewed. TH occurred in 21 (21.4%) of the subjects. 41.9% of subjects over age 10 years had TH, compared to 11.9% of younger children ($p = 0.001$). There was no difference between male and female subjects. Thirteen subjects had BMI ≥95th percentile for age; of these, five (38.5%) had TH, however this was not significantly different from the rest of the population. In addition, there was a non-significant trend toward more TH in patients who received prednisone rather than dexamethasone, but this could not be separated from the confounding effect of age.

Conclusions: The prevalence of TH in this cohort was higher than previously reported. Age ≥10 years was the only significant predictor of TH, though overweight may also increase risk. This study is ongoing and the final results may further elaborate upon these preliminary findings.

Poster 178

Characterization of SP Cells in Pediatric Acute Lymphoblastic Leukemia

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Background: Side population (SP) cells, defined by Hoechst dye exclusion in flow-cytometry, appear to be enriched in hematopoietic stem cells (HSC) and may play a pivotal role in both normal development and cancer biology. SP cells often express high levels of various membrane transporters responsible for drug resistance. SP cells are quiescent and may not be sensitive to agents that act on rapidly dividing cells. Thus, agents that target SP cells may improve therapy for pediatric cancers, including leukemia. SP cells may be involved in human acute myeloid leukemia (AML) transformation. However, SP cells in ALL have not been well characterized.

Objective: The objective of this study was to characterize SP cells in pediatric ALL. We hypothesize that some cases of pediatric ALL relapse are due to the persistence of a relatively rare, chemo-resistant population of leukemia initiating cells.

Design/Methods: After optimizing the SP staining technique for primary ALL cells, we have characterized ALL-SP cells using flow cytometry, cytogenetics, and clonogenic assays.

Results: Our data indicate that SP+ cells in pediatric patients with ALL are less frequent than in patients with AML. SP cells were nearly undetectable

in blood from pediatric ALL patients and comprise <0.1% bone marrow aspirate cells. Similar to HSCs, SP cells in primary pediatric ALL stain weakly for both CD34 and CD38 antigens. Cytogenetic analysis shows that SP cells from patients with ALL can express leukemia-specific translocations.
Conclusions: Our preliminary results suggest that SP cells can be used to further characterize leukemia initiating cells in ALL.

Poster 179

A Combination of Propofol and Ketamine for Painful Procedures in Children with Cancer in a Hospital Outpatient Setting

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Background: Children with cancer undergo repeated painful procedures as part of ongoing treatment. It is essential that these be performed safely while causing minimal pain and anxiety. A prompt recovery will improve patient satisfaction.
Objective: The combination of Propofol and Ketamine administered to pediatric cancer patients in a hospital outpatient setting by trained pediatricians is safe and efficacious.
Design/Methods: All patient records from the oncology service were reviewed from the years 2000 to 2007. The Procedures followed general accepted guidelines for moderate to deep sedation. The following data were obtained: diagnosis, procedure, ASA physical status, duration of sedation, duration of procedure, recovery time, and medications used. Complications or side effects were recorded. Parametric data was analyzed using the unpaired t-test.
Results: 128 procedures were performed. 94 received a combination of Propofol and Ketamine (group a). 34 procedures were done with varying combinations of Fentanyl, Propofol, Ketamine and Versed (group b). The patients ranged from 2 to 21 years of age. Two patients were classified as ASA III and the rest were ASA II. In group a, the median sedation level was 3, mean sedation time was 12 minutes. The average duration of procedures was 7.8 minutes. The average time to full recovery was 10.3 minutes in group a vs 46.7 minutes in group b. Two brief episodes of hypoxia were observed. One patient experienced hallucinations. No episodes of apnea, hypotension or bradycardia were reported. In 63.8% of cases, sedation was provided by specially trained pediatric hospitalists and 36.2% by a pediatric critical care attending. In comparing the two groups, the sedation time and the duration of procedures were comparable. However, the recovery was shorter in the Propofol and Ketamine group ($P<0.05$).
Conclusions: The combination of Propofol and Ketamine administered by trained pediatricians is a safe and effective method of providing sedation with rapid recovery in pediatric oncology patients.

Poster 180

Myelosuppression and Infectious Complications in Down Syndrome ALL

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Background: Children with Down syndrome (DS) are at a 10–20 fold increased risk of acute lymphoblastic leukemia (ALL) compared to children without DS, and suffer more frequent and severe treatment-related toxicities, including increased fatal infectious complications.
Objective: To better understand patterns of myelosuppression and distinctive features of treatment-related toxicities in DS ALL, particularly infectious toxicities, we performed a retrospective case-control analysis of 16 DS ALL patients compared to non-DS ALL controls matched by treatment regimen.
Design/Methods: Absolute peripheral blood counts and incidences of treatment-related toxicities were determined at 13 serial time points during the first six months of therapy.
Results: At nearly all post-induction time points, DS ALL patients had significantly lower absolute lymphocyte counts (ALC) (mean 1049–1388 versus 1719–3001, p values 0.01–0.05). DS ALL patients were hospitalized for significantly more days (mean 36 versus 24, $p<0.03$), and had significantly more grade 3–4 toxicities (37 versus 20, $p<0.014$), particularly mucositis, cellulitis, and a trend toward more infectious complications. Infectious organisms included gram-positive, gram-negative, viral and fungal species.
Conclusions: Increased infectious complications in DS ALL during induction do not appear attributable to disproportionate myelosuppression. Post-induction, DS patients did demonstrate a comparative lymphopenia. Mucositis and cellulitis were the two most frequent toxicities. Relative lymphopenia and increased cellulitis during therapy in DS ALL are novel findings which merit further investigation. Meticulous oral and skin hygiene, as well as vigilance for and aggressive treatment of cutaneous infections, should be practiced in this population. Both quantitative and qualitative immune defects, as well as mucosal and epithelial barrier defects, may all contribute to infectious complications in DS ALL; the relative roles of these factors merit further investigation.

Poster 181

Body Mass Index (BMI) Predicts Insulin Resistance in Survivors of Pediatric Acute Lymphoblastic Leukemia

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Background: Late effects of childhood acute lymphoblastic leukemia (ALL) treatment include obesity, hyperglycemia, and insulin resistance. Hyperglycemia is a recognized, transient side effect of corticosteroids and asparaginase, two agents key to ALL treatment. Limited data is available about whether hyperglycemia or variations in medications during therapy are linked to permanent alterations of glucose metabolism.
Objective: To generate pilot data about whether measures of glucose metabolism (glucose tolerance and/or insulin resistance) in ALL survivors correlate to glucose levels during therapy, total steroid or asparaginase dose, or type of steroid (dexamethasone vs. prednisone) received during treatment.
Design/Methods: Retrospective cohort study with prospective follow-up. Subjects are patients diagnosed with ALL in 1999–2003 at ages 1–18 years. All subjects had completed therapy and are in CR1. One-time oral glucose tolerance testing (OGTT) was performed. Medical records were reviewed for demographic, treatment, and anthropomorphic data. Data was analyzed in linear and logistic regression models.
Results: 15 subjects (9 female) have been evaluated. Mean diagnosis age 5.5 years (range 2.4–14.9) years, mean study age 10.9 years (range 7.2–20.3 years), mean time off therapy 2.8 years (range 1.3–6.0 years). Three subjects had transient hyperglycemia (defined as $\geq$2 random glucose values $\geq$200 mg/dl) during treatment for ALL. No subjects had glucose intolerance by OGTT at the time of the study. Four subjects had insulin resistance, with fasting insulin levels $\geq$15 mg/dl. BMI at study time, evaluated as z-score for age, strongly predicted insulin resistance, based on many measures, including fasting insulin ($p=0.002$), HOMA index ($p=0.002$), Matsuda index ($p=0.01$) and insulin AUC ($p=0.004$). Insulin resistance was not predicted by glucose levels during treatment, cumulative steroid or asparaginase dose, or type of steroid received (dexamethasone or prednisone). Similarly, other demographic factors (age, sex, pretreatment BMI) did not predict insulin resistance independent of BMI at study time.
Conclusions: Based on this preliminary data, ALL therapy does not appear to increase risk of glucose intolerance or insulin resistance in the first few years after completion of therapy. Elevated BMI was a strong predictor of insulin resistance, as it is in the general population. This study is ongoing and further data may elaborate upon these results.

Poster 182

Early Chronic Cardiomyopathy Following Exposure to Anthracyclines Exacerbated by All-Trans Retinoic Acid; Role of B-Type Natriuretic Peptide as an Indicator of Cardiac Dysfunction

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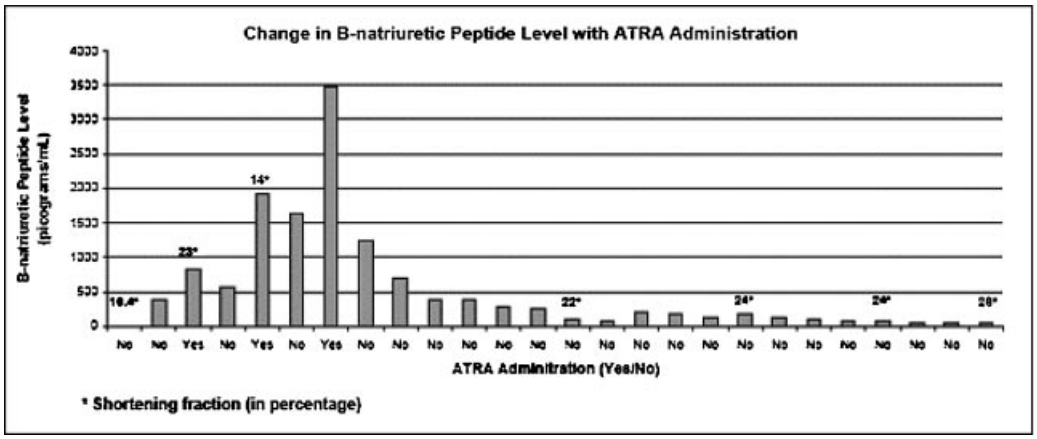
Background: Chronic anthracycline cardiotoxicity presents in an early or late form. The early form occurs within one year of treatment and is directly related to the cumulative dose of anthracyclines (700 mg/m^2 for daunomycin). Literature is incomplete on the cardiotoxic effects of All-Trans Retinoic Acid (ATRA) in combination with anthracyclines and regarding the significance of B-Natriuretic Peptide (BNP) levels in cardiac dysfunction.

Objective: We report the case of a 14-year old female with acute promyelocytic leukemia (APL) who developed symptomatic cardiomyopathy only four months into treatment with a combination of daunomycin and ATRA. Despite cessation of daunomycin, she demonstrated fluctuating cardiac function related to ATRA administration. Improvement and deterioration in left ventricular shortening fraction (LVSF) and serum BNP levels were seen while receiving ATRA one week on and one week off, respectively, during Maintenance chemotherapy.

Design/Methods: A 14-year old previously healthy Caucasian female was diagnosed with APL on morphology, cytogenetics, FISH and RT-PCR for t(15;17) on a bone marrow sample. Routine cardiac evaluation after one cycle of Induction with ATRA, daunomycin and cytarabine, showed normal intracardiac anatomy. She then received two cycles of Consolidation with ATRA, daunomycin and cytarabine, followed by a prolonged Maintenance therapy consisting of oral 6-mercaptopurine, oral ATRA (30 mg/m^2/day for seven days every two weeks) and oral methotrexate.

Results: The patient developed a syncopal episode following course #2 of Consolidation. Cardiology evaluation revealed moderately decreased left ventricular systolic function with markedly reduced LVSF and a high BNP level. She required treatment with digoxin and an angiotensin-converting enzyme (ACE) inhibitor. Despite discontinuation of daunomycin (after cumulative dose of 515 mg/m^2), she continued to have worsening and then improvement of LVSF on serial echocardiograms and waxing and waning of serum BNP levels, depending upon intermittent exposure to ATRA (one week on and one week off) during the Maintenance phase of therapy.



Conclusions: To our knowledge, this is the first report describing worsening cardiac function and rising BNP levels and then improvement with intermittent exposure to ATRA. Large prospective studies to determine the role of ATRA with regards to long-term cardiac sequelae are needed.

Poster 183

Live and Let Die: A Case Report of Spontaneously Resolving Leukemia Cutis with Evidence of Apoptosis

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Background: Congenital leukemia cutis is a rare and poorly understood entity. Infants present with firm erythematous to violaceous "blue berry muffin" skin lesions. These lesions may precede other signs of leukemia by several months. Spontaneous regression of leukemic skin infiltration without leukemic progression has also been described. Mechanisms and predictors of spontaneous regression remain an enigma.

Objective: To describe the evaluation and longitudinal follow up of an infant presenting with congenital leukemia cutis. We describe the spontaneous regression of multiple subcutaneous myelomonocytic blast infiltrations. We document evidence of apoptosis (by histology and Caspase-3 immunohistochemistry) in a representative lesion.

Design/Methods: Case report of an infant with spontaneously resolving leukemia cutis.

Results: A male term newborn without dysmorphic features was noted to have multiple raised, blue-red skin papules on the face and trunk at the time of delivery. TORCH infections were ruled out. A CBC was within normal limits. A bone marrow aspirate showed 5% of nucleated cells with morphologic features consistent with myelomonocytic blasts. Fluorescent *in situ* hybridization showed a 7.2% population within the marrow harboring a deletion of chromosome 7q, but no evidence of rearrangement involving 11q 23. Skin biopsy of a lesion on the patient's trunk revealed a monomorphic population of myelomonocytic blasts with many pyknotic cells consistent with apoptosis. Immunohistochemistry using an antibody for activated caspase-3 was positive in 5–10% of the infiltrating cells. Spontaneous regression of the skin nodules occurred over the first two weeks of life and the decision to continue close monitoring was made. Serial bone marrow aspirates showed resolution of the 7q deletion abnormality by two months of age. The patient remains free of disease at 14 months old and is growing and developing normally.

Conclusions: We describe a case of spontaneous regression of congenital leukemia cutis. Significant caspase-3 dependent apoptosis of the myelomonocytic blasts was clearly evident in the skin lesions as was the absence of the MLL rearrangement. The predictive value of these findings warrants further investigation and may provide guidance regarding the decision to intervene or observe infants with this condition.

Poster 184

Urinary Tract Infections in Pediatric Cancer Patients with Fever and Neutropenia

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Background: Neutropenia increases the risk of serious infections in pediatric cancer patients. However, the relevancy of the urinary tract as a source of infection is not known.

Objective: To determine the incidence of urinary tract infections(UTI) and usefulness of urinalysis and localizing signs(dysuria, frequency, hematuria) in predicting UTI in pediatric cancer patients with fever and neutropenia.
Design/Methods: Urine was collected from a mid-stream void after sterilizing the external genitalia with betadine before the administration of antibiotics. Positive urine culture was defined as growth of a pathogen at $>10^4$ CFU/mL. A diagnosis of UTI was based on a positive urine culture. Neutropenia was defined as an ANC of <500. Demographic, clinical, and laboratory data were collected.
Results: 39 patients had 51 febrile neutropenic episodes eligible for study participation. The 20 boys and 19 girls had a median age of 8.5 years. 14 of the 20 boys were circumcised. The most common diagnosis was acute lymphoblastic leukemia (n = 20). No patient presented with localizing signs. All patients were receiving P.carinii prophylaxis with Septra except 1. The urinalysis was negative in 46 cases and positive in 5 cases. 4 patients had 5 episodes of UTI- 2 patients had 3 E.coli UTI, 1 had vancomycin resistant enterococcus (VRE) UTI, and 1 had Stenotrophomonas multiphila UTI. The E.coli and VRE isolates were resistant to Septra and the patient with trimethoprim/sulfamethoxazole sensitive S.multiphila was not taking Septra. The incidence of UTI was 9.8% (5 of 51). 4 patients had bacteremia none of whom had a UTI. The specificity and negative predictive value of urinalysis was 93.2% and 95.3%, respectively and for localizing signs was 100% and undefined, respectively.
Conclusions: UTI are as common as bacteremia in our pediatric cancer patients with fever and neutropenia. Urinalysis and urine culture should be obtained in the evaluation of patients with fever and neutropenia. Septra prophylaxis may reduce the incidence of UTI in this at-risk population.

Poster 185

Is Chemotherapy Necessary for Secondary HLH with Treatable Etiology?

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Background: Secondary HLH usually treated with HLH-2004 protocol and specific medications if etiology is treatable.
Objective: To study and evaluate the outcome of secondary HLH in a university hospital in Sultanate of Oman.
Design/Methods: 5 children, 3 boys and 2 girls with age ranging from 3 months to 2 years with a diagnosis of secondary HLH with treatable etiology were studied.
Results: All the 5 children fulfilled the criteria for diagnosis of HLH. 3 children had hemophagocytosis in Bone Marrow aspiration. All had confirmed etiology. Two had Leishmaniasis confirmed by Bone Marrow and 3 had CMV infection confirmed by PCR. Positive both in blood and plasma. Genetic studies, history of neonatal deaths and first degree consanguinity were negative. First child with Leishmaniasis was treated with 4 weeks with HLH-2004 protocol and child didn't show significant improvement. Leishmaniasis was diagnosed at that time and treated accordingly and improved well clinically and BM also showed disappearance of hemophagocytosis. Second child with Leishmaniasis got treated only with Ambisome and recovered. Third child received chemotherapy from HLH-2004 and then diagnosed as CMV and got treated with gancyclovir and recovered. Fourth child confirmed as CMV initially and was very sick and started on HLH-2004 and Gancyclovir but by second week child expired with DIC.Fifth child diagnosed initially as CMV and treated with only gancyclovir and improved.
Conclusions: Our study shows that in secondary HLH with treatable etiology child may recover with specific treatment without chemotherapy. The diagnosis should be considered early in patients with unremitting fever, hepato splenomegaly and cytopenias.What constitutes the best treatment approach is still controversial especially with secondary HLH. The HLH protocol should be started without delay for severe disease or primary HLH. For mild non-familial disease, IVIG had been suggested by some authors as the initial treatment although confirmed evidence is still lacking. Further studies are necessary to compare the efficacy of different treatment options. It is hoped that early diagnosis and intervention will increase the chance of survival.

Poster 186

Multiple Lymphocyte Activation Markers in Childhood Oncology Patients with Febrile Neutropenia: Correlates with Culture-Positive Infection

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Background: Febrile neutropenia is a common problem necessitating emergency hospitalisation and empirical broad-spectrum antibiotic therapy due to the risk of bacteremia. Determining whether bacteremia is the cause of febrile neutropenia is difficult with current laboratory methods. Hence, clinicians are searching for a rapid test that may add to the exclusion or diagnosis of infection underlying febrile neutropenia.
Objective: To develop a rapid laboratory test based on markers of lymphocyte activation that may add to the exclusion or diagnosis of infection underlying febrile neutropenia in childhood oncology patients and also to correlate their significance with plasma levels of IL-5 and IL-8.
Design/Methods: 31 pediatric oncology patients with febrile neutropenia were included. Multiple plasma cytokine levels using CBA and range of lymphocyte activation markers were performed in addition to the complete blood counts, blood culture and CRP.
Results: TAB showing percentages of lymphocyte subsets expressing activation markers and CRP for patients with positive and negative blood cultures.

Culture positive pateints	25/3	DR/3	25/DR/3	62L-/45RO/4	45RO/4	CCR5/3	CCR4/3	CRP	IL-5	IL-8	IL-12
1	51	6	12	42	99	230	1	42	99*	230	1
2	48	6	1	12	83	96	0	12	83	96	0
3	52	68	26	118	66	51	1	118	66	51	1
4	40	6	16	64	99	158	0	64	99	158	0
5	8	15	1	<5	77	470	3.3	<5	77	470	3.3
6	11	29	3.9	240	63	231	3.6	240	63	231	3.6
7	24	2	4	ND	69	333	2.5	ND	69	333	2.5
8	18	0	31	5	14	72	0	5	14	72	0
Culture negative range (mean±2sd)	<33	<21	<3.7	<138	<82	229	<2.1	<138	<82	229	<2.1

Conclusions: Elevated T-cell expression of CD25 and HLA-DR proved a relatively sensitive and specific indicator of infection.
A combination of T cell expression of CD25 and HLA-DR and plasma IL5 and IL-8 levels are a very sensitive and specific indicator of infection in childhood oncology patients with febrile neutropenia.

Poster 187

Acute Lymphocytic Leukemia in Relapse: A Retrospective Analysis of 25 Years' Experience at Cook Children's Medical Center

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Background: Long-term remission and survival rates approach 80% for children with ALL treated on contemporary protocols. However, 25% of patients are expected to suffer a relapse. The purpose of this study is to analyze the outcomes of children diagnosed with ALL at Cook Children's Medical Center who have experienced relapse at any site. We seek to identify successes and challenges to improving outcomes and better understanding of the disease. The length of first remission, relapse site, and risk assignment at diagnosis and at relapse influence success in treatment. Patients considered in long-term remission prior to relapse (bone marrow >3 years, extramedullary >18 months), have higher survival rates than do those after early relapse.

Objective: We plan to assess the role of different factors that affected response to therapy after relapse of ALL at our center.

Design/Methods: The study population is derived from patients whose initial diagnosis and subsequent relapse of ALL occurred at Cook Children's from 1982 through September 2007. A total of 617 children were diagnosed with ALL at Cook Children's during these 25 years, of whom 100 (16%) relapsed. Two (2) of the 100 patients are excluded due to incomplete records. The male to female ratio is 63:35 and the range of age at diagnosis is 3 months to 19 years (Median: 5yrs, 7mo). Primary sites of relapse are consistent with national data: 61 bone marrow, 28 CNS, 3 bone marrow and CNS, 2 bone marrow and testicular, 1 bone marrow and mediastinal, 1 nodal, and 2 isolated testicular.

Results: A total of 41 patients underwent bone marrow or stem cell transplantation to manage their relapsed disease, 39 allogeneic and 2 autologous. Transplantation occurred in second complete or partial remission. Twenty-one (21) of the 41 patients are alive in remission, post-transplant. Among the 98 patients who relapsed, 46 are alive, 39 in second complete remission and 7 in third complete remission.

Conclusions: Although these results are encouraging, further studies are necessary to determine the best therapies for relapsed patients. The presentation will include an analysis of clinical, biological, and treatment factors that influence outcome after relapse of childhood ALL at a single institution.

Poster 188

Acute Promyelocytic Leukemia (APL) in a Pediatric Down Syndrome (DS) Patient

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Background: Patients with Trisomy 21 have an increased incidence of leukemia. Although Acute Lymphocytic Leukemia (ALL) is most commonly seen, these patients have a higher incidence of Acute Myelocytic Leukemia (AML). Of the eight subtypes, acute megakaryoblastic leukemia (M7) is the most common. A 12 y/o male presented with diffuse petechial rash and no hepatosplenomegaly or lymphadenopathy on exam. CBC revealed anemia, thrombocytopenia and 25% blasts on smear. Upon further evaluation, he was diagnosed with APL. Overall, only 5- to 10% of patients with AML are diagnosed with acute promyelocytic leukemia (M3).

Objective: To present a pediatric patient with DS and AML, M3.

Design/Methods: Medical records were reviewed following confidentiality guidelines. A literature search and review were performed. Pathology evaluation consisted of bone marrow aspirate and biopsy. Diagnostic testing included immunophenotyping, histochemistry and cytogenetics. This demonstrated a monomorphic population of granular cells with CD13 and CD33 positivity (myeloid cell markers) that confirmed the diagnosis of AML. In addition, myeloperoxidase and chloroacetate esterase stains were positive when compared with standardized controls. FISH revealed translocation (15:17), which is diagnostic of APL. Cerebrospinal fluid evaluation revealed no blasts.

Results: Therapy was started according to CALGB protocol C9710. This regimen consists of all-trans-retinoic acid (45 mg/m^2/day) Day 1 to clinical remission, four days of Daunorubicin (50 mg/m^2) and seven days of Cytarabine (200 mg/m^2). Patient experienced profound myelosuppression following initiation of therapy requiring supportive care accordingly. Resolution of blasts in peripheral blood was documented after four days of therapy. Further evaluation is scheduled at the end of induction with subsequent therapy to follow once Bone Marrow remission is accomplished.

Conclusions: M7 occurs in 1 in 50 to 200 of patients diagnosed with AML. DS patients with APL are very rare. This diagnosis however is associated with higher rates of complete remission and event-free survival. Patients follow standard therapy with side effect profile similar to non-DS. We report 1 case of a patient currently undergoing therapy.

Poster 189

Comparison of Psychosocial Issues in the Treatment of All And AML in Children

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Background: Children with leukemias comprise the largest group of children diagnosed with cancer today. Acute lymphoblastic leukemia (ALL) comprises the majority of this type of malignancy with acute myelogenous (AML)leukemia being less frequent (approximately 7,000 versus 500 children diagnosed in the United States each year). Children with ALL undergo a longer period of treatment due to maintenance therapy whereas children with AML have a shorter but more intense therapy overall often leading to blood or marrow transplantation. This study compares the psychosocial impact of types of therapy, length of hospital admissions and use of BMT on patients and their families. It explores whether the overall stressors for these children and families were significantly different based on type of leukemia diagnosis.

Objective: To evaluate the differences in family/patient experiences with treatment and psychosocial stressors between the group of children with ALL and the group of children with AML at the Children's Hospital of Pittsburgh.

Design/Methods: The study focuses on the patient population diagnosed with ALL or AML leukemia at the Children's Hospital of Pittsburgh since 1992. The author developed a questionnaire to interview medical and psychosocial staff and families who agreed to participate. The questionnaire incorporated specific experiences and general areas such as reaction to diagnosis, hospital and clinic experiences, family stressors and other psychosocial issues.

Results: Of the twenty-five families interviewed those whose children were diagnosed with AML exhibited greater overall stress levels than the families of children who were diagnosed with ALL. These differences were accounted for largely by the more intensive treatments, longer hospital admissions and frequent need for BMT of children with AML experienced. Also the greater relapse rate for children with AML was a significant area of psychosocial stress for these families.

Conclusions: Although the families of children ALL had high levels of psychosocial issues and concerns, including the longer duration of treatment, the families of children with AML treated at the Children's Hospital of Pittsburgh indicated greater overall psychosocial issues such as depression, anxiety and financial concerns than the ALL group.

Poster 190

Subacute Methotrexate Neurotoxicity and Cerebral Venous Thrombosis in a 12-Year Old Patient with Acute Lymphoblastic Leukemia and Homozygous Methyl Tetrahydrofolate Reductase (MTHFR) C677T Polymorphism

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Background: Methotrexate has been associated with many complications involving the Central Nervous System. Methotrexate-induced neurotoxicity is widely classified into three forms. An acute syndrome includes nausea, vomiting, headaches, lethargy and seizures. A subacute syndrome occurs 1–2 weeks following treatment and manifests as encephalopathy with hemiparesis, ataxia, speech disorders, seizures and affective disturbances. Chronic neurotoxicity is characterized by a delayed leukoencephalopathy of variable severity. A clear mechanism for methotrexate-induced neurotoxicity remains to be established.

Objective: We suggest homocysteine as a mediator in methotrexate-induced neurotoxicity via vascular damage.

Design/Methods: We report a 12-year old Caucasian male with pre-B ALL who was treated *as per* the high-risk ALL protocol, CCG 1961. During Delayed Intensification I phase, he developed a generalized tonic-clonic seizure four days after receiving intrathecal methotrexate. He had not received PEG-asparaginase for six weeks and vincristine for four weeks prior to the seizure episode. Subsequently, he developed right hemiparesis, aphasia, altered mental status and persistent seizures. An electroencephalogram showed generalized slowing. Magnetic resonance imaging (MRI) scan of the brain showed evidence of restricted diffusion in the bilateral centrum semiovale with confluent non enhancing T2/FLAIR hyperintensities indicative of white matter injury likely related to methotrexate toxicity and absence of normal flow in the left transverse and sigmoid sinuses.

Results: Thrombophilia evaluation revealed homozygosity for the MTHFR C677T mutation with a serum homocysteine level of 13 μmol/L and undetectable CSF homocysteine. The patient was started on enoxaparin, folic acid and dextrometorphan. Repeat MRI on day # 10 of this episode was significant for the presence of prominent bilateral white matter infarcts involving large portions of the corona radiata and centrum semiovale bilaterally (Right>Left). Following an initially worsening course, he showed dramatic neurological improvement. Dextromethorphan was discontinued shortly after signs of recovery. Subsequent MRI scans showed resolution of the diffusion abnormality and stable left transverse sinus thrombosis.

Conclusions: The combined findings of sinus venous thrombosis and ischemic white matter changes, with mildly elevated plasma homocysteine and homozygous presence of the MTHFR C677T polymorphism, argues for the role of homocysteine as a mediator in methotrexate induced neurotoxicity via vascular damage.

Poster 191

Vanishing Bile Duct Syndrome and Hodgkin's Lymphoma: A Case Series of Two Unique Pediatric Patients

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Background: Vanishing bile duct syndrome (VBDS) is a rare and irreversible disorder characterized by cholestasis and jaundice, as a result of intrahepatic biliary duct obstruction. VBDS is uncommon in pediatrics. Possible etiologies include viral infection, drug toxicity, or an immunodeficiency syndrome. The concurrent diagnoses of VBDS and Hodgkin's Disease (HD) has previously been reported three times in the pediatric literature, each patient presenting with cholestatic liver disease at the time of HD diagnosis.

Objective: We report two cases of VBDS occuring in the setting of pediatric HD. Our goal in this case series is to convey the importance of recognizing VBDS in a patient with HD, and to define optimum treatment strategies for HD therapy in patients with VBDS.

Design/Methods: Retrospective chart review was performed on two cases in which VBDS occurred in combination with HD, at two different institutions.

Results: Both patients in our series developed VBDS preceding the diagnosis of HD. In the first case, the patient had an ill-defined immunodeficiency syndrome, pseudopolyp-colitis, sinopulmonary disease, a seizure disorder, and was diagnosed with EBV+ Stage IIIB HD three months after a diagnosis of VBDS. He achieved complete remission after escalating doses of a modified MOPP-ABV regimen, with transient elevation in bilirubin during times of infection. Overall, his cholestatic picture did not worsen over the course of HD therapy. However, this patient died (likely due to status epilepticus aspiration) three months off therapy, while in remission. In the second case, the patient was misdiagnosed with tuberculosis, received medical management for TB, and developed cholestasis prior to diagnosis with Stage IIIB HD. This patient also completed modified MOPP-ABV therapy and achieved complete remission. Five years later, he is currently awaiting liver transplantation because of progressive VBDS.

Conclusions: In the setting of HD, it is important to consider a diagnosis of VBDS in a patient with severe cholestasis. Liver biopsy is beneficial to ascertain a diagnosis prior to initiating HD therapy. From our series, we show that hepatotoxic chemotherapy can be safely given and complete remission from HD can be achieved with current therapy, but these patients will likely have long-term morbidity from VBDS, which is often life-threatening.

Poster 192

Cerebral Venous Sinus Thrombosis, Infection, Steroids and Pegaspargase

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Background: Cerebral venous sinus thrombosis is an unusual occurrence in the treatment of leukemia or lymphoma. Dehydration, infection, a prothrombotic state, and agents such as steroids and asparaginase have been implicated as factors.

Objective: We report three cases of cerebral venous sinus thrombosis in children being treated for leukemia or lymphoma, and the natural history of their resolution.

Design/Methods: Records were reviewed. Two children (A and B) had acute lymphoblastic leukemia (CNS negative) with new neurologic symptoms. The third child (C) had stage IV T-cell lymphoma (CNS+, BMA+ 15%); the thrombus was found incidentally during re-staging scans. During this 12 month period our service cared for ten other children on similar regimens.

Results: Child A was a 4 year old girl, day 23 of a delayed intensification phase when she developed dehydration, pansinusitis, pancytopenia, fever, and left leg weakness; a scan showed thrombosis. She had received pegaspargase day 3. Her factor 8 was 274%, PTT 50.9 seconds, and D-Dimer elevated, but other studies were normal. Therapy included enoxaparin, then aspirin. Child B was a 6 year old boy, day 22 of induction with pancytopenia when he had a scan showing pansinusitis and thrombosis. There were no specific neurologic symptoms. He had received pegaspargase day 3. Therapy was enoxaparin. Child C was a 20 month old boy, day 35 of induction when he had a restaging scan after an episode of fever and pancytopenia. There were no specific neurologic symptoms and no sinusitis. He had received pegaspargase day 3. He was found to be a Factor V Leiden heterozygote, factor 8 was 231% with elevated D-Dimer. Therapy was enoxaparin then aspirin. Each had been on prednisone or dexamethasone along with other chemotherapy. Each child had clots involving the right transverse and sigmoid venous sinuses. Follow up scanning revealed clot resolution in 5 weeks for child B, 10 months for children A and C.

Conclusions: Cerebral venous sinus thrombosis can be seen during the course of therapy for leukemia or lymphoma. Subsequent neuroimaging revealed resolution of thrombus in these three patients.

Poster 193

Treatment Related Complications in a Young Girl with Acute Lymphoblastic Leukemia May be Due to Uncovered Mutations in the PKD1 And MTHFR Genes

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Background: Underlying genetic polymorphisms and genetic mutations are emerging as important factors that influence outcomes during the treatment of childhood cancer, such as delays in treatment, chemotherapy-related toxicity and potentially, overall survival.

Objective: We describe the case of a 5 year old girl with acute lymphoblastic leukemia (ALL) who experienced multiple complications of therapy leading to the identification of an underlying genetic mutation in the *PKD1* gene and a polymorphism in the *MTHFR* gene, both of which may have contributed to her susceptibility to chemotherapy induced toxicity.

Design/Methods: Descriptive case report.

Results: A 5 year old girl was admitted to hospital with the diagnosis of ALL and a white blood cell count of 134×10^9/L. She received induction chemotherapy with intrathecal cytarabine (Day 0), dexamethasone, vincristine, and daunorubicin. Despite preventive measures, she rapidly developed tumour lysis syndrome and acute renal failure requiring renal dialysis. Serial abdominal ultrasonography documented the gradual development of multiple cysts in the kidneys bilaterally throughout induction treatment. Following intrathecal methotrexate (Day 8), she developed a seizure, and was diagnosed with posterior reversible encephalopathy syndrome (PRES). She subsequently was found to be homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene, a polymorphism associated with increased methotrexate toxicity. During consolidation, following administration of PEG-asparaginase, she was re-admitted to hospital with a large complicated pancreatic pseudocyst, which required multiple drainage procedures and finally a pancreatic-gastric stent, before fully resolving. The combination of bilateral kidney cysts and pancreatic pseudocyst suggested autosomal dominant polycystic kidney disease (PKD). This diagnosis was confirmed by molecular analysis, which found a novel frameshift mutation in one of the patient's *PKD1* genes (1bp deletion of C at codon 633).

Conclusions: This case suggests that chemotherapy may accelerate PKD cyst development and provides evidence that both common genetic polymorphisms and rare mutations may contribute to treatment-related complications. Future treatment approaches will need to incorporate genomic data to individualize therapy in efforts to improve survival and minimize treatment related complications.

Poster 194

Treatment-Refractory Multisystemic Langerhans Cell Histiocytosis with Risk Organ Involvement: A Review of the Pediatric Literature

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Background: Children with multisystemic Langerhans cell histiocytosis (LCH) with hematologic, lung, liver, or spleen involvement are at high risk for progression of their disease. The evidence for how to manage these children with disease progression consists primarily of small case series and anecdotal treatments that are not readily generalized.

Objective: We reviewed the literature to make these observations regarding the management of children with high risk LCH more usable and to direct future therapeutic trials.

Design/Methods: A PubMed search was performed using the following terms: ''Langerhans cell histiocytosis'', ''Letterer-Siwe disease'', ''Hand-Schuller-Christian syndrome'', ''eosinophilic granuloma'', ''infant(s)'', ''neonate(s)'', ''child/children'', ''adolescent(s)'', ''young adult(s)'', ''twin(s)'', and ''treatment''. Articles published between 1954 and March 2007 were reviewed. Inclusion criteria were: An ascertainable disease course, pathologic findings consistent with a diagnosis of LCH, high risk multisystem disease, and failure of initial systemic therapy. These criteria yielded 180 patients, 140 of whom were high risk both at diagnosis and salvage therapy and who failed intensive prior treatment – defined as multiagent therapy or stem cell transplantation.

Results: Salvage therapies used for this high risk group were varied, as were outcomes (**Table 1**):

Table 1: Salvage Therapies and Outcomes

Salvage Outcome	Steroids	Radiation only	Chemo+/− steroids	SCT	Non-steroidal immunosuppressive therapy
Dead	2 (100%)	3 (100%)	29 (38%)	10 (32%)	19 (70%)
Alive with disease	–	–	22 (28%)	–	4 (15%)
No evidence of disease	–	–	26 (34%)	21 (68%)	4 (15%)

Chemo = vinblastine, etoposide, etc.; SCT = stem cell transplantation; Non-steroidal immunosuppressive therapy = cyclosporine, etanercept, etc.

Conclusions: Children with high risk LCH both at the times of initial diagnosis and salvage therapy who fail intensive prior treatment have a high mortality rate. For these children, chemotherapy, stem cell transplantation, and non-steroidal immunosuppressive therapies are the most commonly used salvage regimens. Based on this analysis, non-steroidal immunosuppressive agents appear to be less effective than other treatment modalities for high risk patients with recurrence, and should be avoided outside the setting of clinical trials. Prospective randomized trials are needed to evaluate treatment options in children with high risk LCH.

Poster 195

Success Safety of Trans-Jugular Liver Biopsy (TJLB) As An Interventional Radiology Tool to Investigate Persistent Liver Function Abnormalities in Children with Hematologic Disorders

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Background: Liver function tests (LFT) abnormalities are common during treatment of acute childhood leukemia and hematologic disorders. They may be caused by disease, chemotherapeutic agents, supportive care agents, infections or underlying genetic predisposition. Abnormal LFT may affect our ability to deliver chemotherapy at the intended doses or in a timely fashion. Percutaneous needle, open or laproscopic biopsy may be fraught with risk of bleeding in an acutely ill child with low platelet count and coagulopathy.

Objective: To evaluate safety and utility of TJLB in children with hematologic disorders and liver dysfunction.

Design/Methods: We examined records of 3 children (2 leukemias, one Pearson syndrome) with persistent LFT abnormality, where an interventional radiologist obtained a TJLB. Note was made of diagnosis, indication, complications, pathology and its clinical relevance

Results: The first case was a 14 yr old girl with Down syndrome who had two episodes of hepatic failure associated with coagulopathy, each following a course of consolidation chemotherapy. TJLB showed cirrhosis. She was switched to chemotherapy with low dose mercaptopurine and methotrexate was omitted. The second case was a 15 yr old boy who was on maintenance phase of ALL chemotherapy and had two episodes of fever, diarrhea, direct hyperbilirubinemia and transaminasemia. His TJLB showed hemosiderosis. He was heterozygous for HFE gene mutation. The third case was a 3 year old girl, a known case of Pearson syndrome on chronic transfusions, who had progressive hepatomegaly and worsening synthetic

and detoxifying liver functions. TJLB showed significant hemosiderosis. She was started on oral iron chelation but succumbed to her disease. No complications were encountered in any patients.

Conclusions: Abnormal LFT has varied causes in children with hematologic disorders. Percutaneous liver biopsy may be difficult or dangerous to do because of coagulopathy, low platelet count and ascitis. TJLB yielded clinically relevant information in each of the three cases, helping with therapeutic decisions. TJLB is a safe and effective tool for evaluation of an abnormal liver in children with hematological disorder.

RED CELLS

Poster 196/PLATFORM SESSION 302

Serum and Urine Hepcidin Levels in Patients with Beta-Thalassemia or Sickle Cell Disease

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Background: Hepcidin is a key regulator of systemic iron metabolism and is implicated in the pathogenesis of iron disorders. In normal homeostasis, iron loading and inflammation increase hepcidin levels, and erythropoietic activity and hypoxia decrease hepcidin. Only limited studies measuring urinary hepcidin in thalassemia or sickle cell disease (SCD) have been reported.

Objective: In the present study, we report for the first time serum hepcidin measurements in addition to urinary hepcidin, as well as measurements of comparative bio-markers of erythropoietic drive in transfused β-thalassemia and SCD patients.

Design/Methods: Thirty seven patients with hemoglobinopathies and 24 normal controls were enrolled after approval by IRB and informed consent. Liver iron concentration (LIC) was measured by a SQUID Ferritometer®. Serum and urinary hepcidin were determined by competitive ELISA (Intrinsic LifeSciences, La Jolla, CA) which detects mature bioactive hepcidin peptide.

Results: Transfused thalassemia and SCD patients had higher levels of serum hepcidin compared to controls ($p<0.003$). Serum hepcidin correlated with urinary hepcidin ($r=0.41$, $p=0.031$). There was no significant difference in either serum or urinary hepcidin levels between transfused thalassemia or SCD patients (mean serum hepcidin 265±182 vs. 179±83 ng/ml, respectively). The most significant correlation in transfused patients was found between serum hepcidin and soluble transferrin receptor ($r=-0.52$, $p<0.001$). Also, serum hepcidin was correlated with serum ferritin ($r=0.43$, $p=0.024$). LIC did not correlate with serum hepcidin ($r=0.33$, $p=0.08$). In a step wise multivariate analysis of transfused patients ($n=31$) soluble transferrin receptor ($r=-0.53$, $p=0.004$) and LIC ($r=0.63$, $p=0.054$) were found to be the most robust predictors of serum hepcidin.

Conclusions: In transfusion-dependent patients with β-thalassemia or SCD, iron load and lowered erythropoietic drive, result in relatively higher hepcidin levels than controls. The increased hepcidin levels could be protective by decreasing dietary iron absorption and favoring retention of iron in the reticuloendothelial system. Studies are in progress to examine hepcidin levels in non-transfused β-thalassemia and SCD patients.

Poster 197

Patients with Sickle Cell Disease Have Increased Clearance of Morphine in Steady State of Health

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Background: Standard doses of morphine fail to provide adequate analgesia in many individuals with sickle cell disease (SCD). Although evaluated extensively in non-SCD individuals, morphine pharmacokinetic (PK) parameters have not been well described in SCD population. Unique alterations in hepatic and renal function associated with SCD may alter the morphine PK parameters.

Objective: To study pharmacokinetic parameters of morphine in individuals with SCD in a steady (no pain) state of health.

Design/Methods: Study participants received a single 30-minute infusion of 0.1 mg/kg of morphine sulfate. Post-infusion 24 hour timed blood samples were analyzed by mass-spectroscopy for morphine and its metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Data from 18 participants with SCD with normal serum creatinine and hepatic enzymes were analyzed. The USC*PACK non-parametric population modeling software collection was used to fit candidate PK models to the time-concentration data for morphine, M3G and M6G. Influence of weight, sex, and creatinine clearance (CrCl) on PK parameter estimates was tested.

Results: Mean age and weight of participants was 20 years and 58.9 kg. 83% had Hb SS, and 55% were female. Only one individual had a CrCl <100 ml/min. In the final PK model, overall mean±SD morphine clearance was 2.9±0.75 L/h/kg. Morphine distributed from a central compartment to two peripheral compartments, while M3G and M6G each had a central and one peripheral compartment. PK parameter values were not affected by weight and creatinine clearance. Women had a 40% larger morphine AUC (Area Under the time-concentration Curve) ($P=0.03$) and 32% lower clearance ($P=0.02$) than men, but there was no difference in AUC for either M3G or M6G based on gender.

Conclusions: Mean morphine clearance in SCD individuals is approximately three times previously reported clearance in non-SCD individuals. Many SCD patients particularly men, may require higher and more frequent dosing of morphine to achieve similar systemic exposure to patients without SCD.

Poster 198

Secretory Phospolipase A_2 Releases Pro-Inflammatory Lipids From Sickled RBCs that Cause PMN-Mediated Cytotoxoicity of Pulmonary Endothelial Cells

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Background: The acute chest syndrome (ACS), a form of acute lung injury (ALI) distinct to patients with sickle cell anemia (SCA), is a serious complication of SCA and results in significant morbidity and mortality. Elevated levels of secretory phospholipase A_2 ($sPLA_2$) (healthy 10±8.4, ACS 336±209 ng/ml) predict ACS in SCA patients and cleave phospholipids to release pro-inflammatory lipid mediators. Such lipids are implicated in other forms of ALI, which are postulated to be the result of two events.

Objective: We hypothesize that ACS is caused by $sPLA_2$ production of lipid mediators from sickled red blood cells which cause neutrophil (PMN)–mediated cytotoxicity as the second event in a two-event model.

Design/Methods: $sPLA_2$ levels were measured in pediatric SCA patients when healthy and during hospitalizations for VOC and ACS by ELISA (ng/ml). Heparinized whole blood was collected from pediatric SCA patients when healthy or daily when they were admitted for vaso-occlusive pain (VOC) or ACS. The plasma was separated and the RBCs were incubated with 50 μg/ml $sPLA_2$ for 60 min at 37°C. Lipids were extracted from plasma and the $sPLA_2$-treated PRBCs ($sPLA_2$-lipids). PMN-elicited cytotoxicity was quantified by counting viable human microvascular endothelial cells per mm^2 (HMVECs), which were grown to 80% confluence. The HMVECs were activated with buffer or 2 mg/ml endotoxin (LPS) (6 hours), isolated PMNs were added and allowed to settle, and then the plasma lipids or $sPLA_2$-lipids were added and incubated for 30 min.

Results: Levels of $sPLA_2$ correlated with the development of ACS (healthy: 16±2, VOC 128±36, and ACS: 292±79* ng/ml, * = p<0.05 vs. healthy). The lipids from ACS plasma and the $sPLA_2$-lipids from VOC and ACS patients caused significant cytotoxicity as compared to the controls: buffer, 257±12/mm^2; ACS plasma, 133±14*; ACS $sPLA_2$-lipids, 135±31*; VOC plasma, 193±17; VOC $sPLA_2$-lipids, 171±7*; healthy plasma, 200.5±4.5; healthy $sPLA_2$-lipids, 216±28, * = p<0.01).
Conclusions: We conclude that increased $sPLA_2$ levels were associated with ACS and the $sPLA_2$-lipids from patients with VOC and the plasma lipids and $sPLA_2$-lipids from ACS caused PMN-mediated cytotoxicity as a second event in a two-event model. These data support the hypothesis that ACS is PMN-mediated similar to other types of ALI.

Poster 199/PLATFORM SESSION 302

Prospective Assessment of Laboratory Markers of Cardiovascular Risk in Hereditary Spherocytosis

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Background: Individuals who have undergone splenectomy for various conditions may be at an increased risk of vascular complications such as arterial and venous thrombosis and pulmonary arterial hypertension.
Objective: To determine if splenectomy affects surrogate laboratory measures of cardiovascular risk in persons with hereditary spherocytosis (HS).
Design/Methods: We conducted a prospective cross-sectional study of 21 children >5 years of age and 36 adults with HS. Fasting blood samples were collected for lipid panel, homocysteine, lipoprotein (a), complete blood count, glucose, C-reactive protein (CRP), and fibrinogen. When indicated, values were converted to z-scores based on population norms for age and gender. The means of each variable were compared between the groups with and without prior splenectomy by Mann-Whitney tests.
Results: As expected, subjects with prior splenectomy had higher mean hemoglobin and WBC, platelet, and reticulocyte counts and lower total serum bilirubin (p<0.001). Subjects without prior splenectomy had lower than normal levels of total and LDL cholesterol. These levels were significantly higher, as were fibrinogen and homocysteine, in the post-splenectomy subjects (see Table).
Conclusions: Lipid profiles and other measures of cardiovascular risk are affected by splenectomy in persons with HS. The lower lipid levels prior to splenectomy may suggest a protective influence of hemolysis and, although this benefit is abrogated following splenectomy, the levels do not appear to pose additional cardiovascular harm to the individuals. Further investigations to more clearly define the potential benefits of hemolysis and anemia as well as the risks of splenectomy are indicated.

	No splenectomy (n = 23)		Splenectomy (n = 34)		
Laboratory test	Mean	Std. dev. (±)	Mean	Std. dev. (±)	p-value
Total Cholesterol[1]	−1.55	1.06	−0.65	0.95	**0.001**
HDL[1]	−0.65	0.65	−0.44	0.50	0.162
Triglycerides[1]	−0.07	0.27	−0.12	0.54	0.294
LDL[1]	−1.03	1.08	−0.37	0.92	**0.048**
Lipoprotein (a) (mg/dl)[2,3]	22.6	31.3	30.9	32.9	0.343
Homocysteine (μmol/l)[2,4]	6.25	2.18	8.82	3.78	**0.004**
Fibrinogen (mg/dl)[2]	276	79.5	329	63.0	**0.002**
CRP (mg/dl)[2]	2.46	2.55	2.78	3.67	0.757

[1]Values have been converted to z-scores based on published norms for age and gender.; [2]Values have not been converted to z-scores.; [3]Based on n = 21 splenectomy and n = 15 non-splenectomy.; [4]Based on n = 22 splenectomy and n = 16 non-splenectomy.

Poster 200

An Open-Label, Multi-Center Pilot Study to Evaluate Senicapoc in Pediatric Subjects with Sickle Cell Disease

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Background: The Gardos potassium channel mediates the characteristic erythrocyte dehydration observed in sickle cell disease (SCD). Senicapoc, an inhibitor of the Gardos channel, significantly reduces RBC dehydration and hemolysis in adult SCD patients.
Objective: A pilot study was performed to define the pharmacokinetics and tolerability of senicapoc in a pediatric SCD population.
Design/Methods: Children ages 6–16 years with HbSS, SC, or S$\bullet^{thal}$ were assigned to one of three oral dosing regimens (low, medium and high dose) by age group: 6–12 years (low n = 6 or medium n = 5) and 13–16 years (medium n = 3 or high n = 7). PK parameters were calculated using non-compartmental methods. PD and safety assessments were collected at screening, during, and at the end of the study. The PK and PD relationships were summarized using descriptive statistics.
Results: Twenty-one patients were administrated a 21-day course of drug and assessed at regular interval visits for approximately 77 days. PK values observed in 13–16 year olds were similar to those reported previously in adults. Dose-normalized Cmax was found to be slightly higher in 6–12 year olds compared to 13–16 year olds (min-max: 104–261 ng/ml; 65–158 ng/ml, respectively) and plasma half-life was shorter (5 to 7 days; 12 to 15 days, respectively), primarily due to a lower Vd. Red cell parameters (RBC, Hb, HCT) increased in each dose and age group in a manner similar to adult SCD patients. Concurrently, markers of hemolysis (LDH, I.Bili, Retic) decreased during dosing and returned to baseline levels by study end. The majority of AEs reported were of mild to moderate intensity, and none of them were considered related to study medication or led to drug discontinuation.
Conclusions: Senicapoc was well-tolerated in pediatric subjects at plasma concentrations similar to those observed in adults. Pediatric SCD patients had a comparable, rapid reduction in RBC hemolysis as adult patients, during a 21-day treatment.
Supported by Icagen, Inc and McNeil Pediatrics.

Poster 201

Sickle Red Blood Cells Have Increased Phosphorylation of Adducin and Increased ROS Production Mediated by Signaling Pathways Involving Rac GTPases

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Background: Although sickle hemoglobin (HgbSS) polymerization is the trigger for the sickle deformation of red blood cells (RBCs) containing HgbSS, upon deoxygenation, the concurrent changes in the cytoskeleton have not been fully elucidated. Actin oligomers are a significant structural component of the erythrocyte cytoskeleton. The Rho family small GTPases Rac1 and Rac2 regulate actin structure and mediate reactive oxygen species (ROS) production via activation of the NADPH oxidase system in a variety of cells (Schwartz M., J. Cell Science 2004). Previously we have demonstrated that deficiency of Rac1 and Rac2 in mice alters the RBC cytoskeleton organization, with increased phosphorylation of adducin, an F-actin capping protein, at residue Ser-726, a domain-target of protein-kinase C (PKC) (Kalfa et al., Blood 2006).
Objective: To explore phosphorylation changes in the cytoskeleton of sickle cells and signaling pathways mediated by Rac GTPases, which may modulate the sickle-deformation of HbSS-RBCs.
Design/Methods: RBCs from children with HgbSS or Hemoglobin AA (HgbAA) for control were evaluated for cytoskeleton phosphorylation changes by immunoblotting with phospho-specific antibodies. PKC activity was evaluated by a solid-phase ELISA assay (Stressgen PKC Kinase

Activity Assay). ROS production was evaluated by flow cytometry using 5-(and 6-)-chloromethyl-2,7-dichlorodihydrofluorescein diacetate, a peroxide-sensitive probe.
Results: Phosphorylation of adducin (Ser-726) in RBCs from patients with HgbSS was found to be consistently elevated along with increased PKC activity in these cells. The phosphorylation of adducin increased in a dose dependent fashion by in vitro treatment of HgbSS-RBCs with a Rac-specific small-molecule inhibitor (NSC23766), whereas it decreased when HgbSS-RBCs were treated with a PKC inhibitor (calphostin). In addition, ROS concentration was elevated in HgbSS-RBCs by 150–250% comparing to that in HgbAA-RBC. This difference was more pronounced in the low-density fraction of sickle cells, containing younger red cells, isolated by density gradient centrifugation. Inhibition of Rac activity in HgbSS-RBCs by NSC23766 inhibited ROS production in a dose dependent fashion.
Conclusions: Altered adducin phosphorylation and ROS production mediated by signaling pathways involving Rac GTPases in sickle RBCs may contribute to membrane changes induced by cell sickling. Elucidation of these pathways may offer new therapeutic targets in sickle cell disease.

Poster 202

Transition of Adolescent Patients with Sickle Cell Disease: Baseline Knowledge and Concerns

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Background: Over the past forty years, the life expectancy of patients with sickle cell disease (SCD) has increased from 20 years in the 1960s to over 40 years. Since the life expectancy of patients with SCD is increasing, there is a need to prepare these adolescents for transition to adulthood.
Objective: The goal of this study is to identify and characterize the concerns and baseline knowledge of adolescents with SCD.
Design/Methods: Children 15 years old and older, who are treated in the Duke Pediatric Sickle Cell Center, were enrolled in this IRB approved study after consent was obtained. Subjects completed a 12-question multiple-choice SCD knowledge test and a measure of their concerns about moving to adult care.[1,2]
Results: Fifty-seven African-American children enrolled in the study (18 females, 39 males). Participants' ages ranged from 15–19 years (mean 16.6 years) encompassing grades 8 to secondary education. Scores on the SCD knowledge test ranged from 1 to 12 (mean = 8.05±2.50). Sixteen (28%) incorrectly defined SCD. Thirty-three (58%) thought that only African-Americans are affected by SCD. In addition, 29 (51%) did not understand the inheritance of SCD. Participants' number of concerns about transitioning to the adult clinic ranged from 0 to 8 out of 12 potential concerns (mean = 2.6±2.7 concerns). Thirty-six (70%) of participants identified at least one concern about transitioning to adult care. The most frequent concerns related to issues of abandonment and independence. For example, 17 (31%) were concerned about leaving their current health care providers. Twelve (21%) expressed concern about making decisions on their own.
Conclusions: Gaps in knowledge about SCD and concerns about transitioning to the adult clinic reflect the need for formalized transition programs that will facilitate the transition process.

[1]Baskin *et al*. J Clin Pyschol Med Settings 1998; 5(3): 315–341.
[2]Telfair *et al*. J Adolesc Health 1994; 15(7): 558–565.

Poster 203

Pulmonary Hypertension in Children and Young Adults with Sickle Cell Disease: Lack of Response to Hydroxyurea and Evidence for Familial Clustering

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Background: Pulmonary hypertension (PHTN) in patients with sickle cell disease (SCD) is associated with early mortality during adulthood.
Objective: To determine the prevalence of PHTN and identify factors associated with this complication among children and young adults followed at the Children's Cancer Center of Lebanon.
Design/Methods: Transthoracic Doppler echocardiography was performed during steady state at the time of the initial visit and then yearly. PHTN was defined as a tricuspid regurgitant jet velocity (TRV) of 2.5 m/s or higher. From June 2004 to August 2007, 64 patients were studied. Correlation of TRV $\geq$2.5 m/s with age, mean corpuscular volume (MCV), fetal hemoglobin (HbF) and hydroxyurea use was performed.
Results: 19 of the 64 (29.7%) patients were found to have PHTN. Patients with TRV $\geq$2.5 m/s were older (11.3±2.3 vs 6.5±0.7 years $p = 0.016$), had higher MCV ($p = 0.02$), higher HbF ($p = 0.02$) and a history of hydroxyurea use ($p = 0.02$) compared to patients with TRV<2.5 m/s. Five patients with a normal initial TRV developed PHTN. The median age at the time of the first evaluation was 11.2 years, and abnormal TRV was detected after a median follow up of 24.2 months. All 5 had received hydroxyurea continuously during this period, at a mean dose of 19 mg/kg/day. They experienced significant clinical improvement, manifested by decreased painful crises, hospitalizations and need for transfusions, as well as increase in MCV and HbF levels. Among the 64 patients studied, there were 11 families with more than one sibling affected by SCD. PHTN was found in only 4 of the 11 families and these families contributed 10 of the 19 patients with PHTN. In one consanguineous family 2 young siblings, ages 11 and 13 years and two maternal uncles, aged 24 and 26 years were found to have abnormal TRV. This familial clustering was highly statistically significant ($p = 0.0001$).
Conclusions: PHTN is prevalent among children and young adults with SCD in Lebanon. In our population use of hydroxyurea did not seem to prevent the development of PHTN. The familial clustering of PHTN observed in our patients has not been previously described.

Poster 204

Education and Employment Status of Patients with Thalassemia in North America

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Background: Advances in the management of thalassemia have resulted in increased life expectancy for those affected. As people with thalassemia age, they face new challenges including educational and employment pursuits.
Objective: To investigate the education level and employment status of people with thalassemia in North America, and potential factors affecting these.
Design/Methods: A total of 633 patients (349 adults and 284 school age children) enrolled in the Thalassemia Clinical Research Network (TCRN) registry in Canada and the United State of America, were included in the data analysis. Predictors considered for analysis were age, gender, race/ethnicity, nationality (American vs. Canadian), transfusion and chelation status, serum ferritin, and clinical complications. Multivariate effects of predictors significant in bivariate analysis were modeled using logistic regression for employment and a proportional odds model for education.

Results: Seventy percent of adult patients were employed of which 60 percent reported working fulltime. Sixty percent had a college degree and 14% had achieved some post college education. Of the 284 school age children, 82% were at expected grade level. In a multivariate analysis for adults, patients who defined their race as White (OR = 3.3, 95% CI: 1.43–5.88) were more likely to be employed compared to Asians. Significant predictors of higher education in adults were older age (OR = 1.1, 95% CI: 1.04–1.10), female gender (OR = 2.5, 95% CI: 1.43–3.57) and absence of lung disease (OR = 12.5, 95% CI: 1.96–100). Among children, older patients (OR = 1.2, 95% CI: 1.08–1.32) and patients of Canadian nationality (OR = 5, 95% CI: 1.52–20) were more likely to be at the expected education level. Neither transfusion nor chelation status was associated with employment or education achievements.
Conclusions: Individuals with thalassemia in North America can achieve higher education however fulltime employment remains a problem. Further studies are needed to investigate, understand and overcome barriers. Despite general belief, transfusion and chelation status do not affect employment or education status of patients with thalassemia in North America.

Poster 205

Hemoglobin Louisville (beta 42 Phe-Leu): A New Case Report

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Background: Unstable hemoglobin disorders are characterized by chronic hemolytic anemia. Diagnosis can be confirmed by protein and gene sequencing.
Objective: We describe a 10-year-old boy exhibiting splenomegaly, chronic hemolytic anemia. Patient had history of splenic sequestration at 3-year-old, episodes of severe hemolysis during febrile illness. The peripheral blood on diagnosed showed: Hemoglobin 9.1 g/dL, Hematocrit 30.2%, RBC $3.83 \times 10^6/mm^3$, MCV 89.3 fL, HCH 26.9 pg, MCHC 30.1%, WBC $5.99 \times 10^3/mm^3$, reticulocyte count 9.5%. Heinz bodies were demonstrated in the peripheral blood.
Design/Methods: Hemoglobin was studied by IEF, HPLC reverse phase chromatography, heat stability and isopropranol tests. DNA was sequenced in both coding and non-coding strands.
Results: An unstable Hb was diagnosed on the basis of positive heat stability and isopropranol tests. DNA sequence analysis of beta chain shows that beta hemoglobin mutation present is hemoglobin Louisville. The substitution of TTT to CTT mutation at codon 42, which corresponding to a Phe to Leu.
Conclusions: Hb Louisville is an unstable hemoglobin variant, a decreased oxygen affinity, a marked decrease in heme-heme interaction, and a normal Bohr effect. It can cause mild to moderate hemolytic anemia with reticulocytosis and Heinz bodies.

Poster 206

Red Cell Antibodies in Chronically Transfused Children with Sickle Cell Disease: Red Cell Exchange (REC) Does Not Appear to Increase the Rate Of Allo- and Auto-Immunization

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Background: Chronic red cell transfusion (RCT) for primary and secondary prevention is implemented with increasing frequency in children with sickle cell disease (SCD), but allo- and auto-antibody development is a well recognized complication. Previous studies have shown a reduced incidence of immunization with limited matching of Rh (C, c, D, E, e) and K antigens for these patients.
Objective: Limited red cell antigen matching was implemented uniformly at our institution in December 1996; few patients had Rh and K antigen matching prior to that time. We reviewed our experience with red cell allo- and auto-immunization in pediatric SCD (Hb SS) patients receiving chronic and/or exchange transfusions, to evaluate the rate of immunization after limited red cell antigen matching and specifically during RCE.
Design/Methods: We conducted a retrospective chart review of the patients with SCD followed at our center between 2002 and 2006, who were started on chronic RCT every 3 to 4 weeks for a minimum of 6 months, before or during that time period. Demographics, data regarding transfusions and red cell antibodies were collected.
Results: 93 patients met study criteria. 9 (9%) had antibodies at study entry; 23 (24%) developed antibodies during chronic red cell transfusions.

Table: Red cell antibodies after limited red cell antigen matching

Warm auto-antibody	12
Cold agglutinin	3
Allo-antibody: C	2
D (mosaic)	2
E	1
K	1
S	2
Lewis a,b	4
Others	5

Thirty four antibodies (15 auto-antibodies, 19 allo-antibodies) developed after the institution of limited red cell antigen matching. The rate of immunization (with allo- and auto-antibodies) per unit of red cell exposure after limited phenotyping was 1.69%, comparable to other published data. Fifteen patients underwent RCE, utilizing 2289 red cell units; three had already developed antibodies during chronic RCT. However, none developed antibodies during RCE.
Conclusions: We conclude that limited red cell antigen matching is an effective strategy for reducing the incidence of allo- and auto-immunization in chronically transfused children with SCD. RCE does not appear to increase the risk of allo- or auto-immunization, despite exposure to more blood units.

Poster 207

Acute Splenic Sequestration and Parvovirus Infection in Children with Sickle Cell Disease

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Background: Acute splenic sequestration primarily occurs in young patients with sickle cell disease, most often in HbSS. Splenic sequestration is typically characterized by an enlarging spleen, worsening anemia (>2 gm/dL drop) with adequate reticulocytosis, and thrombocytopenia. In contrast, transient aplastic crisis from parvovirus B19 infection occurs in older children and is characterized by worsening anemia with absence of reticulocytosis; splenomegaly and thrombocytopenia are uncommon. We recently observed 4 cases of acute splenic sequestration in association with parvovirus infection.
Objective: Describe an association between acute splenic sequestration and parvovirus infection.
Design/Methods: With IRB approval, we retrospectively reviewed the charts of four children with sickle cell disease hospitalized between October 2006 and February 2007, all of whom developed acute splenomegaly, severe hypoplastic anemia, thrombocytopenia, and in 2 cases, multisystem dysfunction with acute renal failure and respiratory distress.
Results: Four children (3 females, 1 male; 3 HbSC, 1 HbSS on hydroxyurea; age range 3.9–15.8 years) were identified. No patient had previous splenic sequestration.

Patient	Hb drop from baseline (gm/dL)	Absolute reticulocytes	Platelet change from baseline	Spleen size below LCM	Parvovirus IgM
#1	9.2 to 3.7	2K	148K to 48K	6.5 cm	+
#2	7.3 to 4.3	5K	225K to 108K	palpable	+
#3	12.3 to 3.9	3K	227K to 40K	palpable	+
#4	11.0 to 3.9	8K	226K to 85K	10 cm	+

Patients #3 and #4 required ICU admission with acute renal failure and respiratory distress (Bi-PAP and intubation, respectively). All four patients received PRBC transfusions. Upon recovery from their illness, the hemoglobin concentration and platelet count returned to baseline in all children, along with improvement or resolution of splenomegaly.
Conclusions: Acute splenic sequestration can occur in conjunction with acute parvovirus infection, and may be associated with multisystem dysfunction. These children were older than the typical age for acute splenic sequestration and not considered to be high-risk for an aplastic crisis, yet developed a severe illness. These cases suggest that the definition of acute splenic sequestration may need modification to allow cases without reticulocytosis. Children with acute parvovirus infection should be followed closely for signs of splenomegaly and multisystem dysfunction.

Poster 208
Case Control Study of Modifiers of Stroke in Risk in Patients with Sickle Cell Disease

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Background: Sickle cell disease (SCD) is caused by a point mutation in the hemoglobin β-chain gene. This results in mutant hemoglobin that polymerizes in the deoxy conformation, leading to red blood cell (RBC) deformation and occlusion of, and damage to blood vessels. The mutation is common in African Americans and causes considerable morbidity and mortality. The most feared complication of SCD is damage to the blood vessels of the CNS and resultant stroke occurring in 5–12% of patients. While all patients harbor the same SCD mutation, the phenotype varies considerably between individuals.
Objective: The focus of this project is to find gene polymorphisms or variations in protein expression that contribute to risk of stroke in SCD. We have chosen to evaluate platelet and RBC CD36 expression and a polymorphism in the c-Mpl gene, Mpl Baltimore, as candidate modifiers.
Design/Methods: With approval of the local human investigation committee we have conducted a retrospective case control study, comparing the prevalence of the Mpl Baltimore and the level of CD36 expression between a group of 30 SCD patients with history of stroke and 30 age and sex matched SCD patients without history of stroke. After obtaining informed consent, we collected laboratory and clinical data from patient charts and obtained blood samples for evaluation of CD36 expression by flow cytometry. Genomic DNA was isolated and Mpl Baltimore testing was performed utilizing a TaqMan SNP assay.
Results: Subjects and controls had similar baseline laboratory and clinical parameters. Platelet CD36 expression, measured by mean channel fluorescence, was 78.3±21.3 in the stroke patients and 79.5±16.3 in the controls. The percent of RBCs expressing CD36 was 3.3±1.4 in the stroke patients and 3.1±1.3 in the controls.
Conclusions: Identification of modifiers of SCD phenotype will aid in selection of patients for screening tests and therapies. These modifiers may also lend insight into the pathophysiology of SCD and identify targets for future therapies. Identification of these modifiers will require larger study groups with well defined phenotypes and accurate clinical and laboratory data. This will require multi-center cooperative studies in order to enroll adequate numbers of subjects.

SOLID TUMORS

Poster 209/PLATFORM SESSION 301
Dual VEGF and Trk Signalling Inhibition is an Effective Strategy in Preclinical Models of Neuroblastoma (NBL)

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Background: High-risk neuroblastomas are extensively vascularized and typically express one or more neurotrophin (Trk) receptor. Signalling through VEGF and Trks are oncogenic, and single target inhibition results in tumor growth delay in xenograft models of NBL.
Objective: To determine the efficacy of combined VEGF and Trk signalling inhibition in tractable mouse models of human neuroblastoma.
Design/Methods: We used oligonucleotide microarrays, quantitative RT-PCR and Western blotting to determine the expression patterns of ligands and receptors for both the VEGF and Trk signaling pathways in human NBL-derived cell lines and a genetically engineered murine (GEM) model of NBL during tumor progression. We then performed four arm interventional trials (N = 10 mice/arm) using highly specific inhibitors of VEGF and Trk signalling compared to placebo, as single agents or in combination.
Results: Cell lines NB-1643 and NB-1691 were selected because of robust expression of both VEGFR2 and TrkB. Bevacuzimab (human VEGF inhibitor) and Lestaurtinib (human and murine multi-kinase inhibitor including the Trks) each caused subtle growth delay of xenografts compared to placebo, but the combination showed complete and sustained regression of xenografts in three separate experiments. In addition, single agent DC101 (murine-specific *Vegfr2* inhibitor) caused significant tumor growth delay of spontaneously arising NBL in the *MYCN* GEM model, with eventual escape and rapid regrowth. Combination therapy in this transgenic model is ongoing and will be reported.
Conclusion: Combination therapy with bevacuzimab and lestaurtinib is effective in preclinical models of high-risk NBL and should be prioritized for clinical development.
Acknowledgements: Cephalon, Inc. for providing lestaurtinib and ImClone Systems, Inc. for providing DC101.

Poster 210/PLENARY SESSION 300
Regression of Experimental Osteosarcoma Following Transfer of HER2-Redirected T Cells

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Background: New therapies are needed for osteosarcoma (OS) since the prognosis for patients with metastatic and recurrent disease remains poor despite aggressive multimodal therapies. For immunotherapies, HER2 is an attractive target since it is expressed in approximately 50% of primary OS samples and is associated with worse treatment outcomes.
Objective: While the use of HER2 monoclonal antibodies has been limited by low levels of HER2 expression on OS cells, we show here that T cells expressing HER2-specific chimeric antigen receptors (CAR) have potent anti-tumor activity in animal models. The objective of this project is to develop a novel adoptive immunotherapeutic approach for osteosarcoma.
Design/Methods: T cells were transduced with a retroviral vector encoding a HER2-specific CAR with a CD28. ζ-signaling endodomain (HER2-T cells). We screened 12 OS cell lines for HER2 expression by flowcytometry and analyzed the ability of HER2-T cells to 1) proliferate, 2) produce immunostimulatory cytokines, and 3) kill HER2+ tumor cells in cytoxicity assays. The in vivo function was tested in a murine intraperitoneal xenograft model, which allows for serial imaging by bioluminescence using a representative OS cell line (LM7). Imaging results were confirmed by pathological examination.
Results: Eleven of 12 cell lines had detectable HER2 expression by flowcytometry. HER2-T cells recognized and killed all HER2+ OS cell lines in cytotoxicity assays, whereas HER2-negative tumor cells were not killed. Co-culture of HER2-T cells with HER2+ tumor cell lines resulted in T-cell proliferation, and secretion of IFN-γ and IL-2 in a HER2-dependent manner. In vivo, HER2-T cells eradicated established intraperitoneal xenografts in 80% of animals harboring LM7, resulting in long-term tumor free survival of treated animals. In contrast, delivery of non-transduced T

cells did not change the tumor growth pattern. Survival analysis was done on day 160 and showed a significant survival advantage in animals treated with HER2-T cells.

Conclusions: This study shows that HER2 is a target antigen for adoptive immunotherapy of OS. HER2-redirected T cells not only recognized and killed HER2-expressing tumor cells ex vivo, but also eradicated experimental xenografts in vivo. Hence, adoptive transfer of HER2-redirected T cells may represent a promising immunotherapeutic approach for OS.

Poster 211/PLATFORM SESSION 301

Therapeutic Targeting of Tumor-Initiating Cells Isolated from Neuroblastoma Cell Lines Using a Nestin-Targeted Oncolytic HSV-1

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Background: Oncolytic viruses, engineered to be clinically safe by genetic mutations, are emerging as new tools for killing cancer cells. Importantly, these viruses circumvent typical drug-resistance mechanisms. We and others have demonstrated that neural tumors such as neuroblastoma and MPNST are sensitive to oncolytic HSV-1.

Objective: The ''cancer stem cell'' theory suggests that rare tumorigenic progenitor cells, resistant to conventional therapy, are responsible for disease relapse. If true for neuroblastoma, improved cure rates might only be achieved via identification and therapeutic targeting of the neuroblastoma tumor-initiating cell. Based on cues from normal neural cell ontogeny, we sought to determine if neuroblastoma cell lines contain subpopulations of cells exhibiting characteristics similar to neural stem cells and whether they are susceptible to lytic infection by oncolytic HSV-1 mutants.

Design/Methods: We evaluated human neuroblastoma cell lines for tumorsphere formation, expression of stemness markers (CD34, CD133, ABCG2, nestin), verapamil-sensitive side population, doxorubicin resistance, and tested these subpopulations for tumorigenicity with limiting dilutions. We also determined the susceptibility of these subpopulations to infection by oncolytic HSV mutants, including a vector expressing the critical viral gene ICP34.5 under the control of the nestin promoter.

Results: We identified subpopulations of neuroblastoma cell lines expressing CD34, CD133, ABCG2 and/or nestin, and showed Notch and EGFR-dependent clonal growth as tumorspheres. Tumorsphere cells were relatively chemotherapy resistant and CD133+ cells demonstrated increased tumorigenicity. The nestin-targeted oncolytic HSV-1 mutant efficiently killed neuroblastoma tumorsphere cells. Multi-parameter selections are underway to determine if further isolation of tumor-initiating cells is possible.

Conclusions: Our results suggest that neuroblastoma cell lines contain subpopulations of tumor-initiating cells, which can be therapeutically targeted by an oncolytic HSV-1. These data require validation using primary human specimens.

Poster 212

T Cell Immunotherapy for Murine Medulloblastoma

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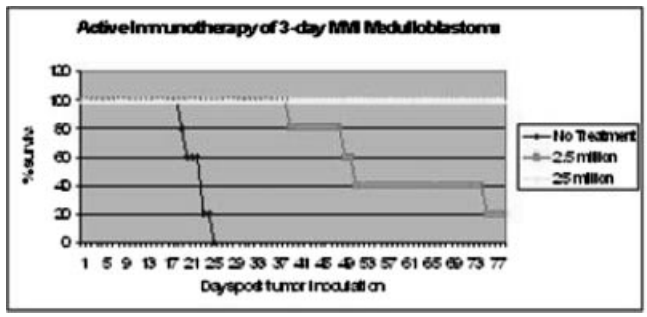
Background: Although the current overall survival for medulloblastoma has increased due to more aggressive therapy, the involved surgery, radiation and high-dose chemotherapy have significant acute and long-term toxicities. T cell immunotherapy is a promising adjuvant for medulloblastoma because the immune response is specific for targeted antigens and is highly anatomically focused, occurring at the level of cell-to-cell interactions, thereby sparing adjacent normal brain tissue. Adoptive transfer of tumor-sensitized T cells has demonstrated therapeutic efficacy in animal tumor models and clinical trials involving transfer of T lymphocytes are ongoing. Priming of T cells using a spontaneously-arising medulloblastoma model may be representative of human cancer.

Objective: To determine if adoptive transfer of tumor-sensitized T cells will demonstrate therapeutic efficacy against spontaneously-arising murine medulloblastoma.

Design/Methods: Spontaneous murine medulloblastoma cell lines were created from knock-out $Ptc^{+/-}p53^{-/-}$ mice. Brain tumors were established by transcranial inoculation of a single-cell suspension of murine medulloblastoma tumor cells in immunocompetent C57BL/6N mice. Murine Medulloblastoma cell lines were tumorigenic and rapidly fatal following intracranial inoculation of 10^4 cells in a dose-dependent manner. T-cells can be obtained surgically from tumor draining lymph nodes (TDLN) in syngeneic mice and after initial purification, antigen-stimulated $CD62L^{low}$ T cells were isolated by depletion of $CD62L^{high}$ cells using MACS beads. T-cells were further activated *ex vivo* by incubation with anti-CD3 monoclonal antibody and exposure to media containing Interleukin-2 and Interleukin-7. Mice carrying 3-day established tumors receive systemic transfer of T cells by injection through the tail vein on day 10 of culture. Mice typically receive sub-lethal total body irradiation (5 Gy) for lymphodepletion several hours before adoptive transfer. Mice were followed for survival and sacrificed when neurological symptoms were apparent as per Institutional Animal Care and Use Committee established guidelines.



Results:

Conclusions: In the above experiment, the activated T cells adoptively transferred to irradiated hosts with 3-day established intracranial tumors resulting in complete regression of established tumors. In addition, the above therapy demonstrated a dose-dependent effect, and the mice were cured without any evidence of neurological dysfunction caused by the anti-tumor response. This research suggests anti-medulloblastoma T cell vaccines may be promising for subsequent clinical testing.

Poster 213

Cooperative Genetic Changes that Temporally Drive Alveolar Rhabdomyosarcoma

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Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescence. Despite advances in therapy, patients with a histologic variant of RMS known as alveolar (aRMS) have a 5-year survival rate <30%. aRMS tissues exhibit a number of genetic changes, including loss of function of the p53 and Rb tumor suppressor

pathways, amplification of MycN, stabilization of telomeres, and most characteristically, reciprocal translocation of loci involving the *PAX3/7* and *FKHR* genes, generating the PAX3/7-FKHR fusion proteins. We previously demonstrated that PAX3-FKHR expression in primary human skeletal muscle myoblasts (HSMMs), cells that can give rise to RMS, cooperated with loss of $p16^{INK4A}$ (encoded at the *INK/ARF* tumor suppressor locus) to promote extended proliferation, an event that potentially initiates aRMS. We hypothesized that additional expression of the above genetic changes, in a precise order, would be necessary to fully drive HSMMs to aRMS.

Objective: To define the cooperative genetic changes required to convert HSMMs to aRMS, and determine whether their order of acquisition was important.

Design/Methods: Using amphotrophic retrovirus encoding PAX3-FKHR, MycN, and the catalytic subunit of telomerase hTERT (or associated empty vectors), we step-wise transduced HSMMs in various combinations, then tested them for tumorigenic capacity in vitro using colony formation in soft agar, and in vivo using subcutaneous xenograft formation in immunodeficient mice. Similarity to control human aRMS tumors was assayed using light microscopic analysis of standard H&E and immunohistochemical stained sections.

Results: The combination of PAX3-FKHR, hTERT, and MycN, in cooperation with downregulation of $p16^{INK4A}/p14^{ARF}$ expression, was necessary and sufficient to convert HSMMs into transformed cells that gave rise to aRMS tumors. However, the order of expression of these transgenes was critical, as only those cells expressing PAX3-FKHR early could form tumors.

Conclusions: We suggest that the translocation of PAX3 to FKHR drives proliferation of myoblasts, and a selection for loss of $p16^{INK4A}/p14^{ARF}$. These early steps, coupled with MycN amplification and telomere stabilization, then drive the cells to a fully tumorigenic state. Defining this set of ordered, cooperative genetic changes provides a blueprint for aRMS formation, and may help in the design of rational drug combinations to treat this disease.

Poster 214/PLATFORM SESSION 301
Polyamines in Neuroblastoma

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Background: Neuroblastoma (NB) is a commonly lethal childhood tumor in which *MYC* gene amplification portends poor outcome. *MYCN* regulates numerous genes implicated in cancer yet the critical targets supporting tumor progression remain poorly characterized. *ODC1* is a *MYCN* target, bona fide oncogene, and the rate-limiting enzyme for polyamine maintenance. We have shown that *MYCN* amplified NBs expand polyamine pools through concerted upregulation of all synthetic polyamine enzymes and downregulation of multiple negative regulators.

Objective: As polyamines are essential to support cell proliferation we assessed depletion strategies in NB.

Design/Methods: In vivo studies used a real-time cell electronic sensor system (RT-CES). In vivo studies utilized the TH-MYCN neuroblastoma-prone mouse model.

Results: Odc inhibition using DFMO led to growth inhibition of 7 of 7 NB cell lines tested (median 42% at 72 hrs) while Amd1 inhibition using SAM486a (Novartis) was more potent (mean 70% inhibition). No synergy was apparent using both agents in vitro where limited extracellular polyamines preclude enhanced uptake. In vivo, single-agent DFMO treatment of NB arising in transgenic mice (*TH-MYCN* GEM) extended survival ($p = 0.002$) while pre-emptive DFMO treatment extended survival and prevented tumor initiation in a subset of tumor-prone mice ($p = 0.035$). NBs arising under DFMO exposure had reduced putrescine ($p<0.005$) but maintained spermidine and spermine levels suggesting partial circumvention of polyamine depletion. We are therefore assessing dual Odc/Amd1 inhibition in vivo using daily DFMO (1% in drinking water) with SAM486a (5 mg/kg IP 3x/wk). In *TH-MYCN* +/+ mice (100% NB penetrance) all treated mice developed tumors to date but latency was extended by DFMO/SAM. ($N = 16$; trial ongoing).

Conclusions: Thus, we provide strong evidence that polyamine depletion may be of therapeutic utility in NB and efforts to integrate such biotargeted agents into clinical trials are warranted.

Poster 215
Selective TRK Inhibitor AZ623 Effective Against Neuroblastoma In Vitro and In Vivo

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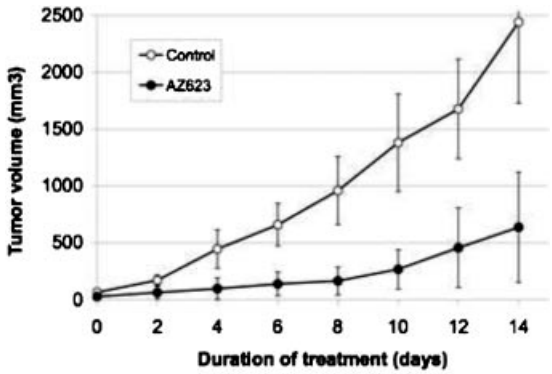
Background: The Trk tyrosine kinase receptors regulate growth, differentiation and survival in neuroblastoma. TrkB expression is associated with disease aggressiveness and poor prognosis, and is an attractive therapeutic target. AZ623 is a potent and selective inhibitor of the Trk family under pre-clinical development.

Objective: To determine the effect of AZ623 on human neuroblastoma.

Design/Methods: A panel of five human neuroblastoma cells lines were used to determine IC50 by MTT assay (SH-SY5Y, SH-EP, SMS-KCNR, CHP-134, SK-N-SH). A flow cytometry-based assay was developed for detection of phospho-TrkB. A xenograft model using the SK-N-SH cell line was treated with 100 mg/kg AZ623 by gavage daily (6 of 7 days per week) for 2 weeks. Tumor volumes were calculated using bidirectional measurements every 2 days.

Results: All five cell lines demonstrated in vitro sensitivity to AZ623 with an IC50 in the 8–12 mcM range. Growth arrest was accompanied by loss of neurites and detachment. A novel flow cytometry-based assay for phospho-TrkB reveals constitutive phosphorylation, despite low levels of TrkB expression. Importantly, the xenograft model demonstrated significant growth inhibition when treated with AZ623 for as little as 6 days (Figure, $p<0.001$), with no toxicities observed.

Conclusions: AZ623 is a new selective Trk inhibitor which induces significant growth inhibition of a panel of human neuroblastoma cell lines. We demonstrate that at non-toxic doses, AZ623 can dramatically inhibit the growth a human neuroblastoma xenograft. Based on these results further pre-clinical development is warranted.



Poster 216
Tissue Inhibitor of Metalloproteinase-3 Expression via Oncolytic Herpesvirus Inhibits Tumor Growth and Circulating Vascular Progenitors

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Background: Malignant solid tumors remain a significant clinical challenge, necessitating innovative therapeutic approaches. Oncolytic viruses are non-mutagenic, biologic anticancer therapeutics shown to be effective against human cancer in early studies.

Objective: Because matrix metalloproteinases (MMPs) play important roles in the pathogenesis and progression of cancer, we sought to determine if ''arming'' an oncolytic herpes simplex virus (oHSV) with a MMP antagonizing transgene increases virus-mediated antitumor efficacy.

Design/Methods: We generated oHSVs that express human Tissue Inhibitor of Metalloproteinases 3 (TIMP3) or firefly luciferase and designated them rQT3 and rQLuc, respectively. We evaluated the antitumor efficacy of these viruses against neuroblastoma and malignant peripheral nerve sheath tumor (MPNST) xenografts.

Results: Relative to rQLuc, rQT3-infected primary human MPNST and neuroblastoma cells exhibited equivalent virus replication but increased cytotoxicity and reduced MMP activity. In vivo, rQT3-treated tumors showed delayed tumor growth, increased peak levels of infectious virus, immature collagen extracellular matrix and reduced tumor vascular density. Remarkably, rQT3 treatment reduced circulating endothelial progenitors (low side scatter, CD133+, Flk-1+), suggesting virus-mediated anti-vasculogenesis.

Conclusions: We conclude that TIMP3 expression by an oncolytic HSV enhanced antitumor efficacy through multiple mechanisms including direct cytotoxicity, elevated virus titer, and reduced tumor neovascularization. These findings support the further development of combined TIMP-3 and oncolytic virotherapy for cancer.

Poster 217

Identifying the Origin and Therapeutic Target in Wilms' Tumor

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Background: The kidney is derived from the metanephritic blastema (forming glomeruli and proximal and distal tubules) and the ureteric bud (forming collecting ducts, calyces and ureter). Wilms' tumor has been thought to arise from the differentiating metanephritic blastema, but details of tumorigenesis remain unclear. Our recent studies had showed 1) activation of mammalian target of rapamycin (m-TOR) pathway in normal podocytes and renal tubules; 2) kidney injury molecule-1 (KIM-1) in renal cell carcinomas [papillary type and clear cell type] derived from proximal tubules and injured proximal tubules; and 3) positive von Hipple-Lindau gene product (pVHL) in all subtypes of renal epithelial tumors and normal tubular components. In this study, we investigated the role of m-TOR pathway and the expression of other markers in Wilms' tumor development.

Objective: Survey p-mTOR expression in Wilms' Tumors

Design/Methods: Immunohistochemical stains using antibodies against KIM-1, pVHL, Wilms' tumor-1 (WT-1), phosphorylated (p)-mTOR (activated form of mTOR) and p-p70S6K (a downstream signal of mTOR) were performed on routine tissue sections of 16 cases of Wilms' tumors and their respective benign kidney tissue. Stains of both Wilms' tumors and benign renal components were graded as negative, 1+ (weak fine granular staining), 2+ (moderate fine granular staining) and 3+ (strong large granular staining).

Results: Podocytes from all 16 cases were stained positively for WT-1, p-mTOR and p-p70S6K but negatively for KIM-1 and p-VHL. All three phases of Wilms' tumors (16/16) showed expressive patterns of all markers identical to the podocytes (Table-1). Except for KIM-1, all renal tubules were stained positively for the remaining 4 markers.

Table 1. Immunostaining results in 16 cases of Wilms' Tumors

Markers (staining location)	Wilms' tumors	Podocytes	Renal tubules
KIM-1 (cytoplasm)	–	–	–
p-VHL (cytoplasm)	–	–	2–3+
WT-1 (nuclei)	3+	3+	+/–
p-mTOR (cytoplasm/nuclei)	2–3+	2–3+	2–3+
p-p70S6K (cytoplasm/nuclei)	2–3+	2–3+	2–3+

Conclusions: Based on the current data, we conclude that Wilms' tumor is most likely derived from the podocyte, but not tubular, differentiation of metanephritic blastema. Activated p-mTOR pathway may play a role in tumorigenesis of Wilms' tumor, and thus represents a potential therapeutic target.

Poster 218

A Novel Signal Modulator in Neuroblastoma: Fibroblast Growth Factor-Binding Protein 3

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Background: Our investigation is focused on a novel Fibroblast Growth Factor (FGF) binding protein, which is selective for neuroblastoma and essential as a cell signal modulator. The FGFs contribute to heightened cellular proliferation and angiogenesis in many tumor types. FGFs bind receptor tyrosine kinases (RTKs) at the cell surface, activating the Ras pathway. We propose that FGF-BP3 functions as an extracellular chaperone of the FGFs, essential for activation and downstream signaling via the Ras pathway. FGF-BP3 may serve as a potential therapeutic target for neuroblastoma.

Objective: The purpose of the current investigation is to characterize and describe the role of recently identified Fibroblast Growth Factor Binding Protein 3 in the context of cellular localization, protein function, and signal modulation. We hypothesize that FGF-BP3 is specific to neuroblastoma, required for extracellular transport and activation of FGFs, and essential for signal transduction.

Design/Methods: Quantitative real-time PCR was performed to evaluate the presence of FGF-BP3 in neuroblastoma cell lines and tissues. Colony formation assays were conducted using siFGF-BP3 transduced SH-SY5Y cells to determine the tumorigenic nature of FGF-BP3. In order to elucidate the signaling pathway of FGF-BP3 and its relationship to bFGF (FGF-2), Western immunoblot was performed on lysed parental, siControl, and siFGF-BP3 SH-SY5Y cells, after incremental treatment time with bFGF. Extracellular signal-regulated kinase (ERK 1,2) served as the downstream marker of signal transduction.

Results: PCR revealed FGF-BP3 to be highly expressed in neuroblastoma cell lines and tissues. Soft agar growth revealed significant inhibition of colony formation in siFGF-BP3 SH-SY5Y cells. Western immunoblot identified a strong pattern of ERK 1, 2 phosphorylation in control groups of SH-SY5Y cells, after incremental treatment time with bFGF, compared to the siFGF-BP3 cells. Rescue supernatant with recombinant FGF-BP3 strongly restores the ERK signal pathway in the siFGF-BP3 cells. This verifies the critical role of the FGF-BP3 protein in the Ras-mediated pathway of neuroblastoma.

Conclusions: FGF-BP3 is a secretory protein, specific to neuroblastoma, important for the tumorigenic nature of cells, and essential for the signal modulation of the Ras pathway. Ultimately, FGF binding protein 3 may represent a potential target of inhibitory therapy in the treatment of neuroblastoma.

Poster 219
Trk Receptor Expression and Signaling in Medulloblastoma

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Background: Despite marked improvement in therapy for medulloblastoma (MB), the majority of high-risk patients still die of disease. Examination of primary tumors and the development of murine models suggest that alterations in developmental signaling pathways contribute to tumorigenesis and clinical heterogeneity of MB. The Trk family of receptor tyrosine kinases are expressed in the developing cerebellum and in MB. High TrkC expression is associated with favorable prognosis in MB. TrkB is also expressed in a subset of tumors, but its significance is unknown. Here we provide a systematic study of the expression, activation and consequences of targeted inhibition of TrkB and TrkC signaling in tumor-derived MB cell lines.

Objective: To profile Trk receptor and neurotrophin expression patterns in primary MB tumors and characterize the biology of Trk-mediated signal transduction pathway activation and targeted inhibition in MB-derived cell lines.

Design/Methods: The medulloblastoma cell line DAOY, which expresses little endogenous Trk protein, was transfected with full-length TrkB or TrkC, and stable cell lines were established. Receptor and signaling pathway effector protein expression and activation was measured by Western blotting with phosphospecific antibodies, and cell proliferation was determined by BrdU incorporation. TrkB and TrkC DAOY cells were treated with MEK and PI3K inhibitors, and the effect on proliferation was measured by BrdU assay.

Results: DAOY-TrkB and DAOY-TrkC cells signaled through both Ras/MAPK and PI3K/Akt pathways when activated by their respective ligands. DAOY-TrkC cells had high proliferative activity that was markedly reduced when treated with NT3, suggesting growth arrest in the presence of active TrkC signaling. However, DAOY-TrkB cells showed a modest increase in proliferation when treated with BDNF compared to untreated cells. Inhibition of the PI3K/Akt pathway decreased cell proliferation in both DAOY-TrkB and DAOY-TrkC cells after ligand activation. Inhibition of MAPK pathway inhibited only DAOY-TrkB but resulted in increased cell proliferation in DAOY-TrkC cells, restoring proliferative activity to non-NT3 treated levels.

Conclusions: TrkB and TrkC signaling in MB results in different biological responses and are differentially sensitive to targeted pathway inhibition. Targeted inhibition of specific Trk-mediated pathways has potent growth inhibitory effects in MB and should be studied further in pre-clinical models.

Poster 220
Novel Method of Using T Cells to Identify Peptides that Stimulate Tumor-Specific T Cells from Baculovirus-Expressed Peptide Libraries

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Background: Mimotope vaccines are being developed to overcome the immune tolerance to tumor-associated antigens (TAAs). Mimotopes are peptide mimics of TAAs, which elicit T cells that recognize the tumor. We have previously identified mimotopes that elicit anti-tumor responses to a colon carcinoma in BALB/c mice from a baculovirus (BV)-expressed peptide library using soluble TAA-reactive T cell receptor (TCR) protein. A drawback to this method is that a single TCR must be chosen and isolated to sort the library. This approach is impractical for clinical applications since patient may have different T cell repertoires and cloning individual TCR is a lengthy process.

Objective: To develop a method using T cell clones or populations of T cells isolated from spleen or tumor infiltrating lymphocytes (TIL) to identify mimotopes from peptide libraries.

Design/Methods: We isolated T cells from transgenic (Tg) mice expressing a tumor-specific TCR or CD8+ TIL from tumor-bearing mice. T cells were incubated with soluble gp64 protein to block non-specific binding between T cells and BV. Unenriched BV-expressed peptide library was added to T cells incubated with gp64 protein. After several washes to remove unbound BV, trypsin was added to separate bound BV encoding mimotope-MHC complexes from T cells. These specific BV were expanded in insect cells for the next round of enrichment. This process was repeated several times until many BV express mimotope-MHC complexes. We used these enriched pools of peptides to stimulate tumor-specific T cells.

Results: T cells isolated from Tg spleen or TIL successfully enriched specific BV. Enriched pools of peptides from both Tg and TIL induced proliferation of tumor-specific T cells while unenriched pools did not.

Conclusions: T cell clones or populations of T cells can be used to identify mimotopes from BV-expressed peptide libraries that stimulate tumor-specific T cells. Future studies will test these peptides in vivo for anti-tumor immunity. Use of BV-expressed peptide library technology is feasible for the design of novel peptide vaccines.

Poster 221
Bevacizumab Enhances Oncolytic HSV Virotherapy for Local Control of Ewing Sarcoma by Inhibiting HSV-Induced VEGF

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Background: Local control for Ewing sarcoma involves surgery, which is not always feasible, or radiotherapy, which can lead to multiple complications. Oncolytic viruses such as those derived from HSV-1 are being developed as anticancer agents. Wild type HSV-1 infection induces local VEGF secretion via reactive inflammatory cells. We hypothesized that HSV infection of tumors also induces a localized VEGF production, which might serve to enhance angiogenesis and tumor growth, thus mitigating the anti-tumor effect of the virus.

Objective: We sought to: (1) test whether Intratumoral injection of oncolytic HSV induces VEGF production in a human Ewing sarcoma xenograft model, and (2) determine whether the inhibition of HSV-induced VEGF with bevacizumab (a monoclonal antibody against human VEGF) is synergistic for the virus-mediated local control of the xenograft tumors.

Design/Methods: rRp450 is an ICP6-deleted HSV-1 mutant engineered to express rat CYP2B1 gene, which activates cyclophosphamide. Because ICP6 encodes the large subunit of ribonucleotide reductase, required by the virus for optimal replication in normal quiescent cells but not rapidly dividing cells, deletion of ICP6 enables cancer-selective virus replication. Established Ewing sarcoma (A673) xenografts were treated with either intratumoral rRp450, intraperitoneal bevacizumab, or the combination and followed for stroma- (mouse) and tumor-derived (human) VEGF levels and animal survival.

Results: Tumor cell-derived VEGF (human) was 17-fold higher than mouse-derived VEGF, but decreased 3.4-fold following virus injection, likely due to destruction of tumor cells. In contrast, the average intratumoral mouse VEGF production increased 4-fold following intratumoral rRp450 injection ($P<0.01$). Median survival was 10, 18 and 28 days for the control, bevacizumab only and the virus only groups, respectively. The combination group showed 6/7 (85%) survival >60 days ($P<0.01$).

Conclusions: Intratumoral oncolytic HSV injection decreased tumor-derived VEGF concentrations, but these changes were partially offset by increased stroma-derived VEGF due to virus infection. These data provide rationale for combining oncolytic HSV with VEGF inhibition. Administration of rRp450 and bevacizumab was synergistic in enhancing the virus-mediated local control in this xenograft model of Ewing sarcoma.

Poster 222
Cancer Stem Cells in Neuroblastoma – A Comparison of Pre-Treatment and Relapsed Models

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Background: Cancer Stem Cells (CSCs) have been identified in many cancers, are capable of unlimited self-renewal, and play an important role in a cancer's pathogenesis. CSCs share many properties with normal stem

cells including resistance to chemotherapeutic agents through multi-drug resistance proteins (MDRPs).
Neuroblastoma Cancer Stem Cells (NCSCs) are identifiable in neuroblastoma specimens including cell cultures. Metastatic and high-risk neuroblastomas have been found to have twice the number of NCSCs compared to non-metastatic and low-risk neuroblastomas. No studies have compared pre-treatment neuroblastoma to relapse.
Objective: We hypothesized that relapsed neuroblastoma would have an increased proportion of NCSCs resulting in enhanced drug resistance and colony forming ability.
Design/Methods: We measured expression levels in a set of 14 genes including stem cell genes and MDRPs with real-time polymerase chain reaction (rt-PCR) using 3 paired pre-treatment and post-relapse neuroblastoma cells lines. We then identified and isolated a side population (SP) consisting of NCSCs from the paired cell lines by flow cytometry for further gene expression and colony formation analyses.
Results: We found increased expression in a majority (86%) of the genes in the relapsed cell lines ranging from a two to nine-fold increase (average 3.5-fold difference). We also found two to five-fold increased expression of the genes in the SP compared to the non-SP cells. The relapsed cell lines maintained a significantly increased proportion of NCSCs compared to pretreatment: 11.1% (9.2–13.4%) versus 6.7% (5.5–8.5%) ($p = 0.0011$). Two weeks post-sorting, the SP cells showed a 3-fold increase in cell number with 98% of the cells differentiated back into non-SP cells. The non-SP number remained stable with no SP cells.
Conclusions: Our study suggests that NCSCs may play an important role in a relapsed neuroblastoma's pathogenesis. The increase in NCSC number and MDRP gene expression may help explain the treatment resistance of relapsed neuroblastoma and suggests that a component of that resistance may have been present prior to initial therapy. Effective therapies that target all components of this disease are therefore urgently needed.

Poster 223

L-MTP-PE Compassionate Access and Aerosol GM-CSF Therapy for Osteosarcoma

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Background: The most common pattern of relapse in osteosarcoma is lung metastases; these have been shown to have under-expression of Fas, thus possibly avoiding immune attack. Both ifosfamide and gemcitabine have been shown to increase Fas. Infection of limb salvage improves osteosarcoma survival (Jeys et al., 2007). INT0133 update shows significantly superior survival after L-MTP-PE addition to chemotherapy: 78% vs 70% with chemo only ($p = 0.03$; Meyers et al. JCO 2008).
Objective: Recent experience (2006- present) with L-MTP-PE given using a compassionate access program will be shared.
Design/Methods: After agreeing to drug supply by IDM Pharma and informed consent of the patient, compassionate IND (CIND) from FDA and local IRB approval for L-MTP-PE was obtained. L-MTP-PE dose was 2 mg/M2 iv over 1 hour twice/week $\times$ 12 weeks, then weekly $\times$ 24 weeks. Aerosol GM-CSF was given at a dose of 250 micrograms in 2 cc saline BID 1 week on/1 week off. Chemotherapy with gemcitabine or ifosfamide was given in a standard manner.
Results: 18 patients (ages 10–21) with history of osteosarcoma metastases and/or local recurrence had compassionate L-MTP-PE; 15 of 18 also received aerosol GM-CSF. Two patients received gemcitabineand 2 ifosfamide. Patients receiving chemotherapy had no unexpected toxicities. Toxicity of L-MTP-PE infusions was minimal; no patient had grade 3 or 4 drug related toxicity except fever grade 3 and flu-like symptoms with first dose. This was prevented with ibuprofen and acetaminophen after subsequent doses. One patient had a pleural and pericardial effusion that was possibly L-MTP-PE and/or GM-CSF related and was removed from study. Three patients completed >40 doses. As of 11/07 eleven had recurred or had progression of known lung metastases; others had stable disease (4) or no evidence of lung metastases (3).
Conclusions: Local control measures such as surgery and alternative methods such as chemo-radiation and thermal ablation will remain the mainstay of control of osteosarcoma. For minimal disease or NED status in high-risk patients, L-MTP-PE and aerosol GM-CSF therapy may have potential benefit to be combined with chemotherapy with no apparent increase in toxicity.

Poster 224

Microarray Gene Expression Data Utilized to Identify Possible Serum Biomarkers in Neurofibromatosis Type 1 Associated Tumors

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Background: Patients with Neurofibromatosis type 1 develop benign plexiform neurofibromas that can progress to malignant peripheral nerve sheath tumors (MPNST). There are currently no reliable methods for detecting plexiform neurofibroma growth or transformation to MPNST in patients with NF1.
Objective: We hypothesized that *NF1 −/−* Schwann cells in plexiform neurofibromas secrete proteins which correlate with neurofibroma tumor burden and that there are unique proteins in serum that correlate with MPNST tumor burden. Identification of secreted proteins might lead to a serum test for neurofibroma burden and/or malignant transformation.
Design/Methods: Gene expression from 9 independent cultures of primary human Schwann cells were compared to 67 NF1 tumor samples including cultured neurofibroma Schwann cells and primary tumors on an Affymetrix platform by the NF1 Microarray Consortium*. The resulting profiles were filtered through the Secreted Protein Database with a confidence rank of 0–3. This yielded a list of genes that encode putative secreted proteins and are up-regulated in NF1 cell cultures and tumor samples compared to normal human Schwann cells. Differential expression of 9 mRNAs encoding putative secreted proteins was confirmed by quantitative real-time PCR.
Results: Protein expression of candidates utilizing ELISA based assays was confirmed. Two candidates were further analyzed in sera from a nu/nu MPNST mouse xenograft model and in human NF1 sera using ELISA based systems. One candidate revealed higher serum levels in NF1 patients with plexiform neurofibromas and malignant peripheral nerve sheath tumors compared to de-identified serum controls.
Conclusions: These results confirm the feasibility of utilizing microarray gene expression patterns to identify possible serum biomarkers of NF1 associated tumors.
*NF1 Microarray Consortium: Nancy Ratner, Shyra Miller, Bruce Aronow, Marco Giovannini, Anat Stemmer-Rachamimov, Peggy Wallace, Conxi Lazaro, and Grier Page.
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Poster 225

The Glycolysis Inhibitor 3-Brop Synergizes with Rapamycin and is a Novel Therapeutic Combination for Neuroblastoma

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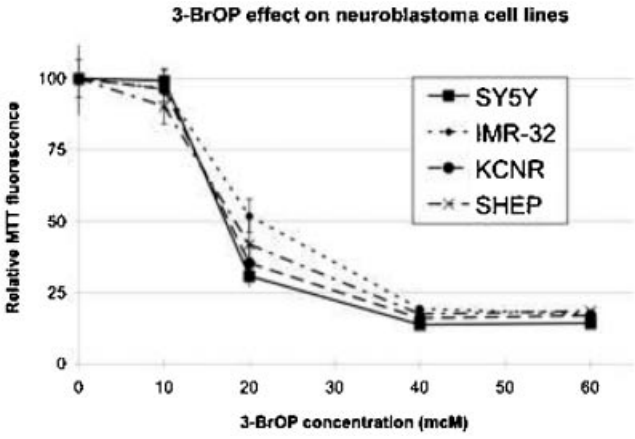
Background: Neuroblastoma is the most common extra-cranial tumor in children. Half of patients have high-risk disease characterized by rapid tumor growth, resistance to chemotherapy, and high likelihood of metastasis. Many cancer cells exhibit dependence on glycolysis for ATP generation (Warburg effect). We have previously shown that a novel glycolysis inhibitor, 3-BrOP, is effective against models of glioblastoma, colon carcinoma, leukemias and lymphoma. In this study, we hypothesized that inhibition of glycolysis may be an effective treatment strategy for neuroblastoma, and that combination with the mTOR inhibitor rapamycin would lead to metabolic dysregulation and cell death.

Objective: To determine if the glycolysis inhibitor, 3-BrOP, effects the growth and survival of neuroblastoma, and whether it synergizes with rapamycin.

Design/Methods: We determined the IC50 of 3-BrOP on a panel of neuroblastoma cell lines by the Alamarblue assay (MTT) and cell cycle analysis. The cell lines included SH-SY5Y (NMYC/1p36 germline), IMR-32 (NMYC+/1p36−), SMS-KCNR (NMYC+/1p36−), and SH-EP (NMYC/1p36 germline) cells. In addition, synergy was determined by serial dilution and combination with rapamycin.

Results: All neuroblastoma cell lines were sensitive to 3-BrOP. Single agent IC50s ranged from 17–21 mcM, suggesting a uniform effect not dependent on NMYC/1p36 status (Figure). At 40mcM of 3-BrOP, viability by MTT was <20% for all cell lines. In combination with 100nM rapamycin, using lower doses of 3-BrOP (25 mcM), we found <1% viability in SH-SY5Y and IMR-32 cells. This combination is highly synergistic with a combination index of 0.001 at 12–25 mcM of 3-BrOP.

Conclusions: These results demonstrate that inhibition of glycolysis via 3-BrOP induces growth arrest and death in a panel of human neuroblastoma cell lines. Furthermore, we observed significant synergy with the mTOR inhibitor rapamycin, suggesting that this dysregulation of multiple metabolic pathways is a promising therapeutic approach for neuroblastoma.



Poster 226

Therapeutic Valproate Levels Induce Osteosarcoma Invasion in Non-Metastatic Lines via Induction of Notch Pathway Activity

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Background: Recurrent osteosarcoma has an extremely poor prognosis, prompting sarcoma experts to turn increasing to new therapies, often without clinical and preclinical osteosarcoma experience. Valproate is a radiosensitizer and chemosensitizer by virtue of its histone deacetylase (HDAC) inhibiting properties. A recent report using carcinoid cell lines indicates that valproate induces Notch pathway expression and activity. We have published recently that Notch-mediated expression of Hes1 induces invasiveness and promotes metastasis in osteosarcoma cells.

Objective: To determine if valproic acid induces Notch pathway activity and promotes invasion of osteosarcoma.

Design/Methods: A panel of invasive and non-invasive osteosarcoma lines were treated with valproate at concentrations of 0.3, 0.6 mM (50 and 100 micrograms/ml), 1, 2 or 4 mM. Cell proliferation was assessed over four days. After 48 hours, expression of DLL1, Notch1, Notch2 and Hes1 was assessed via qPCR. Morphology was assessed via microscopy and invasiveness was measured using Matrigel.

Results: Invasive osteosarcoma lines had endogenous Hes1, indicating Notch pathway activity, and showed little or no change in Notch pathway expression or activity with any valproate concentration. In contrast, expression of DLL1, Notch1 and Hes1 were upregulated in non-invasive osteosarcoma cells exposed to 0.3 mM valproate. DLL1 and Hes1 expression increased in proportion to valproate concentration. Rapidly growing invasive lines showed dramatic cell death in a dose-dependent manner beginning at 1 mM valproate, while cell loss in slow-growing and non-invasive lines was not significant below 4 mM. At 0.3 mM valproate, non-invasive osteosarcoma lines demonstrated increased matrigel invasion (250-fold for Saos-2), but invasion decreased as valproate concentration increased. No increase in invasion was seen for invasive cell lines treated with valproate.

Conclusions: Valproate promotes increased invasiveness and metastatic potential for non-invasive osteosarcoma cell lines by inducing Notch-mediated Hes1 expression. No increase in invasion was seen for invasive lines, presumably because these lines constitutively express Hes1. Rather, invasive lines demonstrated valproate-mediated cell death at concentrations that are likely to be transiently achieved during routine use in patients. Valproate may be indicated for selected osteosarcoma patients with widespread metastatic disease, but should be avoided for patients with non-metastatic osteosarcoma.

Poster 227

Characterization of the 13q31 Amplicon in Alveolar Rhabdomyosarcoma

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Background: Alveolar rhabdomyosarcoma (ARMS), the most common soft tissue sarcoma of children, is an aggressive tumor with a poor prognosis. In addition to known chromosomal translocations, there are multiple areas of genomic amplification in these tumors, which likely contribute to their malignant potential.

Objective: The purpose of this study is to characterize the amplicon of the 13q31 chromosomal region in ARMS, determine the minimal region of amplification, and identify genes within this region that are overexpressed.

Design/Methods: Genomic DNA was extracted from 56 ARMS cases (8 PAX7-FKHR fusion-positive, 32 PAX3-FKHR fusion-positive, and 16 fusion-negative), and was analyzed on 50K Affymetrix arrays. Following normalization of raw data by GeneChip Genotyping Analysis Software (GTYPE), copy number data was analyzed with the Copy Number Analysis Tool (CNAT) viewer. The specific position of cellular genes was localized on the Human Genome map Build 35.1 using the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov). Gene expression was measured in RNA extracted from fusion-positive ARMS cases by quantitative RT-PCR using Taqman MicroRNA Assays (Applied Biosystems). Statistical analyses were performed in SPSS with the Mann Whitney test to determine significant differences in expression.

Results: Eight of the ARMS tumors showed amplification at the 13q31 locus. Six cases were PAX7-FKHR fusion-positive, one was PAX3-FKHR fusion-positive, and one tumor was fusion-negative ($p<0.0001$). The minimum region of amplification was found to contain the miR-17-92 microRNA cluster, and LOC390419, an as yet uncharacterized locus. Expression of 4 of 5 assayed microRNA's (miR-17-5, miR-19a, miR-19b, and miR-92) was significantly higher in the 7 fusion-positive tumors with amplification compared to the non-amplified fusion-positive tumors, with

p values ranging from 0.001–0.027. In contrast, expression of miR-18a was not significantly different in these tumors ($p = 0.467$).
Conclusions: Amplification at 13q31 in ARMS leads to significant overexpression of most microRNAs in the miR-17-92 microRNA cluster. This amplification is more common in PAX7-FKHR-positive tumors, implying a connection that needs to be further analyzed. As overexpression of this cluster has been implicated in the oncogenesis of B-cell lymphoma and small cell lung carcinoma, these findings in ARMS suggest the presence of common biological features and the possibility of common novel therapeutic strategies.

Poster 228
CCN3 Suppresses the Growth of Brain Tumor Cells

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Background: As a whole, brain tumors are the deadliest subgroup of pediatric malignancies. For tumors that cannot be completely resected, less than 20% of children are alive at three years. Neurosurgery and radiation can effectively treat only the local extent of disease; progression, relapse, and, ultimately, mortality result from malignant cells that remain after local treatments. Only through effective chemotherapy can brain tumor growth be targeted at the cellular level. The protein CCN3 was originally described in avian kidney tumors. Recent work has demonstrated that CCN3 is a secreted molecule that coordinates development in a number of tissues, including hematopoietic and neural. Initial findings in cancer have shown indirectly that CCN3 may play a role in cancer growth suppression and that loss or diminution of expression is associated with cancer growth.
Objective: To evaluate CCN3, a protein involved in neuronal differentiation, as a growth suppressor and a potential new therapeutic target in brain tumors.
Design/Methods: We examined the physiologic and molecular changes induced by CCN3 expression in brain tumor cell lines. These examinations consisted of RNA analysis by RT-PCR and quantitative PCR, protein analysis by Western immunoblot, and automated cell growth analysis.
Results: Here we demonstrate that CCN3 can and does have a role in growth of brain tumor cells. We have identified an astrocytoma cell line in which CCN3 is expressed at a very low level. Introduction of CCN3 expression in these cells resulted in significant growth slowing. Furthermore, we have found that expression of CCN3 results in a down-regulation of the Notch signal transduction cascade, providing a mechanism for CCN3-mediated growth suppression. Finally, we have begun investigation into the pharmacologic modulation of CCN3 expression. Taking a cue from a recent report of CCN3 in CML, we show that imatinib treatment of glioma cells results in an increase in CCN3 expression. Further, this imatinib treatment results in growth suppression of these cells.
Conclusions: CCN3 expression results in growth slowing in glioma cell lines, likely through inhibition of the Notch signaling pathway. Pharmacologic modulation of this expression may have a role in the treatment of brain tumors.

Poster 229
MS-275 Counters Decrease in Death Receptor Expression in MB Cell Lines

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Background: Epigenetic deregulation of pro-apoptotic genes is observed in a number of medulloblastoma samples suggesting that evasion of apoptosis may contribute to tumor cell survival. This is significant because agents such as histone deacetylase inhibitors with potential to de-repress gene expression may have therapeutic applications for medulloblastomas.
Objective: Demonstrate that the histone deacetylase inhibitor MS-275 will up-regulate the expression of genes involved in the apoptosis pathways and decrease medulloblastoma cell viability.
Design/Methods: Daoy and D283 medulloblastoma cell lines were treated with MS-275 and subjected to MTT, flowcytometric and western blotting analyses to measure cellular response to drug. Expression of DR4 and DR5 was measured by flowcytometry, immunofluorecence and real-time PCR. DR4 and DR5 real time PCR analysis expression was conducted in 20 paraffin embedded samples of human medulloblastomas. Samples were normalized to 18S RNA levels.
Results: We observed a concentration-dependent decrease in growth of Daoy and D283cells upon exposure to MS-275. This was confirmed by induction of caspase 3 activity and PARP cleavage. Mechanistically, MS-275 induces the expression and cell surface accumulation of the death receptors DR4 and DR5 as demonstrated by real time PCR and flowcytometry. Real time PCR demonstrated a 20 fold increase in *DR 4* gene expression as early as 12 hours and peaked at 20 hours (60 fold) in comparison with untreated controls. *DR5* expression was up-regulated at 12 hours (25 fold) and peaked at 20 hours (110 fold). Interestingly, 2/20 human medulloblastoma samples demonstrated expression of 5 fold *DR4* when compared with normal cerebellum, and 18/20 samples had non-detectable *DR4* expression. *DR5* expression was detected in 18/20 samples. The level of expression *DR5* was different among the samples. 2/20 samples expressed 30 and 40 fold increase when compared with normal cerebellum, while the remaining samples had less than 6 fold increase in gene expression.
Conclusions: *DR4* expression in human medulloblastomas is down-regulated whereas *DR5* levels did not differ significantly from that found in normal cerebellum. Our results indicate that MS-275 may have the potential to up regulate death receptor expression and activate apoptosis in medulloblastoma tumors.

Poster 230
Intravenous Administration of an Oncolytic HSV-1 Mutant Leads to Significant Virus Amplification in Distant Tumors in a Murine Orthotopic Model of Rhabdomyosarcoma

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Background: Effective therapy for metastatic sarcomas remains elusive. Oncolytic viruses have shown promise as anticancer agents and are currently being used in clinical trials mainly for local control of tumors by direct intratumoral injection.
Objective: We sought to determine if the systemic administration of an oncolytic HSV would lead to significant selective amplification of the virus in the tumors compared with other organs.
Design/Methods: The oncolytic virus rRp450 is attenuated by replacement of the ICP6 gene with the rat CYP2B1 gene, which converts cyclophosphamide to its active metabolites. We administered 3×10^8 plaque forming units (pfu) of rRp450 intravenously to mice bearing orthotopic, syngeneic murine rhabdomyosarcoma tumors. Biodistribution time-couses of virus DNA genomes and infectious virus were determined following organ harvest.
Results: The average virus titers in the tumor samples amplifed 1,700-fold over 48 hours. In contrast, the average titers in the spleen, lungs, liver, kidneys and brain at 1 hour were $<20\%$ of those measured in tumors and at 24 or 48 hours were undetectable. Analysis of viral genomes mirrored these results.
Conclusions: Systemically administered virus reached and selectively amplified in distant tumors. In contrast, infectious virus was rapidly cleared from other organs. Our data suggest systemic oncolytic HSV may be useful for therapy of metastatic sarcomas. The antitumor effect of systemic virus is being studied.

Poster 231
A Comparison of Clinical Trial Enrollment Between Adolescent and Young Adult Oncology Patients Treated at Affiliated Pediatric and Adult Oncology Centers

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Background: Over the past thirty years there has been a dramatic increase in the survival rates of pediatric cancer patients, but adolescent and young adult (AYA) patients aged 15–22 years old have not experienced the same improvement. The reasons for this discrepancy are multifactorial but it is clear that clinical trial enrollment correlates with better outcomes. Clinical trial availability in turn, is dependent upon the treatment center.
Objective: To focus on these two factors, we examined the rate of clinical trial accrual of AYA oncology patients when treated at affiliated pediatric and adult cancer centers, the Children's Hospital of Pittsburgh (CHP) and the University of Pittsburgh Cancer Institute (UPCI).
Design/Methods: With IRB approval we retrospectively analyzed all AYA cancer patients diagnosed between 2003 and 2006 at both CHP and the UPCI. We also examined their clinical trial enrollment status.
Results: There were 91 new AYA cancer diagnoses at CHP of which 24 (26%) were enrolled on a clinical trial. During the same time period, there were 121 new AYA cancer diagnoses at UPCI and only 5 patients (4%) were enrolled on a clinical trial. The rates of clinical trial enrollment between CHP and UPCI were significantly different ($p<.001$). When looking at the enrollment rates for specific diseases at CHP, the rate of enrollment was as high as 60% (for Ewing's sarcoma) to as low as 13% (for non-Hodgkin's lymphoma). Of the new AYA cancer diagnoses at UPCI during the same time, the only patients treated on clinical trials had diseases rare in pediatric oncology: 3 were diagnosed with melanoma, 1 with liver cancer, and 1 with colon cancer.
Conclusions: Our data demonstrate that clinical trial enrollment was superior when AYA patients were treated at a pediatric cancer center. To achieve better outcomes, patients need to be enrolled on available clinical trials and more trials need to be made available for this underserved population. One way to accomplish these goals is through the collaboration of pediatric and adult oncologists treating these patients on national cooperative trials within a defined AYA oncology program.

Poster 232

Multi-Targeted Receptor Tyrosine Kinase Inhibitor, ABT-869, Inhibits Proliferation of Ewing's Sarcoma, by Inhibiting PDGFR and C-kit Pathways

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Background: Ewing's sarcoma (EWS) is the second most common primary osseus malignancy in childhood. Despite multimodal approaches to therapy, only 60% of patients with localized disease are cured. Approximately, 30–40% of patients with metastatic disease have long-term survival beyond 5 years. Platelet-derived growth factor receptor (PDGFR) is expressed on EWS cells and its downstream signaling pathways are important for growth of tumor cells. The c-Kit tyrosine kinase receptor pathway has also been shown to be crucial for growth and progression in EWS. ABT-869 is a multi-targeted small molecule inhibitor of several receptor tyrosine kinases. It is a potent inhibitor of FLT3, c-Kit, VEGF and PDGF receptor family members. In vitro, ABT-869 inhibited the growth of 2 different Ewing sarcoma cell lines, A4573 and TC71, at an IC50 of 1.25 uM and 2 uM, respectively. Further investigation revealed that phosphorylation of PDGFR beta and c-Kit was inhibited in both of the cell lines when treated with ABT-869. To examine the effects of ABT-869 in vivo, SCID mice were injected with A4573 or TC71 cells and treated with daily oral preparations of ABT-869 at 40 mg/kg/day. Mice treated from the time of injection did not exhibit any tumor growth. One group of mice was injected with cells and allowed for tumor volume to reach 300 mm^3. Tumors from mice treated with ABT-869 demonstrated no growth in contrast to mice that were treated with corn oil placebo, in which persistent tumor growth was observed. Experiments to examine apoptosis in EWS cells after treatment with ABT-869 or control are in progress. Furthermore, we are studying the effects of ABT-869 on a metastatic disease model in mice injected with EWS cell lines transduced with GFP-Luciferin. Our preclinical studies demonstrate that ABT-869 is effective in Ewing's Sarcoma and provide rationale for the use of this drug in the treatment of patients with EWS, particularly in those patients with metastatic, recurrent, and refractory disease.

Poster 233

Fertility Preservation Practices for Pediatric Cancer Patients at Children's Oncology Group Centers

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Background: Over the past 30 years there has been an increase in the number of long-term survivors of pediatric cancer. Therefore, quality of life outcomes, such as fertility, have become a priority for pediatric oncologists. However, fertility counseling and preservation practices at Children's Oncology Group (COG) institutions are largely unknown.
Objective: The purpose of our survey was to assess the fertility services offered at COG institutions, so studies and interventions can be established to address any shortcomings.
Design/Methods: An internet survey was sent to COG Principal Investigators (PI's) who were asked to complete the first part and designate a fertility expert to fill out the second part.
Results: 70 PI's filled out part I and 57 designated fertility experts completed part II. 63% of part II was completed by the PI, while 28% was completed by another designated physician. 79% thought most post-pubescent males received fertility counseling, mostly performed by a physician. 69% stated that most of these patients were offered sperm cryopreservation while 37% of the centers have a sperm bank within their facility or at an onsite adult facility. 80% estimated 1 to 5 patients banked sperm in the previous year whereas 9% thought none did. Concerning female fertility, 37% thought post-pubertal female oncology patients received fertility counseling and only 9% were offered oocyte or embryo cryopreservation. 9% of respondents said that laboratory research and 18% said clinical research was being done at their institutions to address fertility. 25% thought that the available knowledge on fertility risks is good and 45% of those MD's doing the counseling said their confidence was good to excellent. Of those centers with fellowship programs, only 40% taught at least one lecture regarding risks of infertility and preservation techniques.
Conclusions: Pediatric oncologists are not well educated about the risks of infertility to their patients secondary to their cancer care. Few are referred to fertility experts and less utilize preservation techniques available. The reasons for this lack of comfort and decreased utilization are felt to be secondary to an inadequate body of medical evidence and the lack of available services respectively.

Poster 234

Localization of the Human Dihydroceramide Desaturase in Neuroblastoma Cells

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Background: The synthetic retinoid fenretinide has recently been demonstrated to inhibit the sphingolipid enzyme dihydroceramide desaturase (DEGS-1) and subsequently markedly increase the levels of dihydroceramides (dhcers) within neuroblastoma cancer cell lines (Kraveka, *J Biol Chem* 2007). This enzyme catalyzes the conversion of dihydroceramide to ceramide (Cer). Dihydroceramides have been thought to be biologically inactive molecules however; recent studies have demonstrated the involvement of endogenous dhCers in apoptosis, autophagy, and cell cycle arrest.
Objective: Although DEGS-1 plays an important role in the generation of Cer, there is little information about its biochemical characteristics and

regulation. It is postulated to be part of an enzyme complex common to other lipid desaturases, and located on the cytosolic face of the ER membrane. In this study we investigated its localization in the SMS-KCNR human neuroblastoma cell line.
Design/Methods: SMS-KCNR cells were either 1) transfected with fluorescent protein constructs fused with ER or mitochondrial signal peptides for accurate and specific targeting to sub-cellular compartments (Organelle Lights™ Invitrogen) or 2) permeabilzed and incubated with ER or mitochondrial specific antibodies. The cells were then visualized under a Zeiss LSM 510 confocal microscope. Subcellular fractionation was performed using density gradient fractionation. A LC/MS based *in-situ* assay for DEGS-1 activity was performed with whole cell lysates and subcellular fractions using pyridinium-conjugated dhCer analogues.
Results: We report localization of the human dihydroceramide desaturase protein to the mitochondria. Confocal microscopy in SMS-KCNR human neuroblastoma cells with antibodies against DEGS-1 and mitochondrial markers (MitoTracker® Red CMXRos, Cytochrome C, and Mito-GFP probes) demonstrated co-localization in the mitochondria. Only slight co-localization was observed with ER markers BAP-31 and ER-GFP probes. Furthermore, western blotting of mitochondrial fractions confirmed its location in the mitochondria. Moreover, using a novel *in-situ* assay for DEGS-1 enzyme activity we detected activity in isolated rat liver mitochondria and SMS-KCNR cell fractions.
Conclusions: The localization of DEGS-1 in mitochondria and near the ER, suggest it may be associated with mitochondrial associated membranes (MAMs). This study further implicates the mitochondria as the subcellular site of fenretinide's anti-proliferative effects.

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Successful Treatment of Kaposiform Hemangioendothelioma with Vincristine-Based Therapy

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Background: KHE is a rare vascular anomaly, usually occurring in infants, often presenting with large lesions on the trunk or retroperitoneum (RP). Most pts with KHE have low platelets (TP) or coagulopathy (CG). Surgical resection is usually not possible; corticosteroids (ST) and interferon (Ifn) have had limited success with serious side effects. The vinca alkaloids have proven anti-angiogenesis properties in vitro, and a long history of safety in children with cancer, even infants.
Objective: To describe the successful management of KHE with primarily VCR therapy.
Design/Methods: Pts with KHE were referred to our vascular anomalies program. Diagnosis of KHE was made by biopsy when feasible, or by the presence of characteristic lesions and TP or CG. Treatment was initiated as soon as possible, with VCR 0.05 mg/kg/wk for 12 consecutive wks; courses were repeated as needed. A total of 5 pts (age 5 wks–2 yrs, median 6 wks) were treated (mean 2.8 courses/pt).
Results: All 5 pts responded to VCR; 4 had complete responses without additional intervention. One of these progressed after 3 courses of VCR, and was treated with vinblastine and dactinomycin with CR. One pt is early in therapy and still requires ST for platelet support, but has >50% reduction in mass after one course of VCR. There were no episodes of febrile neutropenia.

Patient	Age	Site	Plt Ct(K)	CG	Prev Rx	# VCR courses	Response (TP/Mass)
1	2 yrs	Chest Neck	20	+	Ifn;ST	3	CR/CR
2	6 wks	Chest	6	+	None	5	CR/CR
3	7 wks	Liver Shoulder	243	+	None	2	CR/CR
4	6 wks	Trunk	14	+	None	3	CR/CR
5	4 wks	RP	4	+	ST	1	PR/CR

Conclusions: VCR therapy is successful in infants with KHE; all 5 of our pts responded; 4 have been off therapy for at least 1 year and are doing well. VCR therapy should be initiated early in the course of KHE; additional therapies may not be necessary.

Poster 236

Transient Complete Response to Chemotherapy and "Anti-angiogenic" (AA) Therapy in Recurrent Alveolar Rhabdomyosarcoma

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Background: Recurrent alveolar rhabdomyosarcoma has a grim prognosis.
Objective: We report a 15 y/o girl whose recurrent alveolar rhabdomyosarcoma (ARMS) had a complete response (CR) to unique combination therapy.
Design/Methods: At initial diagnosis, therapies included conventional vincristine, dactinomycin and cyclophosphamide (VAC) chemotherapy, surgery and radiation therapy to CR on IRSG-D9803. For her first recurrence, 36 months after diagnosis, she received therapy including irinotecan on phase II COG-ARST0121, also achieving partial response (PR), then CR after surgery and radiation. The second recurrence (biopsy: ARMS, PAX3-FKHR by FISH) was 56 months from original diagnosis. On phase I erlotinib, the tumor mass progressed. Chemotherapy then changed to VAC, abandoned after a single cycle because of toxicity. Vinorelbine, irinotecan, and oral cyclophosphamide cycles were given. Celecoxib, doxycycline and isotretinoin, purported AA agents, were added after PR at three months.
Results: Scans showed stable disease for five months; then at seven months, radiographic CR. Second look surgery by a pediatric surgeon and a gynecologic oncologist confirmed CR; peritoneal washings were negative. Patient continued on the same combination therapy for twelve months total, but had a third recurrence six months after surgery.
Conclusions: We cannot ascertain the activity of any of the agents, but the combination was well tolerated and the response was surprising. Toxicities included dysphagia, nausea and pancytopenia, all grade 1–2, without diarrhea.

Poster 237

Multi-Specific Targeting of Polymerized Liposomal Nanoparticles in Pediatric Sarcomas

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Background: Overall only 50–60% of pediatric sarcoma patients are cured with current multi-modality treatment regimens. In fact, there has been little progress in improving patient survival in the past five years and few new therapeutic agents on the horizon. The majority of systemic sarcoma therapy currently in use is not specifically targeted to tumor cells. Organic polymerized liposomal nanoparticles are spherical structures ranging from 1–250 nm composed of a combination of lipid and carbohydrate. They contain hollow cores which are useful for packaging a "payload." By using polymerized liposomal nanoparticles (PLNs) simultaneously expressing two different tumor-associated markers our hypothesis is that the specificity and efficiency of binding and delivery of chemotherapy will be much improved while reducing cytotoxicity to normal cells.
Objective: To demonstrate that multi-targeted nanoparticles bind more specifically to sarcoma cells, thereby enhancing the delivery of chemotherapy and cytotoxicity to these cancerous cells while reducing the bystander effects on normal cells.
Design/Methods: Generation of a model system for testing these multi-specific nanoparticles by creating transgenic sarcoma cell lines, which express heterologous surface markers. PLNs containing irinotecan, which

are coated with ligands to the cell surface markers. To test specificity of cell binding, entry, and cytotoxicity the techniques of flow cytometry, fluorescent microscopy and mixed culture competition assays were utilized.
Results: A model system to test multispecific nanoparticles has been generated by transducing Ewing sarcoma cell lines with the pCCRX lentiviral construct containing genes for carcinoembryonic antigen (CEA) and prostate stem cell antigen (PSCA). By using FACS and fluorescent microscopy, Ewing sarcoma cell lines stably express these heterologous surface markers without altering there behavior. Monoclonal anti-CEA and anti-PSCA antibodies, CT84.66 and 1G8 respectively, were produced and purified. F(ab)2 fragments of these antibodies were produced by pepsin and ficin digestion and then covalently linked to the PLN surface.
Conclusions: So far, we have created an idealized tmodel system to test multi-specific targeting of nanoparticles through the development of transgenic sarcoma cell lines expressing heterologous surface markers. We are currently in the process of testing these multi-targeted nanoparticles in vitro.

Poster 238
Antiangiogenesis in Metastatic Angiosarcoma

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Background: Angiosarcomas are uncommon malignant tumors in adults and are rare in the pediatric population. It is reported angiosarcomas compromised 1.3% of all benign and malignant vascular tumors in children. Unfortunately, angiosarcomas are aggressive tumors with frequent metastases and reported relapse rates as high as 75%. Overall prognosis is poor. Bevacizumab is a monoclonoal antibody that binds and inhibits vascular endothelial growth factor (VEGF). VEGF inhibition by bevacizumab is believed to reduce microvascular growth and slow metastatic progression.
Objective: We present a patient with primary lymph node angiosarcoma, with lung metastates, which progressed on standard chemotherapy. The addition of bevacizumab to the chemotherapy regimen resulted in resolution of the lung nodules and no further progression of disease.
Design/Methods: This 17 year old Caucasian male presented with inguinal lymphadenopathy. The lymph node was almost completely replaced by a spindled and epitheloid richly vascular tumor staining positive for CD31 and CD34. EM showed Weibel-Palade bodies and pinocytosis. With mitoses focally numbering up to 10/10 hpf, this was interpreted as an angiosarcoma, grade III/III, epitheloid-like variant. Metastatic work-up revealed multiple nodal involvement of the right inguinal and periaortic areas. Several bilateral pulmonary metastases were confirmed by biopsy. No other primary site was identified. Chemotherapy (POG 9553) consisted of vincristine, doxorubicin and ifosfamide. After two cycles, reevaluation revealed static lymph node disease but the development of three new pulmonary nodules. Chemotherapy was changed to vincristine (1.5 mg/m^2), topotecan (0.75 mg/m^2), cyclophosphamide (250 mg/m^2) and bevacizumab (15 mg/m^2).
Results: Approximately 6 weeks after initiation of the new regimen, reevaluation showed resolution of all pulmonary nodules and no new abnormalities or changes in lymph node disease. After 8 additional weeks of chemotherapy, surgery was performed to remove remaining nodes in the inguinal area and biopsy confirmed disease in 3 of 9 nodes removed. The patient is currently undergoing radiation treatment. The patient has tolerated his treatments quite well with minimal side effects and no major complications.
Conclusions: Angiosarcoma is a rare and aggressive malignancy. Current treatment with surgery, radiation and standard chemotherapy has largely been unsuccessful. The addition of new therapies such as bevacizumab may prove to be helpful in the future.

Poster 239
First Case Report of C-Myc Oncogene Amplification in Primary Meningeal Melanoma without Neurocutaneous Melanosis

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Background: Primary meningeal melanoma is a rare aggressive tumor, accounting for less than 1% of all melanomas. While various genetic abnormalities have been described in cutaneous and uveal melanomas, none have yet been reported in isolated meningeal melanoma.
Objective: To characterize cytogenetics and expression of the c-Myc and c-KIT oncogenes in pediatric primary meningeal melanoma.
Design/Methods: Cell preparations for chromosome analysis and FISH were obtained from short term cultures of tumor tissue and CSF. On unstained paraffin slides denaturing high-performance liquid chromatography (D-HPLC) was used to look for c-KIT mutations on exons 11, 13, and 17.
Results: A 10 year old Caucasian female presented complaining of three months' history of headache without seizure activity. She had two cutaneous lesions, a 1.3 cm × 2 cm hairy nevus on right forearm and a 0.6 cm round nevus on her anterior trunk, and no nevi of the head/neck. Neurologic exam was normal except for grossly hyporeflexic deep tendon reflexes. MRI of the brain revealed 3.8 cm × 3.8 cm right posterior parietal mass.
Gross total resection revealed primary meningeal melanoma, positive MART-1/melan A, focally positive HMB45. Biopsy of the two cutaneous lesions revealed compound congenital nevi with no atypia. MRI of the spine confirmed leptomeningeal spread. Three subsequent lumbar punctures were positive for melanoma cells with consistent cytogenetic abnormalities.
Cells from resection tissue and CSF displayed a complex karyotype with multiple structural abnormalities and modal number in the near-triploid range. Double minutes (DM) were present in 20–100 copies/cell. FISH revealed extensive c-Myc amplification (>30 copies) relative to a chromosome 8 control probe. c-Myc signals corresponded to positions of DM. FISH was negative for MYCN amplification. There was no c-KIT mutation.
Treatment consisted of focal conformal radiation 56 Gy to the tumor bed with oral temozolomide 90 mg/m^2/day for 42 days. Patient had progression of her disease shortly following radiation and expired 5 months from presentation.
Conclusions: Prognosis of primary meningeal melanoma remains extremely poor. Enhanced biologic understanding of this condition is critical to improving prognosis.

Poster 240
Use of Thalidomide in Adolescent Patients with Plexiform Neurofibromatosis (NFI)

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Background: Thalidomide is a non-vascular endothelial growth factor angiogenesis inhibitor. It's effective in malignancies with underlying abnormal angiogenesis. Tumor reduction and symptomatic improvement of NFI have been reported after thalidomide as a result of antiangiogenesis.
Objective: To present our experience with two adolescent patients with symptomatic neurofibromas of NFI.
Design/Methods: Medical records were reviewed confidentially. Prior to institution of therapy, a survey for thalidomide Education and Patient Safety was completed. Patients were placed on 150 to 200 mg daily of thalidomide.
Results: Patient 1 was a 17-year-old female, wheelchair-bound due to spinal cord compression who presented with paresis of lower extremities

and severe lower back pain. She received 5 narcotics without adequate pain control. One month after thalidomide was started she reported less intensity and decreased episodes of pain with reduction of pain medications to only 2 of the previously prescribed narcotics to achieve pain control. Although repeat CT scan showed no objective evidence of improvement of paraspinous neurofibromas, improvement of lower extremity strength was noted, with movement of the legs—not previously elicited. Patient 2 was a 13-year-old female who complained of back pain with radicular radiation and required 2 narcotics and neurontin for pain control. After thalidomide, pain became less severe and less pain medication was required. She reported better quality of life, with greater activity level. Both patients have been treated for >/ = 18 months. No adverse effects have been reported for either patient.

Conclusions: Plexiform neurofibromas cause significant morbidity in affected patients. Effective pain management is a major concern, with quality of life and function dependent on adequate therapy. We report 2 patients responding to thalidomide therapy. While there are no radiographic signs of improvement, both patients report subjective improvement. Neurologic improvement after thalidomide was reported in one of the patients. Our results are consistent with a previously reported study in which 5 of 8 children, 6.5–16 years, similarly improved with thalidomide.

Poster 241

Pleuropulmonary Blastoma in Children: Five Cases

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Background: Pleuropulmonary Blastoma (PPB) is an aggressive, malignant and extremely rare tumor that affects children. PPB usually presents as a big intrathoracic mass and a family history of cancer is frequently described. This neoplasm is histologically characterized by primitive blastema and a malignant stroma. Despite the introduction of multimodal therapy, the prognosis of patients with PPB remains poor.

Objective: To present a series of cases of PPB in a latin population.

Design/Methods: A retrospective review was carried out of patients diagnosed as having PPB at one institution over a period of 16 years. Clinical data, surgical notes, pathologic findings and treatment are discussed along with a review of the literature.

Results: The series included 5 female patients with a median age of 45 months. Respiratory distress was the most common clinical symptom and all the patients presented with huge pulmonary masses. None of the patients experienced metastatic disease at diagnosis. There were no primary displastic disease or family history of cancer reported. One patient developed a cardiac arrest during surgery and died. The other 4 patients underwent complete resection and received chemotherapy with either cisplatin, vincristine and cyclophosphamide or carboplatine, etoposide, vincristine, ifosfamide, actinomicin- D and doxorubicin. One patient remains free of disease 156 months after diagnosis, 1 patients is in remission 60 months after diagnosis and 1 patient is in remission 16 months after diagnosis. The last patient is lost to follow up.

Conclusions: PPB is an agressive tumor of childhood and because of its rarity no adequate therapy has been defined. After making the diagnosis, the main goal of therapy should be radical surgery, followed by chemotherapy. Some authors recommend adjuvant radiotherapy.

Poster 242

A Challenging Presentation of Generalized Infantile Myofibromatosis: Case Report and Review of the Literature

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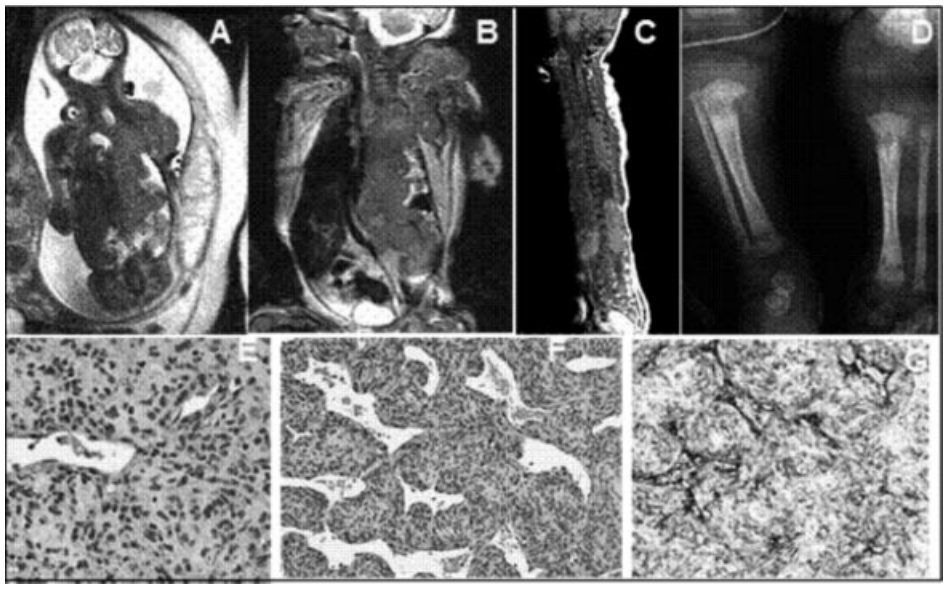
Background: Infantile Myofibromatosis (IM), although rare, is the most common fibrous tumor of infancy. Three types have been described: solitary, multicentric and generalized with visceral involvement and high mortality in the latter form.

Objective: We discuss the clinical, radiological and pathological features of generalized IM in a newborn with a life-threatening presentation.

Design/Methods: Pre- and postnatal Imaging studies as well as tissue biopsies were obtained.

Results: We present a newborn whose prenatal ultrasound and MRI (Fig. A) at 35 weeks gestation revealed a diffuse mass compressing the trachea and heart. After delivery via elective c-section, he was intubated. On physical exam he had a large left shoulder vascular mass protruding through the skin. His abdomen was markedly distended with palpable mass. He had spontaneous movements of his extremities and no evidence of bladder or bowel dysfunction.

Postnatal MRI demonstrated a massive vascular multilobulated lesion extending from the shoulder to the mediastinum, retroperitoneum with intrathecal infiltration to spinal canal from T2–T8 and T11–L2 levels causing cord compression (Fig. B & C). X-rays of long bones showed



multiple lytic lesions (Fig. D). Bone scan consisted with increased radiotracer uptake only for the shoulder soft tissue mass.

Biopsies were obtained demonstrating proliferation of spindle cells with small nuclei and low mitotic rate with condensation of the tumor cells around blood vessels (Fig. E, F). Immunostaining was positive for CD-34 (Fig. G) and smooth muscle actin and negative for cytokeratin or S-100. This was consistent with IM with prominent hemangiopericytoma-like features.

Surgical/neurosurgical management and radiation therapy were not feasible due to the extent of invasive disease. Chemotherapy was initiated with low dose intravenous weekly Vincristine and biweekly Actinomycin D. After two weeks of therapy, we observe a reduction of the shoulder mass. The baby remains in the intensive care unit.

Conclusions: There are few reported cases of Generalized IM in the literature. To the best of our knowledge this is the most severe case presenting prenatally. Multiple therapeutic modalities have been reported with variable success. They ranged from surgical management, radiation therapy and chemotherapy with cytotoxic and noncytotoxic agents. We are encouraged by his preliminary response to chemotherapy however; a large prospective multi-center trial is warranted to standardize treatment.